



**KING ABDULLAH UNIVERSITY**  
**OF SCIENCE AND TECHNOLOGY**

# **Biosafety Manual**

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**Health, Safety and Environment Department**

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# 1. INTRODUCTION

King Abdullah University of Science and Technology (KAUST) is an international research institution with a strong commitment to protecting the health and safety of its faculty, staff, students, and visitors, as well as protecting its assets and the environment. Moreover, it is the objective of KAUST to be a leader among its peers in the international research community for its health, safety, and environmental performance

Due to the special safety concerns related to the use of biological agents/materials, bloodborne pathogens, recombinant/synthetic nucleic acid (r/sNA) molecules and other emerging biotechnologies in research, this Biosafety Manual has been developed as a general guide for the safe conduct of work involving these materials at KAUST. Specifically it is intended to:

- Standardize procedures for the safe handling, containment, and disposal of biological agents, biological materials, bloodborne pathogens, and r/sNA materials;
- Promote compliance with all applicable standards, regulations and guidelines from the Kingdom of Saudi Arabia and other well-recognized international best practices;
- Ensure, to the extent possible, a safe and healthy workplace, campus, and community environment.

The contents of the manual have been developed from, and based upon internationally recognized standards and guidelines, including:

- [The GCC Infection Prevention and Control Manual](#), latest edition
- [Biosafety in Microbiological and Biomedical Laboratories](#) (BMBL), latest edition;
- [National Institute of Health \(United States\) Guidelines for Research Involving Recombinant DNA Molecules](#) (NIH Guidelines), latest edition
- [World Health Organization – Laboratory Biosafety Manual](#), latest edition

***All research activities at KAUST that involves biological agents/ materials, bloodborne pathogens, r/sNA molecules and other emerging biotechnologies shall be conducted in accordance with this Biosafety Manual.***

## 2. STATEMENT OF POLICY

KAUST has adopted the U.S. Public Health Service publication, [Biosafety in Microbiological and Biomedical Laboratories](#) (BMBL), latest edition, as the University standard for the use of biohazards;

All research and teaching involving r/sNA technology shall be treated as prescribed by the NIH's Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines). All projects involving r/sNA molecules require prior approval by the Institutional Biosafety and Bioethics Committee

(IBEC). All work requiring the use of live animals require prior approval by the Institutional Animal Care and Use Committee (IACUC).

Research involving Risk Groups 3 and 4 organisms is NOT permitted at KAUST.

Biosafety Level 3 or 4 containment facilities are currently NOT available in KAUST.

Newly isolated or recognized infectious agents of unknown pathogenicity shall be treated as requiring Biosafety Level 2 or higher (BSL-2+).

The KAUST Biosafety Manual should be regarded as the basis for general biosafety guidelines in the laboratory. Laboratory personnel will be expected to follow practices outlined in this manual, the BMBL publication, the NIH Guidelines, the KAUST [Laboratory Safety Manual](#), as well as the prudent practices specific to the project(s) in which they are involved.

### 3. RESPONSIBILITIES

The responsibility for health and safety within laboratories falls on each individual who works in the laboratory; however, it is the ultimate responsibility of the Principal Investigator or Laboratory Supervisor to ensure that persons working in the laboratory have received all appropriate training and have been provided with all the necessary information to work safely in laboratories under their supervision. Principal Investigators and laboratory supervisors have numerous resources available to them for helping to ensure a safe and healthy laboratory.

#### 3.1. Principal Investigators/Laboratory Supervisors

Principal Investigators and Laboratory Supervisors are accountable for the health and safety of personnel engaged in the use of biohazardous materials under their supervision. While specific tasks related to health and safety can be delegated, overall responsibility for health and safety cannot be delegated. To fulfill this responsibility, they must be familiar with KAUST's HSE policy, this Biosafety Manual, and the KAUST [Laboratory Safety Manual](#).

Their responsibilities include:

1. Adhere to the responsibilities and requirements as outlined in the [Faculty Handbook](#) – specifically section 4.3 - Research Safety;
2. Conduct risk assessments of their experiments;
3. Develop lab-specific standard operating procedures (SOPs) to ensure the safe use of biohazardous agents/materials and r/sNA molecules. The protocols must outline proper emergency procedures in the case of an accidental exposure of students, personnel, and/or the environment;
4. Train laboratory personnel so they understand the hazards involved, safety procedures required, and the emergency protocols in place;



5. Monitor access of laboratory visitors and assure their safety and the security of biohazards;
6. Comply with proper handling of biological waste by following recommendations and guidelines in this manual and the [Laboratory Hazardous Waste Manual](#);
7. Comply with all provisions of the KAUST Biosafety Manual;
8. Obtain prior approval from [institutional committees](#).
9. Ensure that all incidents and near misses occurring in their laboratories are reported to their supervisor and that an incident report is submitted to KAUST HSE using the online reporting system.

***The Principal Investigator/Laboratory Supervisor must ensure that all persons working with biohazards in the laboratory are properly trained, understand the hazards, know how to protect themselves, and are equipped and prepared to work safely.***

## 3.2. Laboratory Personnel

Laboratory personnel consists of those individuals who conduct their work in a laboratory and are at risk of possible exposure to biohazards or other health or physical hazards on a regular or periodic basis. Personnel include laboratory technicians, instructors and researchers, visiting researchers, graduate assistants, and students. The responsibilities of laboratory personnel include:

1. Follow all appropriate laboratory practices and safety requirements for the work being performed as per this Biosafety Manual and the KAUST [Laboratory Safety Manual](#);
2. Attend required health and safety trainings as designated by your supervisor;
3. Inform your principal investigator, supervisor, laboratory manager, or instructor of any safety hazards or unsafe working conditions in the workplace, classroom, or laboratory (e.g., faulty fume hoods, or malfunctions of emergency safety equipment);
4. Follow the standard operating procedures for your laboratory and the experiments being conducted - as provided by your supervisor;
5. Know all emergency procedures as established by the Principal Investigator/Laboratory Supervisor;
6. Report all incidents, near misses, and workplace injuries immediately to your supervisor and by using the online reporting system.

### 3.3. Health, Safety, and Environment (HSE)

The HSE department will provide technical information and program support to assist in compliance with KAUST's Biosafety Manual. This includes developing policies, recommendations, and guidelines (including those found in this Biosafety Manual and the [Laboratory Safety Manual](#)), competency, and serving as consultants in providing health and safety information to laboratory personnel. HSE responsibilities include:

1. Designating a University Biosafety Officer.
2. Provide information to the research community regarding procedures and regulations for the safe use of biohazardous agents/ materials, bloodborne pathogens, and r/sNA molecules in research;
3. Work with campus stakeholders to annually evaluate, implement, review, and make updates, as needed, to the Biosafety Manual;
4. Serve as a resource to review academic research protocols, risk assessments, submission of applications to [university committees](#), and standard operating procedures developed by Principal Investigators, Laboratory Supervisors and laboratory personnel for the use, decontamination, disposal, and spill cleanup of biohazards;
5. Provide Biosafety Training sessions for laboratory personnel and, upon request, assist Principal Investigators, Laboratory Supervisors in developing and conducting hands-on training sessions with laboratory personnel;
6. Investigate accidents and incidents involving biohazards;
7. Keep the senior administration, IBEC and IACUC informed on the progress of continued implementation of the Biosafety Manual and bring campus-wide issues affecting biosafety to their attention.

### 3.4. Guests and Visitors

As per the KAUST [Laboratory Safety Manual](#), due to the potential hazards and liability issues, guests or other persons (in particular children under the age of 16) are not permitted in hazardous work areas, with the exception of KAUST-sanctioned activities, e.g., tours, open houses, or other KAUST- related business as authorized by the Principal Investigator or Laboratory Supervisor. In these instances, all children under the age of 16 must be under careful and continuous supervision.

### 3.5. Visiting Researchers

There are potential risks associated with allowing access to laboratories and equipment by visiting scientists. These risks include theft or questions of ownership of intellectual property, bodily injury, and property damage. Departments should verify that all users of the lab have signed all documents required



by the University to use its research facilities (e.g. visiting faculty agreement) and have the required safety and health training and competence prior to allowing access to the laboratory and/or specialized equipment.

***Non-essential visitors and children must not be allowed access to a laboratory where biohazardous agents/ materials, bloodborne pathogens, or r/sNA molecules may be present.***

## 4. TRAINING REQUIREMENTS

In addition to Laboratory Safety Training, Hazardous Waste Training and Emergency Incident Preparedness Training, which are mandatory for all lab personnel, individuals working with biohazards are required to take Biosafety Training. If individuals are working with human tissues, cell lines, or other potentially infectious materials (OPIM), they are also required to take Bloodborne Pathogens Training annually. As the primary responsible person in the laboratory, the Principal Investigator is also required to take these trainings.

This training must occur **before** any individuals are permitted to work in KAUST laboratories. In addition to KAUST required training courses, the Principal Investigator or Laboratory Supervisor is responsible for providing additional lab specific training including:

- Risk assessments for the biohazards being used;
- Good microbiological techniques;
- Proper use of laboratory equipment and instrumentation;
- Personal protective equipment selection and use;
- Laboratory specific protocols and procedures;
- Decontamination and disinfection procedures;
- Proper waste disposal procedures;
- Spill clean-up and emergency procedures for the specific materials being used.

In order to meet training requirements for specific laboratory activities (i.e. wildlife research, human subjects, etc.), additional training can be recommended through the [Collaborative Institutional Training Initiative \(CITI\) Program](#).

## 5. RISK GROUPS AND BIOSAFETY LEVELS

There are several different systems (US National Institutes of Health, World Health Organization, Health Agency of Canada, European Union, etc.) for the classification of human, animal, and plant biohazards. All are similar in that biohazardous materials are categorized in risk groups based on their relative risk. Depending on the country and/or organization, this classification system might take the following factors into consideration:

- Pathogenicity of the organism;
- Mode of transmission and host range;
- Availability of effective preventive measures (e.g., vaccines);
- Availability of effective treatment (e.g., antibiotics);
- Other factors.

## 5.1. Risk Groups

Biohazards may be classified into Risk Groups (RG) based on their relative hazard and characteristics such as: the capability to infect and cause disease in a susceptible human or animal host, virulence as measured by the severity of disease, and the availability of preventive measures and effective treatments for the disease.

KAUST has adopted the US National Institutes of Health ([NIH](#)) Guidelines categorization of Risk Groups (RG) for infectious agents as follows:

- **RG1** – are not associated with disease in human healthy adults;

*Note: RG1 pathogens may pose harm to immunocompromised or immunosuppressed individuals.*

- **RG2** – are associated with disease which is rarely serious and for which preventative or therapeutics is often available;
- **RG3** – are associated with serious or lethal human disease for which preventative or therapeutics may be available;
- **RG4** – are associated with lethal human disease for which preventative or therapeutics are not readily available.

Classification of Infectious Microorganisms by Risk Group		
Risk Group Classification	NIH <a href="#">Guidelines for Research involving Recombinant DNA Molecules</a>	World Health Organization Laboratory <a href="#">Biosafety Manual</a>
Risk Group 1	Agents not associated with disease in healthy adult humans.	<b>(No or low individual and community risk)</b> A microorganism unlikely to cause human or animal disease.

Risk Group 2	Agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available.	<b>(Moderate individual risk; low community risk)</b> A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.
Risk Group 3	Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available.	<b>(High individual risk; low community risk)</b> A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.
Risk Group 4	Agents likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.	<b>(High individual and community risk)</b> A pathogen that usually causes serious human or animal disease and can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

**Risk Groups correlate with, but do not equate to Biosafety Levels (BSL).** A risk assessment will determine the degree of correlation between an agent's risk group classification and biosafety level.

Relation of risk groups to biosafety levels, practices and equipment				
Risk Group	Biosafety Level	Laboratory Type	Laboratory Practices	Safety Equipment
1	Basic Biosafety Level 1	Basic teaching, research	Good microbiological technique	None; open bench
2	Basic Biosafety Level 2	Primary health services; diagnostic services, research	Good microbiological technique plus protective clothing, biohazard sign	Open bench plus Biosafety Cabinets for potential aerosols
3	Containment Biosafety Level 3	Special diagnostic services, research	As Level 2 plus special clothing, controlled access, directional airflow	Biosafety Cabinets and/or other primary devices for all activities

4	Maximum Containment Biosafety Level 4	Dangerous pathogens	As Level 3 plus airlock entry, shower exit, special waste disposal	Class III Biosafety Cabinets, or positive pressure suits in conjunction with Class II Biosafety Cabinets, double- ended autoclave (through the wall), filtered air
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## 5.2. Biosafety Containment Levels

In contrast to Risk Groups, Biosafety Levels (BSL) prescribe practices, procedures and levels of containment for the particular biohazard. The most important element of containment is strict adherence to standard microbiological practices and techniques. Similar to Risk Groups, BSL are graded from 1 – 4. The four levels of biosafety, designated in ascending order by degree of protection provided to personnel, the environment, and the community, are combinations of laboratory practices, safety equipment, and laboratory facilities. The BMBL recommends that work be done using one of four levels of containment: Biosafety Level 1 (BSL-1), BSL-2, BSL-3, and BSL-4.

