Safety Guidelines on Microbiology and Biotechnology Experiments in School Laboratories

Science Education Section

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Safety Guidelines on Microbiology and Biotechnology Experiments in School Laboratories

The aim of these "Safety Guidelines" is to enhance safety in school laboratories by drawing laboratory users' attention to the necessary safety precautions to take when conducting practical activities in microbiology and biotechnology.

According to the Biosecurity Law of the People's Republic of China, "biosecurity" includes effective prevention of and response to the threat of dangerous biological agents and related factors, promoting the sound development of biotechnology, safeguarding the lives and health of the people, and protecting biological resources and the ecological environment.

It is important to safeguard biosecurity by applying safety precautions and measures to reduce the potential risk of handling microbes and to minimise the biological contamination of our working environment, the community, and the ecosystem. With reference to the "Biosecurity Laws", and the guidelines provided by the World Health Organisation (WHO) and the Centers for Disease Control and Prevention (CDC) of the USA, the major biosafety standards and risk assessment for handling microorganisms and other biotechnological specimens are discussed in these "Safety Guidelines".

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I. Handling Microorganisms

A. Routes of Microbial Transmission

The handling of microbial cultures is considered potentially hazardous due to the possibility of being in direct and/or indirect contact with known and/or unknown pathogens. Pathogenic microorganisms may gain access into the human body via four main routes: (1) the direct exposure to the microbial culture of a body surface with wounds or cuts; (2) the inhalation of aerosols (or airborne particulates) from the microbial culture; (3) the entry of microbes through the mucous membrane of eyes, nose, or mouth; and (4) the ingestion of contaminated food/drink. All microorganisms and their different forms of culture should therefore be handled with great caution.

B. Classification of Biosafety Levels of Laboratories and Risk Groups of Microbes

Before starting any microbial work task, it is important to understand the risks of working with potentially dangerous living microorganisms and the standard requirement of corresponding laboratory settings.

In general, the WHO has classified microorganisms into four risk groups, designated from 1 (the lowest risk) to 4 (the highest risk). The CDC of the US has categorised the requirement of laboratory settings into four biosafety levels (BSL). Each biosafety level has specific measures for controlling the use of microorganisms, the standard of safety equipment and the types of laboratory activity. The safety levels are arranged in ascending order, by degree of protection provided to individuals, the environment and the community. However, risk group levels of microorganisms may not necessarily correspond to the biosafety levels of laboratory settings.

The relationship between risk groups of microorgansims and BSL of laboratory settings and their specific requirements are listed in Table 1.

Table 1

Risk Group of microorganisms	**Biosafety levels (BSL) of laboratory setting**	**Microorganisms involved**	**Laboratory type**	**Laboratory practices**	**Safety requirement**
1	1	• No or low individual and community risk (e.g. nonpathogenic strain of *E. coli*)	• Basic teaching; • research	• Standard microbiological practices	• Open work surface and sink required
2	2	• Moderate individual risk; • low community risk (e.g. *Staphylococcus aureus*)	• Primary health services; • diagnostic services; • research	• Level 1 with limited access; • biohazard sign; • appropriate personal protective equipment (PPE)	• Level 1 with biological safety cabinet (BSC);* • autoclave
3	3	• High individual risk; • low community risk (e.g. *Mycobacterium tuberculosis*)	• Special diagnostic services; • research	• Level 2 with limited access; • special PPE with respirator	• Level 2 with appropriate BSC (e.g. class II); • hands-free sink; directional airflow; • Double self-closing and locking doors
4	4	• High individual and community risk (e.g. Ebola virus)	• Handling dangerous pathogen units	• Level 3 with full PPE with positive pressure, air-supplied; • change clothing before entering; • decontaminate protective suit, and shower before exiting	• Level 3 with isolated zone; • Class III BSC (with equipment for dedicated air supply and exhaust); • vacuum and decontamination systems



Fig. 1 Class II BSC

*Note: A biosafety cabinet (BSC) provides a physical containment between the experimental operators and pathogenic microbes. BSC is divided into three classes. Class I BSC is commonly used for the disposal of infectious waste. Class II BSC (Fig. 1) is often used for cell culture and viral isolation. Class III BSC is used for handling microbes which would lead to diseases with a high mortality rate.