***It is KAUST policy that all laboratories adhere to these [BMBL](#) and [NIH](#) guidelines***

The primary risk criteria used to define the four ascending levels of containment are:

- Infectivity;
- Severity of disease;
- Transmissibility;
- The nature of the work being conducted.

The majority of work at KAUST involves Biosafety Level 1 (BSL-1) and 2 (BSL-2) practices. BSL-2 containment and practices is suitable for work with agents that are infectious to humans or animals where exposure may result in limited to moderate disease. The routes of exposure to these agents are typically through cuts and breaks in the skin, ingestion, and splashes to the mucous membranes (eyes, nose, and mouth). These agents or materials include:

- Microorganisms;
- Human blood, blood components, fluids, unfixed organs, tissues and cell lines (primary and established);
- Biotoxins (with a LD50 of less than 100 micrograms per kilogram of body weight in vertebrates) requiring BSL-2 containment;
- Recombinant DNA activities as described by the [NIH Guidelines](#) for Research Involving Recombinant DNA Molecules.

***Note: BSL-3 work is not permitted at KAUST at this time. There are no BSL-3 or BSL-4 facilities at the University – therefore all work with RG3 and RG4 agents is strictly prohibited.***

### 5.2.1. Biosafety Level 1 (BSL-1)

Biosafety Level 1 (BSL-1) is appropriate for undergraduate and secondary educational training and teaching laboratories, and for other facilities in which work is done with well-characterized agents not known to cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. The laboratory is not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

BSL-1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand washing.

The following standard practices, safety equipment, and facility requirements apply to BSL-1.

#### A) Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory;
2. Long hair is restrained so that it cannot contact hands, specimens, containers or equipment.
3. Gloves are worn to protect hands from exposure to hazardous materials.
  - a. Glove selected is based on an appropriate risk assessment.
  - b. Gloves are not worn outside the laboratory.
  - c. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
  - d. Do not wash or reuse disposable gloves, and dispose of used gloves with other contaminated laboratory waste.
4. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.
5. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory;
6. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area.
7. Mouth pipetting is prohibited; mechanical pipetting devices must be used;
8. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken

glassware must be developed, implemented, and followed. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions must always be taken with sharp items which include:

- a. Plasticware is substituted for glassware whenever possible.
  - b. Use of needles and syringes or other sharp instruments is limited in the laboratory and is restricted to situations where there is no alternative (e.g., parenteral injection, blood collection, or aspiration of fluids from laboratory animals or diaphragm bottles). Active or passive needle-based safety devices are to be used whenever possible.
    - Uncapping of needles is performed in such a manner to reduce the potential for recoil causing an accidental needlestick.
    - Needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
    - If absolutely necessary to remove a needle from a syringe or recap a needle, a hands free device or comparable safety procedure must be used.
    - Used, disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal immediately after use. The sharps container is located as close to the point of use as possible.
  - c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
  - d. Broken glassware is not handled directly. Instead, it is removed using a brush and dustpan, tongs, or forceps.
9. Perform all procedures to minimize the creation of splashes and/or aerosols.
10. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the laboratory.
11. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local, and state requirements. Depending on where the decontamination will be performed, the following methods are used prior to transport:
- a. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak-proof container and secured for transport. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label.
  - b. Materials to be removed from the facility for decontamination are packed in accordance with applicable local, state, and federal regulations.



12. A sign must be posted at the entrance to the laboratory when infectious materials are present. The sign may include the laboratory's Biosafety Level, name of the agent(s) in use, the name and phone number of the laboratory supervisor or other responsible personnel, PPE requirements, general occupational health requirements, and entering and exiting procedures. Agent information should be posted in accordance with the Biosafety Manual;
13. An effective integrated pest management program is required;
14. The laboratory supervisor/principal investigator must ensure that laboratory personnel receive appropriate training regarding their duties, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures and that appropriate records are maintained. **Personnel must receive annual updates and additional training when equipment, procedures or policies change.**
15. Personal health status may impact an individual's susceptibility to infection and ability to receive available immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk of infection, should be provided with information regarding immune competence and susceptibility to infectious agents. Individuals having these conditions are not prohibited from work, but are encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.
16. Animals and plants not associated with the work being performed are not permitted in the laboratory

#### **B) Special Practices**

None required.

#### **C) Safety Equipment (Primary Barriers and Personal Protective Equipment)**

1. Special containment devices or equipment, such as biosafety cabinets (BSCs), are not generally required.
2. Protective laboratory coats, gowns, or uniforms are worn to prevent contamination of personal clothing.
3. Protective eyewear is worn by personnel when conducting procedures that have the potential to create splashes and sprays of microorganisms or other hazardous materials. Eye protection and face protection are disposed of with other contaminated laboratory waste or decontaminated after use.
4. In circumstances where research animals are present in the laboratory, the risk assessment considers appropriate eye, face, and respiratory protection, as well as potential animal allergens.

#### **D) Laboratory Facilities (Secondary Barriers)**

1. Laboratories have doors for access control.

2. Laboratories have a sink for handwashing.
3. An eyewash station is readily available in the laboratory.
4. The laboratory is designed so that it can be easily cleaned.
  - a. Carpets and rugs in laboratories are not appropriate.
  - b. Spaces between benches, cabinets, and equipment are accessible for cleaning.
5. Laboratory furniture can support anticipated loads and uses.
  - a. Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
  - b. Chairs used in laboratory work are covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
6. Laboratory windows that open to the exterior are fitted with screens.
7. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.

### **5.2.2. Biosafety Level 2 (BSL-2)**

Biosafety Level 2 (BSL-2) builds upon BSL-1. BSL-2 is suitable for work with agents associated with human disease and pose moderate hazards to personnel and the environment. BSL-2 differs from BSL-1 primarily because:

1. laboratory personnel receive specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures;
2. access to the laboratory is restricted when work is being conducted; and
3. all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

The following standard practices, safety equipment, and facility requirements apply to BSL-2.

#### **A) Standard Microbiological Practices**

1. The laboratory supervisor enforces the institutional policies that control safety in and access to the laboratory.
2. The laboratory supervisor ensures that laboratory personnel receive appropriate training regarding their duties, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization) and that appropriate records are maintained. Personnel receive annual updates and additional training when equipment, procedures, or policies change. All persons entering the facility are advised of the potential hazards, are

instructed on the appropriate safeguards, and read and follow instructions on practices and procedures. An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered.

3. Personal health status may affect an individual's susceptibility to infection and ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. Individuals having such conditions are encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.
4. A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated as necessary.
  - a. The safety manual contains sufficient information to describe the biosafety and containment procedures for the organisms and biological materials in use, appropriate agent-specific decontamination methods, and the work performed.
  - b. The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, and other potential emergencies. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.
5. A sign incorporating the universal biohazard symbol is posted at the entrance to the laboratory when infectious materials are present. Posted information includes: the laboratory's Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the laboratory. Agent information is posted in accordance with the institutional policy.
6. Long hair is restrained so that it cannot contact hands, specimens, containers, or equipment.
7. Gloves are worn to protect hands from exposure to hazardous materials.
  - a. Glove selection is based on an appropriate risk assessment.
  - b. Gloves are not worn outside the laboratory.
  - c. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
  - d. Do not wash or reuse disposable gloves, and dispose of used gloves with other contaminated laboratory waste.
8. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.

9. Persons wash their hands after working with potentially hazardous materials and before leaving the laboratory.
10. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in laboratory areas. Food is stored outside the laboratory area.
11. Mouth pipetting is prohibited. Mechanical pipetting devices are used.
12. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware are developed, implemented, and followed; policies are consistent with applicable state, federal, and local requirements. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions are always taken with sharp items. These include:
  - a. Plasticware is substituted for glassware whenever possible.
  - b. Use of needles and syringes or other sharp instruments is limited in the laboratory and is restricted to situations where there is no alternative (e.g., parenteral injection, blood collection, or aspiration of fluids from laboratory animals or diaphragm bottles). Active or passive needle-based safety devices are to be used whenever possible.
    - Uncapping of needles is performed in such a manner to reduce the potential for recoil causing an accidental needlestick.
    - Needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
    - If absolutely necessary to remove a needle from a syringe (e.g., to prevent lysing blood cells) or recap a needle (e.g., loading syringes in one room and injecting animals in another), a hands-free device or comparable safety procedure must be used (e.g., a needle remover on a sharps container, the use of forceps to hold the cap when recapping a needle).
    - Used, disposable needles and syringes are carefully placed in puncture-resistant containers used for sharps disposal immediately after use. The sharps disposal container is located as close to the point of use as possible.
  - c. Non-disposable sharps are placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
  - d. Broken glassware is not handled directly. Instead, it is removed using a brush and dustpan, tongs, or forceps.
13. Perform all procedures to minimize the creation of splashes and/or aerosols.
14. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained

and equipped to work with infectious material. A spill procedure is developed and posted within the laboratory.

15. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local, and state requirements. Depending on where the decontamination will be performed, the following methods are used prior to transport:
  - a. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak-proof container and secured for transport. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label.
  - b. Materials to be removed from the facility for decontamination are packed in accordance with applicable local, state, and federal regulations.
16. An effective integrated pest management program is implemented.
17. Animals and plants not associated with the work being performed are not permitted in the laboratory.

## **B) Special Practices**

1. Access to the laboratory is controlled when work is being conducted.
2. The laboratory supervisor is responsible for ensuring that laboratory personnel demonstrate proficiency in standard microbiological practices and techniques for working with agents requiring BSL-2 containment.
3. Laboratory personnel are provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.
4. Properly maintained BSCs or other physical containment devices are used, when possible, whenever aerosol generating activities are conducted. These activities include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
  - a. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotors or centrifuge safety cups with loading and unloading of the rotors and centrifuge safety cups in the BSC or another containment device.
  - b. If it is not possible to perform a procedure within a BSC or other physical containment device, a combination of appropriate personal protective equipment and administrative controls are used, based on a risk assessment.
5. Laboratory equipment is decontaminated routinely; after spills, splashes, or other potential contamination; and before repair, maintenance, or removal from the laboratory.

6. A method for decontaminating all laboratory waste is available (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).
7. Incidents that may result in exposure to infectious materials are immediately evaluated per institutional policies. All such incidents are reported to the laboratory supervisor and any other personnel designated by the institution. Appropriate records are maintained.

**C) Safety Equipment (Primary Barriers and Personal Protective Equipment)**

1. Protective laboratory coats, gowns, or uniforms designated for laboratory use are worn while working with hazardous materials and removed before leaving for non-laboratory areas (e.g., cafeteria, library, and administrative offices). Protective clothing is disposed of appropriately or deposited for laundering by the institution. Laboratory clothing is not taken home.
2. Eye protection and face protection (e.g., safety glasses, goggles, mask, face shield or other splatter guard) are used for manipulations or activities that may result in splashes or sprays of infectious or other hazardous materials. Eye protection and face protection are disposed of with other contaminated laboratory waste or decontaminated after use.
3. The risk assessment considers whether respiratory protection is needed for the work with hazardous materials. If needed, relevant staff are enrolled in a properly constituted respiratory protection program.
4. In circumstances where research animals are present in the laboratory, the risk assessment considers appropriate eye, face, and respiratory protection, as well as potential animal allergens.

**D) Laboratory Facilities (Secondary Barriers)**

1. Laboratory doors are self-closing and have locks in accordance with the institutional policies.
2. Laboratories have a sink for handwashing. It should be located near the exit door.
3. An eyewash station is readily available in the laboratory.
4. The laboratory is designed so that it can be easily cleaned.
  - a. Carpets and rugs in laboratories are not appropriate.
  - b. Spaces between benches, cabinets, and equipment are accessible for cleaning.
5. Laboratory furniture can support anticipated loads and uses.
  - a. Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
  - b. Chairs used in laboratory work are covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.



6. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they are fitted with screens.
7. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
8. 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.
9. There are no specific requirements for ventilation systems. However, the planning of new facilities considers mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
10. BSCs and other primary containment barrier systems are installed and operated in a manner to ensure their effectiveness.
  - a. BSCs are installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs are located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
  - b. BSCs can be 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.
  - c. BSCs are certified at least annually to ensure correct performance.

### **5.2.3. Biosafety Level 2+ (BSL-2+)**

Biosafety Level 2+ (BSL-2+) or Biosafety Level 2 – enhanced, is used to describe laboratories where work with microorganisms is conducted with select biosafety practices and procedures that are found at BSL-3. This enhanced containment level is used for biological agents that may pose a higher risk than standard Risk Group 2 agents due to changes at the molecular level, procedures being used, amount of agent being propagated, etc. BSL-2+ is not a recognized containment level in biosafety guidance documents such as the US Centers for Disease Control’s (CDC) Biosafety in Microbiological and Biomedical Laboratories (BMBL) or the National Institute of Health’s Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules.

There is no current KAUST standardized list of microorganisms, viral vectors, or research projects that should be conducted at BSL-2+. This level of containment will be determined by the Biosafety Officer and Institutional Biosafety and Bioethics Committee (IBEC) based on a risk assessment of the agent(s) in use and the procedures being conducted.

#### 5.2.4. Biosafety Levels 3 and 4 (BSL-3 and BSL-4)

Biosafety Level 3 (BSL-3) is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious harm. Lab personnel must receive specific training in handling these pathogenic and potentially lethal agents and must be supervised by scientists competent in handling infectious agents and associated procedures. All procedures involving the manipulation of infectious materials must be conducted within BSCs or other physical containment devices.

Biosafety Level 4 (BSL-4) is applicable for work with exotic agents that pose a high individual risk for life-threatening disease by infectious aerosols and for which no current treatment is available (e.g., laboratories working with Ebola, Marburg, and pox viruses). These are high-containment laboratories have very complex and advanced facility requirements.

***IMPORTANT: Work that requires BSL-3 or BSL-4 containment is not permitted at KAUST at this time. There are no BSL-3 or BSL-4 facilities at the University – therefore all work with RG3 and RG4 agents is strictly prohibited.***

### 5.3. Animal Biosafety Levels (ABSL)

Similar to the biosafety levels mentioned in the previous sections, there are biosafety levels that describe work with vertebrate animals exposed to biological agents. These Animal Biosafety Levels provide guidance on practices, equipment, and facilities that are comparable to the laboratory biosafety levels. However, there are unique hazards associated with infected animals that must be understood by personnel who are working with the animals and addressed in the animal facility. Animal biosafety levels are designed to protect personnel from exposure to potentially infectious materials. Quarantine facilities and procedures must be utilized to prevent spread of infectious materials from animal to animal. Though there are four ABSL levels. Since ABSL and BSL levels are similar, the following sections will cover the additional items that are required at each level. The sections will only discuss levels 1 and 2 as these are the only levels permitted at KAUST.

#### 5.3.1. Animal Biosafety Level 1 (ABSL-1)

Animal Biosafety Level 1 (ABSL-1) is suitable for animal work involving well-characterized agents that are not known to consistently cause disease in immunocompetent adult humans and present minimal potential hazard to personnel and the environment. Special containment equipment or facility design may be required as determined by risk assessment.

Personnel receive specific training in animal facility procedures and are supervised by an individual with adequate knowledge of potential hazards and experimental animal procedures. The following standard practices, safety equipment, and facility specifications are recommended for ABSL-1.

##### **A) Standard Microbiological Practices**

1. The animal facility director/manager establishes and enforces policies, procedures, and protocols for biosafety, biosecurity, and emergencies within the animal facility.

2. Access to the animal room is limited. Only those persons required for experimental, husbandry, or support purposes are authorized to enter the facility.
3. Each institution ensures that worker safety and health concerns are addressed as part of the animal protocol review process. Consideration is given to specific biohazards unique to the animal species and protocol in use. Prior to beginning a study, animal protocols are reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) as appropriate.
4. The facility supervisor ensures that animal care, facility, and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization). Personnel receive annual updates and additional training when equipment, procedures, or policies change. Records are maintained for all hazard evaluations, training sessions, and staff attendance. All persons, including facility equipment personnel, service workers, and visitors, are advised of the potential hazards (e.g., naturally acquired or research pathogens, allergens); are instructed on the appropriate safeguards; and read and follow instructions on practices and procedures. An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered.
5. Facility supervisors ensure that medical staff are informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care, and manipulations.
6. Appropriate occupational medical services are in place, as determined by risk assessment.
  - a. An animal allergy prevention program is part of the medical surveillance.
  - b. Personnel using respirators for animal allergy prevention are enrolled in an appropriately constituted respiratory protection program.
7. A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated, as necessary.
  - a. The safety manual contains sufficient information to describe the biosafety and containment procedures for the experimental animals, organisms, and biological materials in use, appropriate agent specific decontamination methods, and the work performed.
  - b. The safety manual contains or references protocols for emergencies including exposures, medical emergencies, facility malfunctions, escape of animals within the animal facility, and other potential emergencies. A plan for the disposition of animals during emergency situations is included. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.

8. A sign is posted at the entrance to the animal room when infectious agents are present. Posted information includes: the room's Animal Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the animal room. Agent information is posted in accordance with the institutional policy.
9. Gloves are worn to protect hands from exposure to hazardous materials and when handling animals.
  - a. Glove selection is based on an appropriate risk assessment.
  - b. Consider the need for bite and/or scratch-resistant gloves.
  - c. Gloves worn inside the animal facility are not worn outside the animal facility.
  - d. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
  - e. Do not wash or reuse disposable gloves, and dispose of used gloves with other contaminated animal facility waste.
10. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.
11. Persons wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or manipulated.
12. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
13. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the animal facility.
14. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local and state requirements. Depending on where the decontamination will be performed, the following methods are used prior to transport:
  - a. Materials to be decontaminated outside of the immediate animal room are placed in a durable, leak-proof container and secured for transport. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label.

- b. Materials to be removed from the facility for decontamination are packed in accordance with applicable local, state, and federal regulations.

15. An effective integrated pest management program is required.

**B) Special Practices:**

None required.

**C) Safety Equipment (Primary Barriers and Personal Protective Equipment)**

1. Specialized devices or equipment for restraint or containment may be required as determined by appropriate risk assessment.
2. Laboratory coats, gowns, or uniforms are the minimum recommended to prevent contamination of personal clothing. Protective outer clothing is not worn outside areas where infectious materials and/or animals are housed or manipulated. Gowns and uniforms are not worn outside the animal facility.
3. Eye protection and face protection (e.g., safety glasses, goggles, mask, face shield, or other splatter guard) are used for manipulations or activities that may result in splashes or sprays of infectious or other hazardous materials. Eye protection and face protection are disposed of with other contaminated facility waste or decontaminated after use.
4. Persons having contact with Non-Human Primates assess the risk of mucous membrane exposure and wear protective equipment (e.g., face shield, surgical mask, goggles), as appropriate.
5. Additional PPE is considered for persons working with large animals.

**D) Animal Facilities (Secondary Barriers)**

1. ABSL-1 facilities should be separated from the general traffic patterns of the building and restricted as appropriate. Consider placing animal areas away from exterior walls of buildings to minimize the impact from the outside environment temperatures.
  - a. External facility doors are self-closing and self-locking.
  - b. Access to the animal facility is restricted.
  - c. Doors to areas where infectious materials and/or animals are housed open inward, are self-closing, are kept closed when experimental animals are present, and never propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.
2. The animal facility has a sink for handwashing.

- a. Emergency eyewash and shower are readily available, easily accessible, and appropriately maintained.
  - b. Sink traps are filled with water and/or appropriate disinfectant to prevent the migration of vermin and gases.
  - c. If open floor drains are provided, the traps are filled with water and/or appropriate disinfectant or sealed to prevent the migration of vermin and gases.
- 3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (e.g., walls, floors, ceilings) are water-resistant.
  - a. Floors are slip-resistant, impervious to liquids, and resistant to chemicals. Floors with drains are sloped toward drains to facilitate cleaning.
  - b. It is recommended that penetrations in floors, walls, and ceilings be sealed, including openings around ducts, doors, doorframes, outlets, and switch plates to facilitate pest control and proper cleaning.
  - c. Internal facility fixtures, such as light fixtures, air ducts, and utility pipes, are designed and installed to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.
  - d. External windows are not recommended; if present, they are resistant to breakage. Where possible, windows are sealed. If the animal facility has windows that open, they are fitted with fly screens.
  - e. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
- 4. Furniture can support anticipated loads and uses.
  - a. Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
  - b. Chairs used in animal areas are covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant and sealed to prevent harboring of insects/vermin.
  - c. Equipment and furnishings are carefully evaluated to minimize exposure of personnel to pinch points and sharp edges and corners.
- 5. Ventilation is provided in accordance with the Guide for the Care and Use of Laboratory Animals.
  - a. Ventilation system design considers the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.



6. Cages are washed manually or preferably in a mechanical cage washer. The mechanical cage washers have a final rinse temperature of at least 82°C (180°F). If manual cage washing is utilized, ensure that appropriate disinfectants are selected.

### **5.3.2. Animal Biosafety Level 2 (ABSL-2)**

Animal Biosafety Level 2 (ABSL-2) builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1. ABSL-2 is suitable for work involving laboratory animals infected with agents associated with human disease and posing a moderate hazard to personnel and the environment. It also addresses hazards from ingestion and from percutaneous and mucous membrane exposure. ABSL-2 requires that, in addition to the requirements for ABSL-1, a BSC or other physical containment equipment is used when procedures involve the manipulation of infectious materials or where aerosols or splashes may be created. Appropriate PPE is worn to reduce exposure to infectious agents, animals, and contaminated equipment. An appropriate occupational health program is in place, as determined by risk assessment. The following standard and special practices, safety equipment, and facility specifications are recommended for ABSL-2.

#### **A) Standard Microbiological Practices**

1. The animal facility director establishes and enforces policies, procedures, and protocols for biosafety, biosecurity, and emergencies within the animal facility.
2. Access to the animal room is limited. Only those persons required for experimental, husbandry, or support purposes are authorized to enter the facility.
3. Each institution ensures that worker safety and health concerns are addressed as part of the animal protocol review process. Consideration is given to specific biohazards unique to the animal species and protocol in use. Prior to beginning a study, animal protocols are also reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) as appropriate.
4. The supervisor ensures that animal care, facility, and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization). Personnel receive annual updates and additional training when equipment, procedures, or policies change. Records are maintained for all hazard evaluations, training sessions, and staff attendance. All persons, including facility equipment personnel, service workers, and visitors, are advised of the potential hazards (e.g., naturally acquired or research pathogens, allergens); are instructed on the appropriate safeguards; and read and follow instructions on practices and procedures. An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered.
5. Personal health status may affect an individual's susceptibility to infection and ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. Individuals

having such conditions are encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance. Facility supervisors ensure that medical staff are informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care, and manipulations.

6. Appropriate occupational medical services are in place, as determined by risk assessment.
  - a. An animal allergy prevention program is part of the medical surveillance.
  - b. Personnel using respirators for animal allergy prevention are enrolled in an appropriately constituted respiratory protection program.
7. A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated as necessary.
  - a. The safety manual contains sufficient information to describe the biosafety and containment procedures for the experimental animals, organisms, biological materials in use, and the work performed.
  - b. The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, escape of animals within the animal facility, and other potential emergencies. A plan for the disposition of animals during emergency situations is included. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.
8. A sign is posted at the entrance to the animal room when infectious agents are present. Posted information includes: the universal biohazard symbol, the room's Animal Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunization, respiratory protection), and required procedures for entering and exiting the animal room. Agent information is posted in accordance with the institutional policy.
9. Gloves are worn to protect hands from exposure to hazardous materials and when handling animals.
  - a. Glove selection is based on an appropriate risk assessment.
  - b. Consider the need for bite and/or scratch-resistant gloves.
  - c. Gloves worn inside the animal facility are not worn outside the animal facility.
  - d. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
  - e. Do not wash or reuse disposable gloves, and dispose of used gloves with other contaminated animal facility waste.

10. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.
11. Persons wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or manipulated.
12. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Decontaminate all potentially infectious materials before transport or disposal using an effective method. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the animal facility.
13. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local and state requirements. Depending on where the decontamination will be performed, the following methods are used prior to transport:
  - a. Materials to be decontaminated outside of the immediate animal room are placed in a durable, leak-proof container and secured for transport. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label.
  - b. Materials to be removed from the facility for decontamination are packed in accordance with applicable local, state, and federal regulations.
14. An effective integrated pest management program is required.
15. Animals and plants not associated with the work being performed are not permitted in the areas where infectious materials and/or animals are housed or manipulated.