C. Standard Microbiological Practices

Standard microbiological practices described in this chapter are applicable to normal biology laboratories in Hong Kong secondary schools. These practices may include:

1. Wear laboratory gowns to prevent contamination of personal clothes.

2. Wear safety goggles for procedures in which splashes of microorganisms or other hazardous materials are anticipated.

3. Wear latex or nitrile gloves before handling any chemical or biological samples. Cover cuts and wounds with sterile dressings. **Do not wash or reuse disposable gloves.**

4. Wear PPE such as surgical mask if instructed by teachers or laboratory technicians.

5. Tie up long hair and fix your tie.

6. Do not eat or drink in the laboratory.

7. Always employ aseptic technique when handling microbial cultures. Before and after work, clean the work surface with disinfectant and wash hands. Sterilise and dispose of all unwanted cultures appropriately after the experiment.

8. Use pipette fillers to help transfer liquid cultures. **Mouth pipetting is strictly forbidden in the laboratory.**

9. Discard unwanted culture media in disposal container with 10% chlorine bleach.

D. Microbial Culture

a) Preparation of Culture Media

Culture media should be prepared according to the instruction manual from the supplier. If the medium needs to be autoclaved, the container must not be filled with more than half the total volume.

b) Labelling and Taping

Using a permanent pen, label around the edge of the base (the part for holding the agar) of the Petri dishes, rather than the lid. This is to prevent mix-up in case the bases are separated from the lids. Always hold the whole Petri dish set (the base and the lid together) when transferring it.

Before incubation, stick adhesive tape on the dish, as shown in Fig. 2, to avoid accidental separation of the base from the lid (Fig. 2). Holding the dish in this manner allows gaseous exchange for the cultured microbes. Complete sealing of the plate is not preferred, as this would prevent air flow and create anaerobic conditions to favour the growth of anaerobic microbes.

After the incubation period, the agar plates can be sealed with parafilm as shown in Fig. 3 and stored at 4°C before observation. The sealing can help retain moisture of the culture media and reduce contamination introduced during observation.

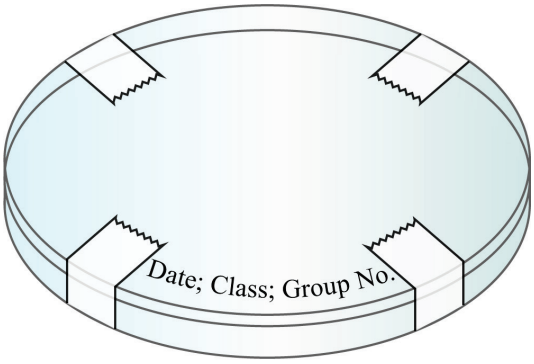


Fig. 2

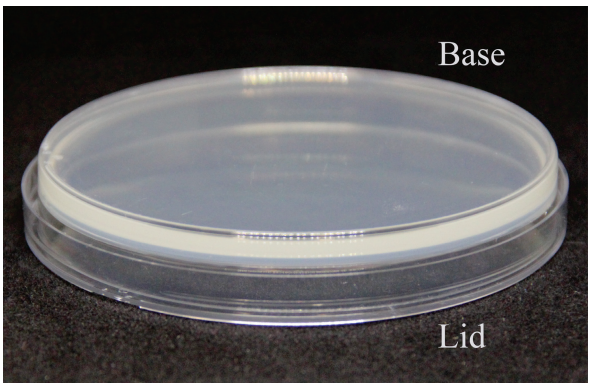


Fig. 3

c) Aseptic Technique

The use of aseptic technique in the transfer of microorganisms is vital in microbiological experiments, to ensure there is no contamination of cultures by microorganisms from the environment. Also, the environment is not contaminated by the organisms being handled.