## **B) Special Practices**

1. Animal care staff are provided 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 facility.
2. All procedures involving the manipulation of infectious materials that may generate an aerosol are conducted within a BSC or other physical containment device, when possible. If it is not possible to perform a procedure within a BSC or other physical containment device, a combination of appropriate personal protective equipment, administrative and/or engineering controls (e.g., downdraft table) are used, based on a risk assessment.
  - a. Restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint, chemical restraint) are used whenever possible.

- b. Equipment, cages, and racks are handled in a manner that minimizes contamination of other areas. Cages are decontaminated prior to washing.
- 3. Develop and implement an appropriate decontamination program in compliance with applicable institutional, local, and state requirements.
  - a. Equipment is decontaminated before repair, maintenance, or removal from the animal facility. A method for decontaminating routine husbandry equipment and sensitive electronic or medical equipment is identified and implemented.
  - b. Decontamination of an entire animal room is considered when there has been gross contamination of the space, significant changes in usage, and for major renovations or maintenance shutdowns. Selection of the appropriate materials and methods used to decontaminate the animal room is based on the risk assessment.
  - c. Decontamination processes are verified on a routine basis.
- 4. Incidents that may result in exposure to infectious materials are immediately evaluated per institutional policies. All such incidents are reported to the animal facility supervisor and any other personnel designated by the institution. Appropriate records are maintained.

**C) Safety Equipment (Primary Barriers and Personal Protective Equipment).**

- 1. Properly maintained BSCs and other physical containment devices or equipment are used whenever conducting procedures with a potential for creating aerosols, splashes, or other potential exposures to hazardous materials. These include the necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals. A risk assessment dictates the type of other physical containment devices used when BSCs may not be suitable.
  - a. When indicated by risk assessment, animals are housed in primary biosafety containment equipment appropriate for the animal species, such as solid wall and bottom cages covered with micro-isolator lids or other equivalent primary containment systems for larger animals.
  - b. If used, actively ventilated caging systems are designed to contain microorganisms. Exhaust plenums for these systems are sealed. Safety mechanisms are in place to prevent the cage and exhaust plenums from becoming positively pressurized if the exhaust fan fails. The system is also alarmed to indicate operational malfunctions. Exhaust HEPA filters and filter housings are certified annually.
- 2. Protective clothing, such as gowns, uniforms, scrubs, or laboratory coats, and other PPE are worn while in the areas where infectious materials and/or animals are housed or manipulated.
  - a. Scrubs and uniforms are removed before leaving the animal facility.
  - b. Reusable clothing is appropriately contained and decontaminated before being laundered. Animal facility and protective clothing is never taken home.

- c. Disposable PPE and other contaminated waste are appropriately contained and decontaminated prior to disposal.
- 3. Eye protection and face protection (e.g., safety glasses, goggles, mask, face shield, or other splatter guard) are used for manipulations or activities that may result in splashes or sprays from infectious or other hazardous materials when the animal or microorganisms is handled outside the BSC or another containment device. Eye protection and face protection are disposed of with other contaminated facility waste or decontaminated after use.
- 4. Persons having contact with NHPs assess the risk of mucous membrane exposure and wear protective equipment (e.g., face shield, surgical mask, goggles), as appropriate.
- 5. Additional PPE is considered for persons working with large animals.
- 6. Based on the pathogen and work performed, respiratory protection may be considered for staff enrolled in a properly constituted respiratory protection program.

#### **D) Animal Facilities (Secondary Barriers)**

- 1. ABSL-2 facilities should be separated from the general traffic patterns of the building and restricted, as appropriate. Consider placing animal areas away from exterior walls of buildings to minimize the impact from the outside environment temperatures.
  - a. External facility doors are self-closing and self-locking.
  - b. Access to the animal facility is restricted.
  - c. Doors to areas where infectious materials and/or animals are housed open inward, are self-closing, are kept closed when experimental animals are present, and are never to be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.
- 2. A handwashing sink is located at the exit of the areas where infectious materials and/or animals are housed or manipulated. Additional sinks for handwashing are located in other appropriate locations within the facility. If the animal facility has segregated areas where infectious materials and/or animals are housed or manipulated, a sink is also available for handwashing at the exit from each segregated area.
  - a. Emergency eyewash and shower are readily available, easily accessible, and appropriately maintained.
  - b. Sink traps are filled with water and/or appropriate disinfectant to prevent the migration of vermin and gases.
  - c. If open floor drains are provided, the traps are filled with water and/or appropriate disinfectant or sealed to prevent the migration of vermin and gases.

3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (e.g., walls, floors, and ceilings) are water resistant.
  - a. Floors are slip-resistant, impervious to liquids, and resistant to chemicals. Floors with drains are sloped toward drains to facilitate cleaning.
  - b. Penetrations in floors, walls, and ceiling surfaces are sealed, including openings around ducts, doors, doorframes, outlets, and switch plates to facilitate pest control and proper cleaning.
  - c. Internal facility fixtures, such as light fixtures, air ducts, and utility pipes, are designed and installed to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.
  - d. External windows are not recommended; if present, they are sealed and resistant to breakage. e. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
4. Furniture is minimized and can support anticipated loads and uses.
  - a. Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
  - b. Chairs used in animal areas are covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant and sealed to prevent harboring of insects/vermin.
  - c. Equipment and furnishings are carefully evaluated to minimize exposure of personnel to pinch points and sharp edges and corners.
5. Ventilation is provided in accordance with the Guide for the Care and Use of Laboratory Animals.
  - a. Ventilation system design considers the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.
  - b. The direction of airflow into the animal facility is inward; animal rooms maintain inward directional airflow compared to adjoining hallways.
  - c. A ducted exhaust air ventilation system is provided.
  - d. Exhaust air is discharged to the outside without being recirculated to other rooms.
6. Mechanical cage washers have a final rinse temperature of at least 82°C (180°F). The cage wash area is designed to accommodate the use of high-pressure spray systems, humidity, strong chemical disinfectants, and 82°C (180°F) water temperatures during the cage/equipment cleaning process.



7. BSCs and other primary containment barrier systems are installed and operated in a manner to ensure their effectiveness.
  - a. BSCs are installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs are located away from doors, windows that can be opened, heavily traveled areas, and other possible airflow disruptions.
  - b. BSCs can be connected to the animal facility 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 animal facility environment if no volatile toxic chemicals are used in the cabinet.
  - c. BSCs are certified at least annually to ensure correct performance.
8. Vacuum lines in use are protected with liquid disinfectant traps and in-line HEPA filters or their equivalent. Filters are replaced, as needed, or on a replacement schedule determined by a risk assessment.
9. An autoclave is present in the animal facility to facilitate decontamination of infectious materials and waste. A validated alternative process (e.g., alkaline digestion, incineration) may be used for decontamination and disposal of carcasses.

## **5.4. Plant Biosafety Levels (BL-P)**

Similar to the biosafety levels mentioned in the previous sections, there are biosafety levels that describe work with plants exposed to or transformed with biological agents. These Plant Biosafety Levels provide guidance on practices, equipment, and facilities that are comparable to the laboratory biosafety levels. However, there are unique hazards associated with genetically modified plants that must be understood by personnel who are working with the plants and addressed in the greenhouse facility. Plant biosafety levels are designed to protect the environment from potential release of genetically modified plant materials. Since BL-P and BSL levels are similar, the following sections will cover the additional items that are required at each level. The sections will only discuss levels 1 and 2 as these are the only levels permitted at KAUST.

### **5.4.1. Plant Biosafety Level 1 (BL1-P)**

BL1-P is used if there is no likelihood that the transgenic plant would survive and reproduce in nature, or if released would not cause any type of serious environmental risk. BL1-P research can be conducted in most modern research greenhouses and labs as well as in any growth chamber or growth room. BL1-P requires only a moderate level of containment and suffices for the vast majority of genetically engineered plant research.

#### **Greenhouse Access (BL1-P)**

Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, when experiments are in progress. Prior to entering the greenhouse, personnel shall be required to read and

follow instructions on BL1-P greenhouse practices and procedures. All procedures shall be performed in accordance with accepted greenhouse practices that are appropriate to the experimental organism.

#### **Records (BL1-P)**

A record shall be kept of experiments currently in progress in the greenhouse facility.

#### **Decontamination and Inactivation (BL1-P)**

Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.

#### **Control of Undesired Species and Motile Macroorganisms (BL1-P)**

A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens), by methods appropriate to the organisms and in accordance with applicable laws. Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.

#### **Concurrent Experiments Conducted in the Greenhouse (BL1-P)**

Experiments involving other organisms that require a containment level lower than BL1-P may be conducted in the greenhouse concurrently with experiments that require BL1-P containment, provided that all work is conducted in accordance with BL1-P greenhouse practices.

#### **Greenhouse Design (BL1-P)**

The greenhouse floor may be composed of gravel or other porous material. At a minimum, impervious (e.g., concrete) walkways are recommended. Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to contain or exclude pollen, microorganisms, or small flying animals (e.g., arthropods and birds); however, screens are recommended

### **5.4.2. Plant Biosafety Level 2 (BL2-P)**

A higher level of containment level, BL2-P, is used when the consequence of containment loss is predictably minimal. This means that research utilizing BL2-P can operate in standard greenhouse and laboratory facilities. An important consideration for BL2-P facilities is the location of research. For instance, special attention is required to work with disease resistance in wheat in an area adjacent to wheat production, e.g., by stipulating either higher containment or conducting the experiments when wheat is not actively growing in fields. Documentation, signage, and access to an autoclave may be needed. A growth chamber can generally satisfy BL2-P requirements.

#### **Greenhouse Access (BL2-P)**

Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, to individuals directly involved with the experiments when they are in progress. Personnel shall be required to read and follow instructions on BL2-P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms. A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility. A record shall be kept of experiments currently in progress in the greenhouse facility. The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Greenhouse Director, Institutional Biosafety and Bioethics Committee, and other appropriate authorities immediately (if applicable). Documentation of any such accident shall be prepared and maintained.

#### **Decontamination and Inactivation (BL2-P)**

Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility. Decontamination of run-off water is not necessarily required. If part of the greenhouse is composed of gravel or similar material, appropriate treatments should be made periodically to eliminate, or render inactive, any organisms potentially entrapped by the gravel.

#### **Control of Undesired Species and Motile Macroorganisms (BL2-P)**

A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable laws. Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.

#### **Concurrent Experiments Conducted in the Greenhouse (BL2-P)**

Experiments involving other organisms that require a containment level lower than BL2-P may be conducted in the greenhouse concurrently with experiments that require BL2-P containment provided that all work is conducted in accordance with BL2-P greenhouse practices.

#### **Signs (BL2-P)**

A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following:

1. the name of the responsible individual,
2. the plants in use, and
3. any special requirements for using the area. If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated on a sign posted on the greenhouse access doors. If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

#### **Transfer of Materials (BL2-P)**

Materials containing experimental microorganisms, which are brought into or removed from the greenhouse facility in a viable or intact state, shall be transferred in a closed non-breakable container.

#### **Greenhouse Practices Manual (BL2-P)**

A greenhouse practices manual shall be prepared or adopted. This manual shall:

1. advise personnel of the potential consequences if such practices are not followed, and
2. outline contingency plans to be implemented in the event of the unintentional release of organisms.

#### **Greenhouse Design (BL2-P)**

A greenhouse floor composed of an impervious material. Concrete is recommended, but gravel or other porous material under benches is acceptable unless propagules of experimental organisms are readily disseminated through soil. Soil beds are acceptable unless propagules of experimental organisms are readily disseminated through soil. Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to exclude pollen or microorganisms; however, screens are required to exclude small flying animals (e.g., arthropods and birds).

#### **Autoclaves (BL2-P)**

An autoclave shall be available for the treatment of contaminated greenhouse materials.

#### **Supply and Exhaust Air Ventilation Systems (BL2-P)**

If intake fans are used, measures shall be taken to minimize the ingress of arthropods. Louvers or fans shall be constructed such that they can only be opened when the fan is in operation.

#### **Other (BL2-P)**

BL2-P greenhouse containment requirements may be satisfied by using a growth chamber or growth room within a building provided that the external physical structure limits access and escape of microorganisms and macroorganisms in a manner that satisfies the intent of the foregoing clauses

## **6. BIOLOGICAL RISK ASSESSMENT**

Conducting a risk assessment of the experimental protocol using biohazardous materials is the responsibility of Principal Investigators. Risk assessment is a process used to identify the hazardous characteristics of a known infectious or potentially infectious agent or material, the activities that can result in a person's exposure to an agent, the likelihood that such exposure will cause a Laboratory Acquired Infection (LAI), and the probable consequences of such an infection. The information identified by risk assessment will determine the appropriate biosafety levels, microbiological practices, safety equipment, and facility safeguards that can prevent LAIs from occurring.

The information gained from a risk assessment needs to be communicated to all laboratory personnel working under the supervision of the Principal Investigator. This information includes the hazards of working with biohazardous materials, safe microbiological practices, and proper containment equipment.

Risk assessment is a subjective process relying on the expertise and knowledge of the Principal Investigator. Where there is insufficient information to make a clear determination of risk, it is the responsibility of the Principal Investigator to implement additional safeguards until more data is available.

The primary factors to consider in risk assessment and selection of precautions fall into three broad categories:

- The known hazards of the biohazardous materials;
- The hazards associated with the laboratory procedures to be used during the experiment;
- The training, competency, and good habits of the laboratory personnel working with the biohazardous materials.

The [BMBL](#) describes a five-step approach that gives structure to the risk assessment process which includes:

- Identify the hazards of the biohazardous material and perform an initial assessment of risks;
- Identify laboratory procedure hazards;
- Make a determination of the appropriate biosafety level and select additional precautions indicated by the risk assessment;
- Evaluate the proficiencies of staff regarding safe practices and the integrity of safety equipment;
- Review the risk assessment with a biosafety professional, subject matter expert, and the IBEC.

Additional information on conducting risk assessments, an example risk assessment form, and risk group classifications can be found on the [Biosafety webpage](#).

## 6.1. Routes of Exposure

The primary routes of exposure for biohazardous materials are:

- Direct skin, eye, or mucosal membrane exposure to an agent;
- Injection by a syringe needle or other contaminated sharp, or by bites from infected animals and arthropod vectors;
- Ingestion of liquid suspension of a biohazardous material, or by contaminated hand-to-mouth exposure;
- Inhalation of infectious aerosols - this hazard requires special caution because aerosols may not be a recognized route of transmission for the natural disease.

Aerosols are very small droplets of fluid that are formed during a number of routine laboratory operations and can spread through the air. The formation of aerosols should be avoided as much as possible. When working with organisms that hold a certain risk, all aerosol generating activities should be performed in a certified biosafety cabinet. Examples of aerosol generating activities include:

- Pouring, blending, grinding, homogenizing, sonication, and vortexing fluids;
- Falling droplets, cell sorters;
- Emptying a pipette by blowing;
- Opening of wet caps, lyophilized cultures, containers not at ambient pressure (e.g. freezer vials);
- Centrifugation by means of open tubes;
- Heating a wet inoculation needle or wire loop in a flame.

It is important to note that the severity of disease and the probable route of transmission caused by a laboratory acquired infection may be different than the route of transmission and severity associated with the naturally-acquired disease.

## 6.2. Personal Protective Equipment (PPE)

Personal protective equipment (PPE) can be an effective way – in combination with good microbiological techniques and containment – to prevent exposure to biohazardous materials. The following are applicable to Biosafety Levels 1 and 2:

1. Laboratory clothing (laboratory coat, gloves, etc.) must be worn at all times for work in the laboratory. These items should not be worn outside of the laboratory (i.e. canteens, coffee rooms, offices, libraries, staff rooms, restrooms, etc.);
2. Appropriate gloves must be worn for all procedures that may involve direct or accidental contact with blood, body fluids, and other potentially biohazardous materials. After use, gloves should be removed aseptically and hands must then be washed with disinfectant soap and water;
3. Personnel must wash their hands with disinfectant soap and water after handling biohazardous materials and before leaving the laboratory working areas;
4. Safety glasses, face shields (visors), or other protective devices must be worn when it is necessary to protect the eyes and face from splashes, impacting objects, and sources of artificial ultraviolet radiation;
5. Shorts and open-toed footwear are not allowed to be worn in KAUST laboratories;
6. Eating, drinking, smoking, applying cosmetics, and handling contact lenses are prohibited in the laboratory working areas;
7. Storing human foods or drinks anywhere in the laboratories is prohibited; empty bottles and food

containers should not be trashed within the laboratory;

8. Protective clothing that has been used in the laboratory must not be stored in the same lockers or cupboards as street clothing. Laboratory coats need to be washed separately from street clothing. [The Chemical Warehouse](#) provides this service.

Additional information on PPE selection and use can be found in the [Laboratory Safety Manual](#).

### 6.3. Good Microbiological Techniques

Basic working practices that everyone working with any level of biohazardous materials should incorporate as part of the daily routine for working in a laboratory include:

1. Laboratories using biohazardous materials must have a posted standard biohazard sign indicating BSL-1 or BSL-2 on the laboratory door. Other laboratories without biohazards should not use this sign;
2. Keep doors closed when experiments are in progress;
3. Wear a laboratory coat, safety glasses, and gloves;
4. Do not wear any jewelry and/or watches, and keep your hands clean and your nails short at all times;
5. Decontaminate any biohazard spills immediately;
  - a. Remove fluids with tissues or paper towels and dispose of them into the biohazardous waste bin;
  - b. Decontaminate the surface on which the material was spilled with 70% isopropanol or ethanol, or another proper disinfectant such as bleach solution (as identified within the lab's risk assessment);
  - c. Wash your hands with disinfecting soap and water afterwards, even if you were wearing gloves;
6. Minimize the creation and spread of aerosols by:
  - a. Only using closed tubes for centrifugation;
  - b. Preventing caps from becoming wet;
  - c. Heating wet inoculation needles the proper way - first heat the shaft, then the eye;
  - d. Allowing inoculation needles to cool down before putting them back into the fluid;
  - e. Never using force to empty pipettes - allow gravity to do its work;
  - f. Pouring out fluids in a gentle manner and never from a great height.

7. Mouth pipetting is prohibited:
  - a. Always use a pipetting bulb or other mechanical pipette;
  - b. Use a mechanical micro pipette for small amounts.
8. Decontaminate used materials before washing and reusing them:
  - a. This can be done by autoclaving them, or by immersing them in a proper disinfectant.
9. Biological waste:
  - a. Put the material in a biological waste container. The waste can be autoclaved or incinerated.
10. Wash your hands with disinfecting soap and water after the experiment and before leaving the laboratory – even though you were wearing gloves.

## 7. EMERGENCY PROCEDURES

All laboratories handling biohazards must establish written emergency procedures based on the biohazards used, as well as other hazards that may be present. This information needs to be included in the [Lab Safety Plan](#) as established in the [Laboratory Safety Manual](#).

The following items should be noted for the type of biohazards used in the laboratory in the event of an accident, exposure, and/or spill:

- Attend to any injured personnel;
- Call 911 from a landline (012-808-0911 from a mobile) for emergency assistance, and inform responders of biohazards that may be a threat;
- For spills in BSL-2 laboratories, evacuate the room and close the doors;
- After evacuating the area, wait to assist emergency responders;
- Notify [HSE@kaust.edu.sa](mailto:HSE@kaust.edu.sa) about a spill or exposure to a biohazards. Report spills, exposures, and injuries to your Principal Investigator or Laboratory Supervisor;
- Report the incident using the online reporting system.

### 7.1. Biohazard Spill Kit

A biological spill kit should be kept in each laboratory where work with biohazards is conducted. Basic components of a spill kit include:

- Concentrated appropriate disinfectant according to risk assessment (e.g. chlorine bleach, 70% isopropanol or ethanol);



- Note: 10% chlorine bleach solution must be freshly prepared at the spill time since it decomposes over time.
- Empty spray bottle (for diluting the concentrated bleach or other appropriate disinfectant);
- Package of paper towels;
- Several pairs of nitrile gloves (different sizes);
- Face protection: safety goggles and disposable face mask (couple of each);
- Disposable shoe covers;
- Biohazard bags;
- Small disposable broom with dustpan (or autoclavable broom and dust pan); tongs or forceps for collection of sharps and of disinfectant-soaked paper towels;
- Sharps container (medium size);
- Spill door sign (to be posted on the door to alert others about the spill);
- Copy of biohazard spill response SOP in a plastic sleeve.

## 7.2. Biohazard Spill Cleanup

The degree of risk involved with a spill depends on the volume of material spilled, the potential concentration of organisms in the material spilled, the hazard of the organisms involved, the route of infection of the organisms, and the diseases caused by the organisms.

Spills of biological agents can contaminate areas and lead to infection of laboratory workers. Prevention of exposure is the primary goal in spill containment and cleanup – the same as with chemical spills. In evaluating the risks of spill response, generation of aerosols or droplets is a major consideration.

If an accident generates droplets or aerosols in the laboratory room atmosphere, especially if the agent involved requires containment at Biosafety Level 2, the following steps should be taken:

1. Evacuate all personnel from the immediate area;
2. Wait approximately 30 minutes for the aerosols to settle before entering the spill area;
3. Remove any contaminated clothing and place in biohazard bag to be treated (autoclave or dispose);
4. Wear a disposable gown, shoe covers, eye protection, and gloves;
5. Initiate cleanup with disinfectant as follows:

- a. Place paper towels over spill;
  - b. Pour an appropriate disinfectant solution onto paper towels – from the outside of the spill to the center;
  - c. Allow sufficient contact time for the disinfectant to work. Contact time is a function of the disinfectant used, disinfectant concentration, and the agent being cleaned up. This information should be included in the Lab Safety Plan;
  - d. Place cleanup materials into appropriate biohazard waste container(s) for disposal;
  - e. If broken glass is involved, use forceps or other mechanical device to collect the broken pieces. Do not use hands to pick up broken glass. Place in biological sharps container (if contaminated) or broken glass container (if not contaminated).
6. Report the spill to your supervisor and report the incident using the online reporting system.

### **7.3. Biohazard Spills during Transport**

In the event of a spill involving a biohazardous material while transporting outside of the laboratory, the guidelines below should be followed:

- Transport biohazardous materials in an unbreakable sealed primary container, placed inside a second unbreakable container with a screw top lid. Label the outer container with the biohazard symbol;
- Should a spill occur in a public area, do not attempt to clean it up without appropriate personal protective equipment. Call 911 from a landline (012-808-0911 from a mobile) for assistance;
- As an interim measure, wear gloves and place paper towels, preferably soaked in disinfectant, directly on spilled materials to prevent spread of contamination. Keep other people away.

### **7.4. Spills of Human Blood or Other Potentially Infectious Materials**

When a spill of human blood or other potentially infectious material occurs, use the following guidelines to clean up the spill:

- Wear gloves and laboratory coat to clean up spill;
- If broken glass is present, use forceps or broom and dustpan to pick up and place directly in the biohazard sharps container (Never place sharp materials in biohazard bags). Do not use your hands to pick up broken glass or other sharp materials;
- Absorb blood with paper towels;
- Pour disinfectant from edge of spill to the center (careful not to splash) or place disinfectant soaked paper towels, such as bleach diluted 1:10 (vol/vol), on the spill site. Note: For large spills contact 911 from a landline (012-808-0911 from a mobile);

- After at least 30 minutes contact time, pick up the paper towels with forceps or broom and dustpan and re-wipe the spill area with disinfectant;
- Discard all contaminated non-sharp materials into biohazard bags, properly close and promptly dispose into yellow biohazard containers located at you waste accumulation area in the service corridor;
- Decontaminate your spill cleanup equipment with the appropriate disinfectant;
- Wash hands thoroughly with soap and water or use alcohol based sanitizer until you reach a location that has soap and water;
- Report the spill as soon as possible to your supervisor, LSR, biosafety specialist, and report the incident using the online reporting system.

## **7.5. Injuries Involving Biohazardous Materials**

In the event of injury or illness where medical assistance is required or large spill, call 911 from a KAUST landline or 012-808-0911 from a mobile phone. KAUST Health will provide ambulance transport, if necessary. Follow these steps:

- Call 911 from a landline (012-808-0911 from a mobile phone) for assistance and transportation to the KAUST Health emergency room;
- Accompany the injured person to the clinic and provide information to medical personnel about the accident/exposure.

### **7.5.1. Splashes to the Eyes**

When a splash involving a biohazardous material or a chemical occurs, it is important to use an eyewash immediately. Eyewash stations are designed to deliver continuous flushing of tepid water under gentle pressure to ensure that the material is flushed from your eye.

- First remove your gloves immediately.
- Remove any contact lenses if a biohazardous material, chemical, or other substance gets into your eye;
- Immediately go to the nearest eyewash and push the activation handle all the way on;
- Forcibly hold your eyes open and put your eyes or other exposed area in the stream of water and begin flushing for at least 10-15 minutes;
- Have one of your co-workers call 911 from a campus phone or 012-808-0911 from a mobile phone while you continue to irrigate your eyes;
- If you are alone, call 911 from a campus phone or 012-808-0911 from a mobile phone - after you have finished flushing your eyes;

- After seeking medical attention, report the incident to your supervisor and report the incident using the online reporting system.