1. The sterile field:

- All microbial work should be carried out within the sterile field around the flame where air convection currents (an updraft) are maintained.
- Light the Bunsen burner. Allow it to burn for 20 seconds before carrying out a procedure, to ensure sufficient time for sterilising the surrounding air.

2. Sterilising the mouth of the vessels:

- When removing the lid of a vessel (e.g. bottle, broth tube or culture flask), hold it at ~45^{o} or a slant as far as possible without spilling the contents (Fig. 4). This prevents airborne particulates from entering. While holding the lid in one hand after opening, pass the mouth of the vessel over the flame in a circular motion 3–4 times with the other hand, to burn off any plausible contaminants (Fig. 4).

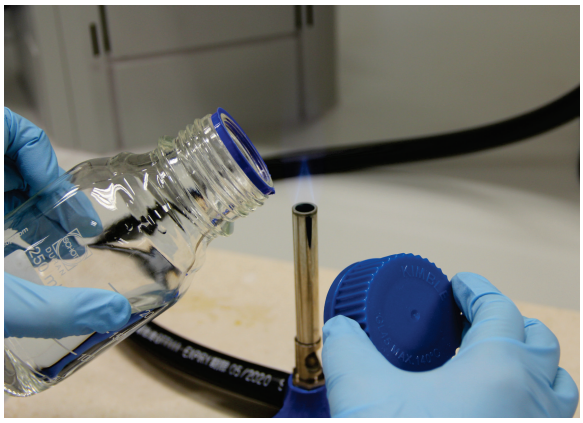


Fig. 4

- When adding or removing liquids, hold the vessel at a slant.
- Flame the mouth of the vessel again. Allow it to cool slightly, and recap the vessel.

3. Transferring microorganisms:

- When transferring microorganisms to a broth tube or culture plate, do so in as short a time as possible, to reduce the time of exposure.
- When handling culture-ware with a wide opening, such as a Petri dish, raise the lid of the dish at ~45° or with as little exposure as possible (Fig. 5).

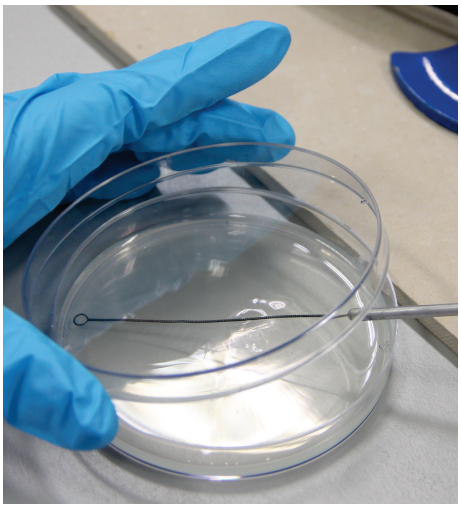


Fig. 5

4. Sterilising the inoculating loop:

- When using a reusable inoculating loop, place it at the tip of the blue cone (Fig. 6). This is the hottest part of the flame. Flame the loop to red hot (Fig. 6) and allow it to cool. Disinfect the loop after each use by flaming it to red hot. The loop can be placed in storage after cooling.
- When using a disposable inoculating loop, use a new one for handling different samples. Disinfect it with 10% chlorine bleach before disposal.