### 7.5.2. Needlesticks and Cuts

Injuries resulting from a needle stick or cutting accident involving a biohazardous material should be handled as follows:

- Wash the exposed area immediately with soap and water. If there is a cut, wash the area with soap and water and allow the area to bleed freely. If blood or other potentially infectious body fluids enter the eye, nose, or mouth, flush with water for at least 15 minutes;
- If known, get the name or ID number of the person whose blood or infectious materials you came into contact with. If an object, e.g., needle, broken glass, etc., was part of the incident, save the object by placing it in a plastic bag or other container;
- Report immediately to KAUST Health for additional support and medical attention. Arrangements will be made for you to consult with the occupational health nurse or physician for confidential medical evaluation. There are certain medical procedures required for follow-up;
- Report the incident to your supervisor and the online reporting system.

### 7.5.3. Hepatitis B Vaccination

Laboratory personnel working with any human or human derived materials (cell lines, tissues, bodily fluids, etc.) are eligible to receive the hepatitis B vaccination. The hepatitis B vaccine and the subsequent titer testing is made available free of charge to all employees and students with occupational exposure. The hepatitis B vaccination review or declination form is mandatory to be completed and can be found on the [Biosafety webpage](#).

## 8. BIOSAFETY EQUIPMENT

In order for certain pieces of equipment used in a laboratory with biohazards to protect and contain the hazards, the equipment must be properly used, decontaminated, and maintained. Principal Investigators must provide training to laboratory personnel on how to properly use equipment found in laboratories under their supervision. Always follow manufacturer's instructions for use and maintenance of all equipment.

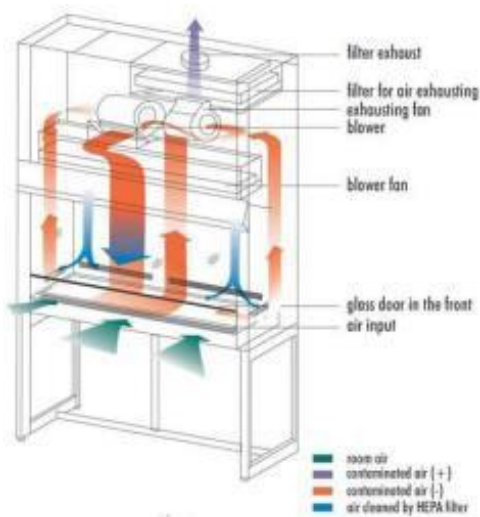
### 8.1. Biological Safety Cabinets (BSCs)

The Biological Safety Cabinet (BSC) is designed to provide protection to the product, the user, and the environment when appropriate practices and procedures are followed. Three types of BSCs (Class I, II, and III) and the horizontal laminar flow cabinet are described below.

The common element to all classes of BSCs is the high efficiency particulate air (HEPA) filter. This filter removes particulates of 0.3 microns with an efficiency of 99.97%. **However, it does not remove vapors or gases that are associated with chemical use.**

There are different classes of biosafety cabinets for various operations, the different classes and their features are described below:

1. **Class I BSCs** protect personnel and the environment, but not research materials. They provide an inward flow of unfiltered air, similar to a chemical fume hood, which protects the worker from the material in the cabinet. The environment is protected by HEPA filtration of the exhaust air before it is discharged into the laboratory or to the outside via the building exhaust;
2. **Class II (Types A1, A2, B1, and B2) BSCs** provide personnel, environment, and product protection. Air is drawn around the operator into the front grille of the cabinet, which provides personnel protection. In addition, the downward laminar flow of HEPA-filtered air within the cabinet provides product protection by minimizing the chance of cross-contamination along the work surface of the cabinet. Because cabinet air passes through the exhaust HEPA filter, it is contaminant-free (environmental protection). Cabinet exhaust may be discharged back into the laboratory (Type A cabinets) or ducted out of the building (Type B cabinets);



*Class II Biological Safety Cabinet*

***In KAUST, the most used BSC is Class II Type A2 non-canopy connected that exhausts 30% of the air within the laboratory and recirculates 70% within the cabinet.***

3. **Class III BSCs** (sometimes called Class III glove boxes) are designed for work with infectious agents that require BSL-4 containment, and provide maximum protection to the environment and the worker. The cabinet is gas-tight with a non-opening view window, and has rubber gloves attached to ports in the cabinet that allow for manipulation of materials in the cabinet. Air is filtered through one HEPA filter as it enters the cabinet, and through 2 HEPA filters before it is exhausted to the outdoors. This type of cabinet provides the highest level of product, environmental, and personnel protection;
4. **Horizontal or Vertical laminar flow "clean air benches"** are not BSCs. They discharge HEPA-

filtered air across the work surface and toward the user, providing only product protection. They can be used for certain clean activities, such as dust-free assembly of sterile equipment or electronic devices. However, they should never be used when handling cell culture materials or potentially infectious materials, chemicals, or as a substitute for a BSC in research laboratories.

Biosafety Cabinet	Protection Level		
	Worker	Environment	Experiment
Class I	Good	Good	Bad
Class II	Good	Good	Good
Class III	Excellent	Excellent	Excellent

Appendix A of the [BMBL](#) contains detail description and guideline on selecting the different types of BSCs.

### 8.1.1. Chemicals Use

Work with biohazardous materials often requires the use of different chemicals, many of which are volatile and hazardous. Therefore, the use of any chemicals and the associated hazards must be included as part of the risk assessment and when selecting which class of BSC to use. Flammable chemicals should not be used in Class II, Type A1 or A2 cabinets since vapor buildup inside the cabinet presents a fire hazard.

This is important because the electrical systems of Class II BSCs are not spark-proof. Therefore, a chemical concentration approaching the lower explosive limits of the compound must be prevented. Additionally, since non-exhausted Class II, Type A1 and A2 cabinets return chemical vapors back to the BSC work space and to the lab, they may expose the operator and other room occupants to toxic chemical vapors.

A chemical fume hood should be used for procedures using volatile chemicals instead of a BSC – unless the BSC is hard ducted/thimble connected, does not recirculate the air within the laboratory and you are using a small amount of the volatile chemical. However, use of small quantities of ethanol and isopropanol for disinfection purposes is acceptable. Many liquid chemicals, including nonvolatile antineoplastic agents, chemotherapeutic drugs and low-level radionuclides, can be safely handled inside Class II, Type A cabinets.

### 8.1.2. Certification

Class II BSCs require regular maintenance and certification by a professional technician for proper airflow and filter integrity to ensure it is working properly and that it protects you, your experiments, and the environment.

Certification must be performed on a Class II BSC:

- When it is first installed: damage or maladjustments to both the cabinet and HEPA filter can occur during shipment and installation;

- On an annual basis and the BSC must have a label indicating the date it was last tested;
- When the BSC is moved to a new location;
- When the HEPA filters are changed or other repairs are performed.

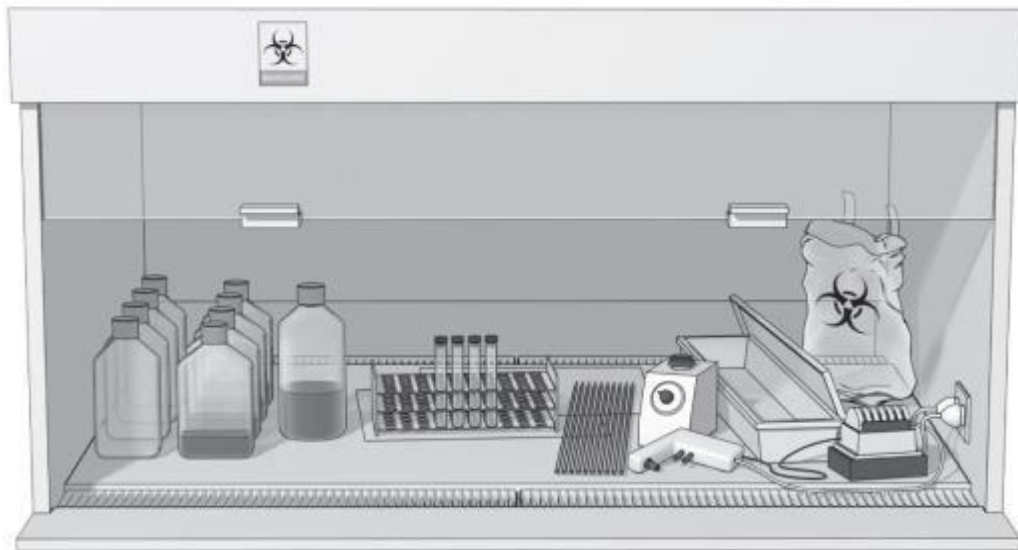
To have your BSC included as part of the annual certification cycle or if the date on the label of your BSC indicates it has been longer than one year since last being certified, Contact [HSE@kaust.edu.sa](mailto:HSE@kaust.edu.sa).

### **8.1.3. Proper Operation of Class II Biological Safety Cabinets**

Biosafety cabinets can be an important piece of equipment to protect against exposure to biohazardous materials, but must be properly used and operated. The following procedures should be used for proper operation of BSCs.

1. Prepare an experiment thoroughly and collect all necessary materials before beginning work;
2. Turn on the cabinet fan 15 minutes before beginning work;
3. Do not work in a BSC while a warning light or alarm is signaling;
4. Disinfect the cabinet work surface with 70% isopropanol, ethanol, or other disinfectant;
5. Only place materials and equipment in the cabinet which are required for the immediate work;
6. Keep the work area of the BSC free of unnecessary equipment or supplies. Clutter inside the BSC may affect proper air flow and the level of protection provided;
7. Never place objects over the front or rear intake grilles;
8. Wear gloves whenever working with biohazardous materials;
9. Remember to always work from a “clean” to a “dirty” side. On the dirty side you should place a small container for contaminated items such as pipette tips;
10. Move your arms slowly in a manner that will minimize the disruption of the airflow. Movement of hands in and out of the cabinet to discard pipettes into a container located outside of the cabinet creates turbulence and disrupts the air barrier that maintains sterility inside the cabinet;
11. Work as far to the back (beyond the air split) of the BSC workspace as possible;
12. Always use mechanical pipetting aids. Never use mouth pipetting due to the substantial risk of exposure to the biohazardous material you are working with;
13. Avoid using open flames inside BSCs. Besides the fire hazard, flames disrupt the airflow and contribute to the heat load inside the BSC. Flames have burned holes through HEPA filters and have caused explosions in BSCs. Instead of using an open flame, consider using a Bacti- Cinerator (carried by Fisher Scientific and VWR) - a better solution is to use disposable inoculation loops;
14. Locate liquid waste traps inside cabinet and use a hydrophobic/HEPA filter to protect the vacuum line;

15. When work is completed, remove equipment and supplies from the cabinet. Wipe the work area with 70% isopropanol or ethanol, and allow cabinet to run for 15 minutes;
16. Some BSCs are equipped with ultraviolet (UV) lights. However, if good procedures are followed, **UV lights are not needed**. *If a UV lamp is used, due to the limited penetrating ability of UV light, the tube should be wiped with 70% isopropanol every two weeks, while turned off, to remove dust. Follow manufacturer's instructions for cleaning the UV tube.* UV radiation should not take the place of disinfection using 70% Isopropanol or other suitable disinfectant of the cabinet interior. See the section on [Ultraviolet Lights](#);
17. The UV lamp should never be on while an operator is working in the cabinet;
18. Minimize traffic around the biosafety cabinet and avoid drafts from doors and air conditioning.



*A typical layout for working “clean to dirty” within a Class II BSC. Clean cultures (left) can be inoculated (center); contaminated pipettes can be discarded in the shallow pan and other contaminated materials can be placed in the biohazard bag (right).*

## 8.2. Vacuum Lines

All vacuum lines used to aspirate supernatants, tissue culture media, and other liquids that may contain microorganisms should be protected from contamination by the use of a collection flask and overflow flask. In addition, at BSL-2 and above, a hydrophobic vacuum line filter should be used.





*The left suction flask (A) is used to collect the contaminated fluids into a suitable decontamination solution; the right flask (B) serves as a fluid overflow collection vessel. An in-line HEPA filter (C) is used to protect the vacuum system (D) from aerosolized microorganisms.*

A hydrophobic filter (C-above) will prevent fluid and aerosol contamination of central vacuum systems or vacuum pumps. The filter will also prevent microorganisms from being exhausted by a vacuum pump into the environment. Hydrophobic filters such as the Gelman Vacushield are available from several scientific supply companies such as Fisher Scientific and VWR.