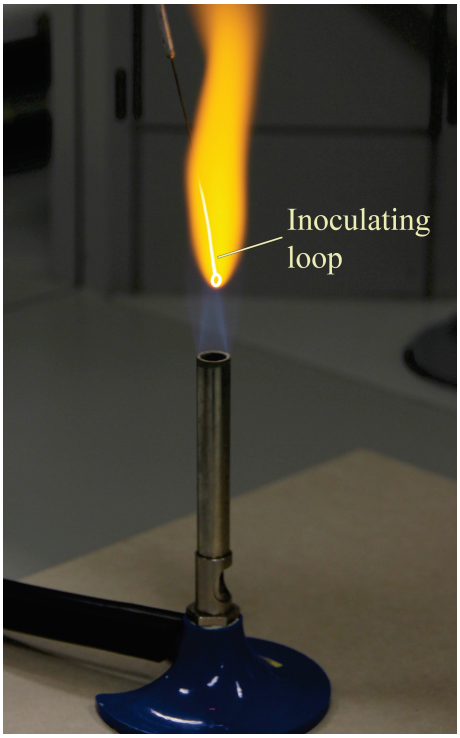


Fig. 6

5. Sterilising the spreader:

- When using a reusable spreader (glass or metal), immerse it in a beaker of 70% ethanol*. Pass it through the flame gently to burn off any clinging microorganisms (Fig. 7). Allow it to cool. Disinfect the spreader after each use by the mentioned procedures before placing it in storage.
- When using a disposable spreader (i.e. a plastic spreader), use a new one for different samples. Disinfect it with 10% chlorine bleach before disposal.

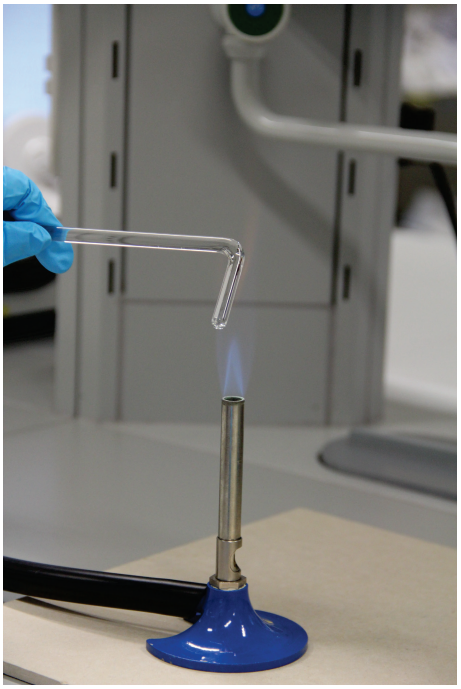


Fig. 7



***Caution: Do not put a hot spreader into ethanol, as the ethanol may catch fire. If the ethanol catches fire, cover the beaker with a fireproof board (larger than the beaker) to cut off the oxygen supply, thus extinguishing the fire.**

d) Incubation of Microbial Culture

Most microorganisms used in school microbiological experiments grow well under 30°C. Incubating microbes at or close to 37°C should be avoided, as this may encourage growth of potentially pathogenic microbes that thrive at human body temperature.

Cultures of microorganisms should be incubated in an enclosed environment, e.g. an incubator or in a locked preparatory room. During incubation, the covered agar dishes should be placed upside down (Fig. 8). This ensures that any condensation that may form on the lid of the dish will not drip onto the agar and will hence avoid the risk of leaking out.

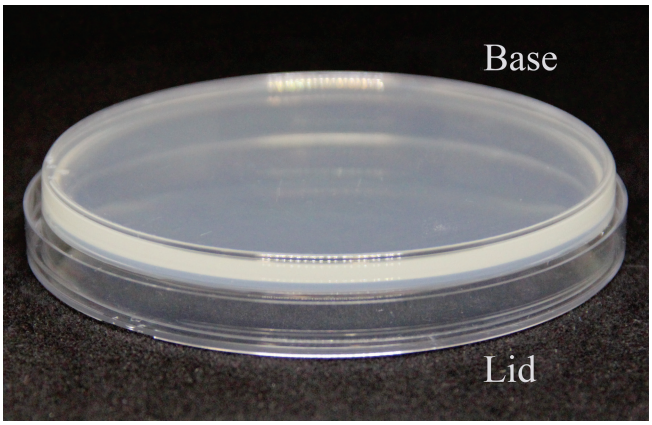


Fig. 8

Incubators must be disinfected by 70% ethanol or 10% chlorine bleach before and after incubation. If incubators are used continuously, disinfection should be periodically carried out, e.g. every week.

e) Examination of Microbial Culture

Examine the specimens in a sealed state, e.g. Petri dishes sealed with sealing tape or parafilm, or put into sealed transparent plastic bags. **Opening the Petri dishes containing cultures of microorganisms for inspection is not recommended.**

E. Sterilisation and Disposal of Unwanted Cultures and Contaminated Materials

Unwanted cultures should be collected in biohazard bags and destroyed by steam under pressure (autoclave) at 121°C, 15 psi pressure for at least 30 minutes or by immersing in 10% chlorine bleach for at least 2 hours before disposal. All laboratory consumables contaminated with microorganisms or waste materials should be treated in the same way before disposal.

The use of autoclaves (Fig. 9) in schools is governed by the Code of Practice for Owners of Boilers and Pressure Vessels, issued by the Labour Department (https://www.labour.gov.hk/eng/news/pdf/CoPOwners.pdf). The autoclave must be registered with the Labour Department and examined and inspected regularly by Appointed Examiners. Autoclaves should be operated by competent persons unless the autoclaves are granted exemption in accordance with Section 9 of the Boilers and Pressure Vessels Ordinance (https://www.elegislation.gov.hk/hk/cap56). The operation of autoclaves by students is prohibited.



Fig. 9

F. Safety Precautions in Handling Ethanol

Ethanol is flammable. In microbiological experiments, we often use ethanol and Bunsen flame for sterilising purposes. Do not panic if ethanol is accidentally set on fire. Notify the teacher / laboratory technician immediately and

1. if the ethanol in a container catches fire, cover the container with a fireproof board (larger than the opening of the container);
2. if the ethanol fire is on the work surface and is small, turn off the Bunsen burner and remove any flammable objects or fuel. The fire will burn itself out quickly; or
3. if the fire seems large, use a fire extinguisher or a fire blanket.

G. Handling of Microbial Spillage

Microbial spillage should be reported immediately and handled by teachers or laboratory technicians. When cleaning up the mess, wear protective gloves and laboratory gowns. Wear masks when appropriate. It is important not to inhale the aerosol cloud formed above the spill.

The spillage should be covered with paper towels soaked in disinfectant (e.g. 70% ethanol or 10% chlorine bleach). The paper towels should be left in place for 15 minutes and then swept into a biohazard or chemical waste bag. The contaminated area should also be disinfected as appropriate. If the skin comes into contact with the spillage, wash with liquid soap and water immediately and thoroughly. Seek medical help if necessary.

H. Sterilisation after Microbial Work

After each microbiology practical session, the work surface and experimental area should be wiped with disinfectant (e.g. 70% ethanol or 10% chlorine bleach) immediately. **Ensure all sources of flames are shut off before applying the disinfectant.**

Wash hands thoroughly with liquid soap and water after microbiological work. Paper towels are preferred for drying the hands. They should be disposed of in a waste container with a lid.

II. Experiments in Biotechnology

A. General Precautions for Biotechnological Experiments

Precautionary measures should be taken when working with experiments in biotechnology, especially when handling deoxyribonucleic acid (DNA), cells and tissue culture. This is for laboratory safety as well as to avoid contamination. Aseptic technique should be employed if appropriate.

Cover all cuts and wounds with sterile dressings. Wear protective gloves. Laboratory gowns are recommended to prevent contamination of personal clothes. Wash hands before and after experiments.

Manipulation of bacterial cells may be involved in biotechnological experiments. Take the necessary precautions as mentioned in Part (I) "Handling micro-organisms" whenever appropriate. Before handling experimental kits and delicate equipment, read the operating manuals in order to get familiar with the operation procedures. Handle all pipetting devices with special care, to avoid formation of aerosols during transfer. **Mouth pipetting is strictly prohibited.**

B. Working with Deoxyribonucleic Acid (DNA) and Other Biomolecules

Isolation, analysis and manipulation of both DNA and other biomolecules are always involved in the study of biotechnology/molecular biology. Schools should ensure that all samples used are biologically safe. The use of potentially hazardous DNA fragments, such as highly biologically active molecules and full-length viral genomes, is prohibited.