### 8.3. Centrifuges

Centrifuges are commonly used in laboratories using biohazardous materials. The following procedures should be used whenever operating a centrifuge:

- Examine centrifuge tubes and bottles for cracks or stress marks before using them. If any cracks or stress marks are present, then do not use the centrifuge tubes or bottles and properly dispose of them. Only use centrifuge tubes and bottles that are free of cracks or stress marks;
- Always use centrifuge safety buckets and sealed rotors to protect against release of aerosols;
- Do not cap tubes with aluminum foil as the foil may rupture or detach during centrifugation;
- When possible, fill centrifuge tubes and rotors in the biological safety cabinet;
- Never overfill centrifuge tubes since leakage may occur when tubes are filled to capacity. Fill centrifuge tubes no more than 3/4 full;
- Always ensure tubes, buckets, and rotors are properly balanced;
- After filling and sealing, wipe tubes and rotors with a disinfectant to remove any external contamination;
- After centrifugation, transport rotors to a biological safety cabinet to safely open rotors and remove tubes;

- When appropriate, use a vacuum system with in-line reservoirs and filters to remove supernatants from tubes. Avoid decanting or pouring off as this can cause aerosols;
- Work in a biological safety cabinet when pellets are re-suspended. Use a swirling rotary motion rather than shaking or vortexing. Let aerosols settle before the tube is opened;
- Always follow manufacturer's recommendations for maintenance and operation of centrifuges, including keeping use logs to establish appropriate maintenance intervals.

## 8.4. Lyophilizers and Ampoules

Production of aerosols may occur when ampoules are loaded or removed from the lyophilizer units. Use a biosafety cabinet when filling or opening ampoules with suspensions of biohazardous materials in order to contain aerosols. After use, decontaminate all surfaces of the unit after lyophilization. When handling ampoules, wrap them in a disinfectant-soaked towel, held upright, and snapped open at the neck. To reconstitute lyophilized samples, slowly add liquid to avoid creating aerosol particles from the dried material. Only use ampoules made of pyrex-type materials to prevent implosion under vacuum and breakage during handling or storage.

## 8.5. Microtomes and Cryostats

Hazards of microtomes and cryostats include the potential for aerosol generation and cutting hazards. Prepared materials should be considered potentially biohazardous and handled using good microbiological techniques. The following procedures should also be followed when using microtomes/cryostats:

- Always keep hands away from blades;
- Always retrieve samples, change blades, dislodge blocks, or clean equipment with appropriate engineering controls (i.e. forceps, tweezers, dissecting probes, and small brushes);
- Use caution when aligning and loading blocks, ensure the block holder is in a locked position;
- Use protectors/guards for knife-edges that may extend beyond microtome knife holder;
- Keep blocks wet when in the microtome to minimize airborne shavings during slicing;
- Wear appropriate PPE such as gloves, lab coat or gown, mask, safety glasses or goggles. Consider the use of surgical grade Kevlar gloves to provide additional protection from cuts and scrapes;
- Avoid freezing propellants that are under pressure and could cause splattering or droplets of biohazardous materials;
- Decontaminate equipment on a regular schedule using an appropriate disinfectant;
- Consider trimmings and sections of tissue as contaminated and dispose of as biohazardous waste;
- Do not move or transport microtome with the knife in position;

- Secure knives in containers when not in use;
- Do not leave motorized microtomes running unattended;
- Always follow manufacturer's recommendations for maintenance and operation of microtomes and cryostats.

## 8.6. Other Equipment

Ultra-low freezers, liquid nitrogen, and dry ice chests as well as refrigerators should be periodically checked and cleaned out to remove any broken ampoules, tubes, plates, etc. that contain biohazardous materials. Any spills must be cleaned up and decontaminated.

Other pieces of equipment used in a biosafety laboratory includes homogenizers, shakers, blenders and sonicators – all of which are capable of causing aerosols and should be operated in biological safety cabinets or covered with shields during use. Domestic (kitchen) homogenizers are not sealed and release aerosols. Only equipment designed for laboratory use must be used to minimize or prevent the release of such aerosols. Shields and the outsides of equipment should be decontaminated after use.

Laboratory personnel are responsible for disinfecting and decontamination equipment **before** allowing any maintenance work to be performed on the equipment.

The [World Health Organization – Laboratory Biosafety Manual](#), chapters on laboratory equipment, lists equipment and operations that may create hazards and suggests how such hazards may be eliminated or reduced.

## 9. TRANSPORTATION OF BIOLOGICAL MATERIALS

Transportation of biohazardous materials (infectious substances) or substances that are known or suspected to contain said materials, are regulated as dangerous goods by national and foreign governmental agencies and are subject to strict regulatory controls. For transport purposes, the term “infectious substance” is understood to include the term “etiologic agent.”

International and domestic transport regulations for infectious substances are designed to prevent the release of these materials in transit to protect the public, workers, property, and the environment from the harmful effects that may occur from exposure to these materials.

### 9.1. Importing/Exporting Biological Materials

Shipments of certain biological materials are regulated domestically and by the International Air Transportation Association (IATA), if sent by air. Any students or University employees involved in packaging materials, preparing samples for shipping, handling such packages, preparing related paperwork, or signing to authorize shipments must arrange for assistance from Research Materials Logistics Team ([RMLogistics@kaust.edu.sa](mailto:RMLogistics@kaust.edu.sa)).

Researchers need to coordinate the import or export of research materials with the Materials Transfer Office ([MTA@kaust.edu.sa](mailto:MTA@kaust.edu.sa)) in the Office of Sponsored Research (OSR). If you need to ship any infectious substance, biohazardous material, or other hazardous material, or if someone other than a commercial

company with the appropriate permits, training, packaging, paperwork, etc., plans to ship you an infectious substance, then please contact Research Materials Logistics Team ([RMLogistics@kaust.edu.sa](mailto:RMLogistics@kaust.edu.sa)) to arrange for appropriate shipping, permits and proper receipt of these materials.

As in all aspects of research and compliance, Principle Investigators are responsible to ensure that proper and appropriate shipping practices are followed when shipping biological materials and specimens. Failure to comply with these practices will put the Principle Investigator and the University at risk of substantial fines and penalties for improper shipping of infectious substances or other hazardous materials.

## 9.2. Transporting Biological Materials on Campus

The following is required for transporting biological materials throughout the KAUST campus:

- Materials must be transported in a secondary container that is shatterproof and leak-proof. Materials should never be carried in hands or pockets;
- The secondary container should be closeable and easy to decontaminate;
  - An absorbent pad (or similar material) can be placed inside the secondary container to absorb any spills.
- Label information must include the identity of the biological material or agent, the universal biohazard symbol;
  - Labels and signs can be found on HSE's [Signs and Labels webpage](#).
- The container should be carried directly to the intended laboratory and not taken to offices, cafeterias, or other public locations.
- The container must be disinfected before and after use.

***Please keep in mind that the transport of biological materials on roads by personal vehicle, public transportation – including University buses is strictly prohibited.***

## 10. DECONTAMINATION AND DISINFECTION

In order for a laboratory acquired infection (LAI) to happen, there are a number of requirements necessary for environmental transmission to occur. These requirements are referred to as the “chain of infection” and include:

- Presence of a pathogen of sufficient virulence;
- Relatively high concentration of the pathogen (i.e., infectious dose);
- A mechanism of transmission of the pathogen from environment to the host;
- A correct portal of entry to a susceptible host.

All of these requirements for the “chain of infection” must be present in order for an infection to occur. The removal of any one requirement will prevent transmission. Additionally, the pathogen in question must overcome environmental stresses to retain viability, virulence, and the capability to initiate infection in the host.

This “chain of infection” can be broken via antisepsis, decontamination, disinfection, and sterilization.

The definition of each term is as follows:

- **Antisepsis** - a substance that inhibits the growth and development of microorganisms without necessarily killing them. Antiseptics are usually applied to body surfaces;
- **Decontamination** – any process for removing and/or killing microorganisms. The same term is also used for removing or neutralizing hazardous chemicals and radioactive materials. The purpose of decontamination is to protect the laboratory worker, the environment, and anyone who enters the laboratory or handles laboratory products away from the laboratory.
- **Disinfection** – a chemical or mixture of chemicals used to kill microorganisms, but not necessarily spores. Disinfectants are usually applied to inanimate surfaces or objects;
- **Sterilization** - any item, device, or solution is considered to be sterile when it is completely free of all living microorganisms and viruses. The definition is categorical and absolute (i.e., an item is either sterile or it is not). A sterilization procedure is one that kills all microorganisms, including high numbers of bacterial endospores. Sterilization can be accomplished by heat, ethylene oxide gas, hydrogen peroxide gas, plasma, ozone, and radiation (in industry);

Disinfection is generally a less lethal process than sterilization. It eliminates nearly all recognized pathogenic microorganisms but not necessarily all microbial forms (e.g., bacterial spores) on inanimate objects. Disinfection does not ensure an “overkill” and therefore lacks the margin of safety achieved by sterilization procedures. The effectiveness of a disinfection procedure is controlled significantly by a number of factors, each one of which may have a pronounced effect on the end result. These factors include:

- The nature and number of contaminating microorganisms (especially the presence of bacterial spores);
- The amount of organic matter present (e.g., soil, feces, and blood);
- Type and condition of instruments, devices, and materials to be disinfected;
- Temperature;

Decontamination renders an area, device, item, or material safe to handle (reasonably free from a risk of disease transmission). The primary objective is to reduce the level of microbial contamination so that infection transmission is eliminated. The decontamination process may be ordinary soap and water cleaning of an instrument, device, or area. In laboratory settings, decontamination of items, spent laboratory materials, and biohazardous wastes is often accomplished by a sterilization procedure such as steam autoclaving, perhaps the most cost-effective way of decontaminating a device or an item. The

presence of any organic matter requires longer contact time with a decontamination method if the item or area is not pre-cleaned.

Decontamination of surfaces is never 100% effective; however, it can substantially reduce the number of viable microorganisms. It is important to use hot water and soap to clean the work surfaces, floors and door knobs during the weekly cleaning of the laboratory. Best practice dictates that you need to decontaminate twice in order to decontaminate successfully.

There are a number of disinfectants available and each has their specific uses and requirements for use such as, temperature, concentration, contact time, etc. Disinfectants include:

- Ethanol and/or Isopropanol (70% strength; **do not use 100%**);
- Sodium hypochlorite (household bleach diluted to a level of 10%);
- Formaldehyde (note: this is a known carcinogen);
- Hydrogen peroxide (6% solution);
- Iodophors
- Modern wide-spectrum disinfectants – examples include oxidizing chemicals, quaternary ammonium salts, surfactants, and other substances.

Biohazardous liquids and liquids that contain human blood or body fluids should be decontaminated in the laboratory using an appropriate chemical disinfectant then flushed down the drain. If liquid disinfectants are used, they must be shown to be effective against the organism(s) present.

The [World Health Organization – Laboratory Biosafety Manual](#), the monograph on decontamination and waste management, has detailed descriptions of various disinfectants, their uses and limitations, as well as descriptions of decontamination procedures.

## 10.1. Chlorine (Household Bleach)

Chlorine compounds can be effective disinfectants, however the following must be kept in mind:

- Working dilution is 10-fold (1 part bleach : 9 parts water), to 100-fold in water (1 part bleach : 99 parts water);
- Effective against vegetative bacteria, fungi, most viruses at 100-fold dilution;
- Effective against bacterial spores at 10-fold dilution;
- Very corrosive;
- Rapidly inactivated by organic matter;
- Solutions decompose rapidly - fresh solutions should be made daily.

Dilution in Water	% Available Chlorine	Available Chlorine(mg/l or ppm)
Not Diluted	5.25	50000
10-fold	0.5	5000
100-fold	0.05	500

Bleach solutions decompose at room temperature and should be made fresh daily. However, if stored in tightly closed brown bottles, bleach solutions retain activity for 30 days. The use concentration is dependent on the organic load of the material to be decontaminated. Use a 1% solution to disinfect clean surfaces, and 10% solution to disinfect surfaces contaminated with a heavy organic load. To disinfect liquid biological waste before disposal, add concentrated bleach to a final concentration of 10% (0.5% available chlorine).

## 10.2. Autoclaving

Bench top autoclaves are available in some labs to sterilize (treat) small quantities of biological waste materials. To ensure proper decontamination, it is generally recommended that a minimum temperature of 121°C (250°F) and pressure of 1.03 bar (15 psi) for 15 minutes be achieved, however, the actual time needed is dependent on the material being autoclaved. In addition to proper temperature, pressure and time, material to be sterilized must come in contact with steam and heat so it important not to overload the autoclave. Temperature indicators (e.g., autoclave tape) must be used with each load placed in the autoclave. More information can be found on the [Autoclave Safety webpage](#).