C. Biological Expression Systems

Biological expression system generally refers to vectors and host cells. Routine biological engineering experiments at biosafety level 1 (BSL-1) using non-pathogenic bacterial cells, explicitly *Escherichia coli* (*E. coli*), and yeasts are recommended. The common examples of BSL-1 vector and host cells of *E. coli* include plasmid pUC18 and strain K12, respectively. pUC18 has been completely sequenced for the greatest convenience of use. K12 is a non-pathogenic strain of *E. coli.* Besides, *Saccharomyces cerevisiae* is a common example of yeast used in molecular cloning.

D. Electrophoresis

Electrophoresis of DNA fragments or other biomolecules should be operated at low voltage (e.g. ≈ 75−100 V). Make sure the wire connection is correct and secured before switching on the power supply. Do not touch electrical devices with wet hands or gloves. Switch off the power supply before removing the gel from the electrophoresis tank for staining. If homemade electrophoresis equipment is used, make sure it is carefully constructed for safe handling.

To visualise the fluorescent markers used in the electrophoresis gels using an ultraviolet (UV) transilluminator, UV-absorbing PPE, e.g. nitrile or latex gloves and UV protective eyewear or face shield, should be used, as UV radiation is hazardous to both skin and eyes.

E. Handling of Reagents

Toxicity or harmfulness of reagents, such as restriction enzymes and stains, should be checked and proper precautions taken. For instance, we should select appropriate DNA staining dyes. **Ethidium bromide** and **propidium iodide**, which bind specifically to nucleic acids, are mutagens and should not be used in schools. For the purpose of staining, the non-specific dye methylene blue is commonly used. In addition, non-mutagenic DNA stain or equivalence, such as GelRed and GelGreen, may be used. Light-sensitive chemicals (e.g. silver bromide and silver chloride) should be stored in amber bottles or containers covered with aluminum foil. Temperature-sensitive chemicals should be stored according to the storage conditions suggested by the manufacturer.

F. Tissue Culture

Whenever tissue cultures are manipulated, an aseptic environment is of great importance. In tissue culture, plant cells/tissues are commonly used. If animal cells are cultured, ensure that they do not harbor any pathogens. Reagents involved in the preparation of tissue culture may be harmful and should therefore be handled with care.

G. Disinfection after Practical Work

All glassware and plasticware that have been in contact with DNA, biomolecules, bacterial cells and tissue culture should be considered contaminated. The items should be soaked in disinfectant (e.g. 10% chlorine bleach) for at least two hours or autoclaved (sterilised using steam under pressure at 121°C, 15 psi pressure in an autoclave) for at least 30 minutes before cleaning or disposal.

After each practical session, the work surface should be wiped with disinfectant (e.g. 70% ethanol or 10% chlorine bleach) immediately. Wash hands thoroughly with liquid soap and water. Clean and disinfect reusable safety goggles according to the manufacturer's instructions.

III. Risk Assessment

Risk assessment should be conducted when preparing a microbiological or biotechnological experiment. The following factors should be considered:

Factors	Explanation
1. The type of microorganism to be studied and the suggested biosafety level	Estimate the risk level of microbial culture
2. Laboratory procedures	Well-planned laboratory procedure minimises the potential risk.
3. Volume of cultures	The smallest possible volume of liquid culture should be used, to minimise the potential risk
4. Composition of culture media	Selective or differential culture media for the growth of desired microbes is preferred, to reduce the risk of culturing unwanted microbes
5. Incubation conditions	Special culturing conditions for the growth of desired microbes is preferred, to reduce the risk of culturing unwanted microbes
6. Laboratory facilities and equipment	Must be adequate for the experiment (by groups or by individuals)
7. Proper disposal procedures	Minimise contamination
8. Expertise of teachers and laboratory technicians	Equipped with knowledge and skills required for conducting the experiment
9. Study level of the students	Sufficient pre-requisite knowledge and skills for conducting the experiment

Isolation and culturing microorganisms from potentially dangerous sources such as sewage, animal or human mucus, pus and faeces is not recommended. Samples collected from these sources may contain a large quantity of pathogenic microbes. If the culture of microbes from an unknown source is taken, follow the experimental procedures of BSL-2.

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