***Note: Autoclaves should not be used for the treatment of chemically contaminated materials which can cause adverse chemical exposures when the door of the autoclave is opened.***

Precautions for the use of autoclaves include:

1. Always follow manufacturer's instructions and recommendations for operation, care, and maintenance;
2. Responsibility for operation and routine care should be assigned to trained individuals in the laboratory;
3. A preventive maintenance program should include regular inspection of the chamber, door seals, and all gauges and controls by qualified personnel;
4. The steam should be saturated and free from chemicals (e.g. corrosion inhibitors) that could contaminate the items being sterilized;
5. All materials to be autoclaved should be in containers that allow ready removal of air and permit good heat penetration;
6. Materials should be loosely packed in the chamber for easy steam penetration and air removal. Bags should allow the steam to reach their contents;

7. For autoclaves without an interlocking safety device that prevents the door being opened when the chamber is pressurized, the main steam valve should be closed and the temperature allowed to fall below 80°C **before** the door is opened;
8. Slow exhaust settings should be used when autoclaving liquids, as they may boil over when removed due to superheating;
9. Operators should wear suitable gloves and visors for protection when opening the autoclave, even when the temperature has fallen below 80°C;
10. In any routine monitoring of autoclave performance, biological indicators or thermocouples should be placed at the center of each load. Regular monitoring with thermocouples and recording devices in a “worst case” load is highly desirable to determine proper operating cycles;
11. The drain screen filter of the chamber (if available) should be removed and cleaned daily;
12. Care should be taken to ensure that the relief valves of pressure cooker autoclaves do not become blocked by paper, plastic, etc. in the load.

#### 10.2.1. Biological Indicator Testing

The use of autoclave tape alone is not an adequate monitor of efficacy. Autoclave sterility monitoring indicators (*Bacillus stearothermophilus*) should be placed inside the center of the waste to verify performance. The results of the indicator tests should be recorded in a logbook. Biological indicator tests work to make sure that the autoclave achieves the appropriate sterilization temperature and maintains it long enough to fully decontaminate the items within the autoclave. To test autoclaves, indicators typically use bacterial spores which are considered hard to decontaminate and a color changing media indicator. This means that if autoclave fails the test the bacterial spores will be allowed to grow and consume the liquid media. The consumption will change the color of the media which can be observed by the researcher.

For KAUST, all autoclaves are required to have a biological indicator test performed monthly or at least with every load if the autoclave is used less than monthly. The validation standard operating procedure and testing log can be found on the [Autoclave Safety webpage](#).

### 10.3. Ultraviolet Light in Biological Safety Cabinets

Ultraviolet (UV) lamps are not recommended in BSCs or clean benches - nor are they necessary. If installed, UV lamps must be cleaned weekly per manufacturer's instructions to remove any dust and dirt that may block the germicidal effectiveness of the ultraviolet light. The lamps should be checked weekly with a UV meter to ensure that the appropriate intensity of UV light is being emitted. UV lamps must be turned off when the room is occupied to protect eyes and skin from UV exposure, which can burn the cornea and may increase the likelihood of causing skin cancer. When operating at optimum effectiveness, disinfection is achieved in a period of about 15 minutes, so lamps should not be left on for extended periods longer than this. Leaving the lamp on for unnecessarily long periods results in the lamp aging faster and results in the lamp needing to be replaced more frequently. If the cabinet has a sliding sash, close the sash when operating the UV lamp to prevent UV exposure to laboratory occupants. Please note, the sash does not completely block all the UV, so it is necessary to avoid the area within a meter or two



of the cabinet, while the UV light is on to minimize potentially harmful exposure.

Two articles that discuss UV lamps in BSCs and associated issues with their use include:

- [Position Paper on the Use of Ultraviolet Lights in Biological Safety Cabinets](#)
- [Use of Ultraviolet Lights in Biological Safety Cabinets: A Contrarian View](#)

## 11. BIOLOGICAL WASTE DISPOSAL

In the research laboratory or field environment, any non-sharp item that is contaminated with biohazardous materials is assumed to present an infectious disease transmission risk or an environmental release risk and is to be treated as biohazardous waste. These biohazardous materials include recombinant DNA, human or animal diagnostic specimen material, any microbiological culture material, etc. Any biological waste that is generated on the KAUST campus must be placed in a yellow biohazard labeled bag.

Examples of non-sharp items include, but are not limited to:

- Gloves and other disposable PPE that has been contaminated with specimen or culture material;
- Plastic-ware such as pipettes or pipette tips, culture plates, specimen vials, etc. that are contaminated with biohazards;
- Cultures and stocks of infectious agents (including specimen cultures from medical and pathological labs) from research and industrial laboratories, waste from the production of biological, discarded live and attenuated vaccines, and culture dishes and devices used to transfer, inoculate and mix cultures;
- Towels and bench paper that are biologically contaminated (Note: Bench paper that is used in areas where samples or cultures are opened and manipulated must be regarded as biologically-contaminated and therefore removed and managed as solid biohazardous waste);
- All culture or sample containers that are contaminated with biohazardous materials;
- Human and non-human primate blood, tissue, body fluids, and cell lines;
- Pathogenic agents (bacteria, fungi, viruses, protozoa, parasites, and prions);
- Recombinant DNA, cultures, stocks and cell lines containing recombinant DNA;
- Carcasses, tissues and bedding from animals exposed to biohazardous agents;
- Human pathological waste;
- Laboratory waste that has come in contact with the above listed biohazardous agents; and sharps waste.

## 11.1. General Disposal Guidelines

When disposing of biohazardous waste, the following procedures must be completed:

- All biohazardous waste should be disinfected or decontaminated appropriately before being disposed in the yellow biohazard bins typically located in the service corridors;
- Decontamination and disposal are the responsibility of the person/laboratory generating the waste;
- Collect disposable, solid materials contaminated by an infectious agent, excluding sharps, or broken or unbroken glass, into an autoclave bag within a sturdy container. Full bags should be labeled with a hazardous waste tag and placed in the large yellow biohazard waste collection bins located in the service corridor;
- Liquids containing a biohazardous material should be decontaminated by the addition of a disinfectant such as 1% - 10% bleach (depending on the content of biological material), held for at least 30 min to one hour, and then disposed of by pouring down the sink if it does not contain any other hazardous chemicals.

For additional information on biohazardous waste disposal, see the [Hazardous Waste Webpage](#).

## 11.2. Animal Carcasses

When disposing of animal carcasses, they must be placed in a biohazard bag large enough to contain the whole carcass. An “[Incinerate Only](#)” label must be affixed to the biohazard bag before finally being placed in the large biohazard bin in the service corridor.

## 11.3. Plant Materials

All plant materials, including items contaminated with plant materials, must be placed in an autoclavable biohazard bag and autoclaved. The autoclave time must be long enough to ensure that all biological materials have been fully decontaminated. Finally, place the autoclaved bag into the yellow biohazard bin in the service corridor.

## 11.4. Safe Handling and Disposal of Sharps

To prevent needle stick injuries:

- Avoid using needles whenever possible. If needles are required, try to use blunt tip needles over hypodermic needles. Blunt needles are readily available, they should be used for all applications except skin puncture is intended – puncturing skin is only intended use of hypodermic needles;
- Do not bend, break, or otherwise manipulate needles by hand;
- Do not recap needles by hand. Do not remove needles from syringes by hand;
- Immediately after use, **discard needle and syringe (whether contaminated or not) into puncture resistant sharps containers;**

- Never discard sharps into regular trash;
- Never discard sharps into bags of biological waste;
- Use care and caution when cleaning up after procedures that require the use of syringes and needles;
- Do not overfill the sharps containers. Completely close sharps containers when they are 3/4 full, and securely tape shut;
- Place sharps containers in a secondary tray in the lab's waste Satellite Accumulation Area designated in the service corridor for pickup. Place sharps containers in areas in which needles are commonly used in close proximity to the users. Sharps containers of various sizes are available at the [Chemical Warehouse](#).

## 12. LARGE SCALE BIOLOGICAL EXPERIMENTS

When working with biological agents in large-scale quantities, there are unique considerations that must be addressed in order to ensure worker and environmental protection. To help protect workers and the environment from hazards associated with large-scale biological research, the KAUST Health, Safety and Environment Department (HSE) has developed a [Large Scale Biosafety Manual](#). The manual complements principles of the general biosafety program stated in this Biosafety Manual, and applies to all large-scale work with biological materials (e.g. genetically modified organisms [GMO] and non-GMO, human, animal/zoonotic and plant pathogens). In addition to requirements of the laboratory biorisk assessment, the utilization of larger equipment and volumes of chemicals or raw materials requires risk management strategies beyond biological safety alone.

## Appendix A – Definitions

Term	Definition
Aerosols	Very small droplets of fluid formed during a number of routine laboratory operations and can spread through the air increasing the potential for a laboratory acquired infection
Biohazardous Materials	Any biological material (i.e. plants, animals, microorganisms, or their by-products) that may present a potential risk to the health and / or well-being of humans, animals or plants.
Biohazard Waste	Any waste material (liquid, semi-liquid, or solid waste) which may contain biohazardous materials.
Bloodborne Pathogens	Microorganisms in the blood or other body fluids that can cause illness and disease in people. These microorganisms can be transmitted through contact with contaminated blood and body fluids from humans or animals.
Containment	Used to describe safe methods, facilities and equipment for handling and managing biohazardous materials in the laboratory environment.
Established Cell Lines	Cell cultures that have been growing for extended periods of time and which are immortalized, so that they keep dividing infinitely.
Genetically Modified Organism (GMO)	Organisms whose genetic material has been altered in a way that is not possible by reproduction or natural recombination.
Infectious Substance	Material known to contain or reasonably expected to contain a pathogen.
Pathogen	A microorganism (including bacteria, viruses, rickettsiae, parasites, fungi) or other agent, such as a proteinaceous infectious particle (prion), that can cause disease in animals, plants, or humans.
Primary Cells	Cell cultures created by growing cells directly from a biopsy.
	Defined as either:
Recombinant/Synthetic Nucleic Acid (r/sNA) Molecules	<ol style="list-style-type: none"> <li>1. Molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or</li> <li>2. Molecules that result from the replication of those described above.</li> </ol>

## Appendix B – Biosafety Containment Level Table

<b>Biosafety Level</b>	<b>Risk Assessment</b>	<b>Practices and Techniques</b>	<b>Safety Equipment</b>	<b>Examples</b>
<b>BSL-1</b>  <i>Basic Laboratory</i>	Individual risk: <b>Low</b>  Community risk: <b>Low</b>	Standard Microbiological Practices.	None: primary containment provided by adherence to standard lab practices during open bench operations.	E. coli K12;  S. cerevisiae;  culture of most non-primate mammalian cells.
<b>BSL-2</b>  <i>Basic Laboratory with physical containment devices as required</i>	Individual risk: <b>Moderate</b>  Community risk: <b>Low</b>	BSL-1 practices plus: <ul style="list-style-type: none"> <li>• lab coats;</li> <li>• autoclaving biological waste preferred;</li> <li>• limited access;</li> <li>• biohazard warning signs on doors and equipment.</li> </ul>	Partial containment (i.e., Class I or II biosafety cabinets) for procedures which produce aerosols.	E. coli O157; Hepatitis B virus;  Salmonella typhimurium;  human blood;  many common human pathogens.
<b>BSL-3</b>  <i>Containment Laboratory with special engineering and design features</i>	Individual risk: <b>High</b>  Community risk: <b>Low</b>	BSL-2 practices plus: <ul style="list-style-type: none"> <li>• special protective clothing;</li> <li>• controlled access through entrance room;</li> <li>• biological waste must be autoclaved, within the laboratory.</li> </ul>	Partial containment equipment used for <u>all</u> manipulations of infectious materials; directional airflow.	Yellow fever virus;  M. tuberculosis;
<b>BSL-4</b>  <i>Maximum Containment Laboratory</i>	Individual risk: <b>High</b>  Community risk: <b>High</b>	BSL-3 practices plus: <ul style="list-style-type: none"> <li>• entrance through change room;</li> <li>• complete change of clothing to laboratory gear;</li> <li>• shower at exit;</li> <li>• all wastes decontaminated on exit from facility.</li> </ul>	Maximum containment equipment (i.e., Class III biosafety cabinet or partial containment in combination with full-body, air-supplied positive-pressure personnel suit) used for all procedures and activities.	Ebola virus;  Marburg virus;  propagation of herpes virus simiae.