

Basic Standard Operation Procedure and Safety Precaution in Bioscience Laboratories

生命科学实验室安全与操作规范 (英文版)



Editors ▶ Li Su Xiaomei Zeng Zhen Wang

主编 ▶ 苏莉 曾小美 王珍



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内 容 简 介

实验室安全是研究者的身体健康以及教学和科研活动顺利开展的重要保障。本书采用全英文编写,以生命科学基础实验、综合性自主实验、开放性研究实验以及科学研究活动为对象,以用于基础教学的一级生物安全水平基础实验室为参照,针对实验过程中易发生的安全事故隐患及防范措施进行分类陈述,图文并茂,通俗易懂。

本书可作为高等院校相关专业留学生或双语教学的推荐教材,也可供开展实验室工作的科研和管理人员参考。

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Preface

This book was developed by Experimental Teaching Center for Life Science and Technology of Huazhong University of Science and Technology, College of Life Science and Technology to provide sufficient information on safety protection of workers and the surrounding environment in bioscience laboratories. This guideline is expected to be revised up to date guaranteeing that regulations, policies and procedures obey applicable and safe standards.

All users in bioscience laboratories of educational institutions, including researchers, teachers and students, are required to study and implement safety precautions to avoid safety accidents. This book aims to protect operators, experiment equipment and the research environment to reduce or eliminate the potential of biohazard. Neither any harms to the health of the operators nor any intentional failure of equipment is expected during bioscience experiments. Also, the research environment shall be kept fine for continuous work. Especially targeted to be applied to laboratories in general bioscience laboratories for undergraduate and postgraduate education, this manual illustrates detailed procedure and guidelines for laboratory water, electricity, gas, sound, light usage and for some common apparatus.

The contents are formulated on fundamental laboratory safety knowledge, standard equipment operations, the main properties of chemicals, and past laboratory experiences. The contents are concise and practical. In addition to the text description, there are also standard operation procedure figures in standard operations of the equipment part. Safety precautions are illustrated with figures. Therefore, this guideline is excellent with pictures and texts that are easy to understand. In addition, this guideline is scripted mainly based on Laboratory Biosafety Manual (3rd Edition, WHO), Laboratories-general requirements for biosafety (GB19489—2008), the Control of Substances Hazardous to Health (COSHH) (<http://www.hse.gov.uk/>), the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) etc. Moreover, this book is edited by first-line researchers in bioscience laboratories who have rich research and teaching experience, so the contents are specific and have practice guiding significance for users of bioscience laboratories.

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Chapter 1 Introduction to Basic Safety in Bioscience Laboratories

Infectiousness and toxicity of some biomaterials to the users and the environments in bioscience laboratories shall always be notified. In accordance to the seriousness of the potential infectiousness or toxicity of these biomaterials, bioscience laboratories are officially classified into four levels. The higher the level is, the greater the potential dangers there are. In this chapter, biosafety levels of bioscience laboratories are introduced; and the fundamental safety guidelines are illustrated on water, electricity, gas, sonic, and laser usage.

1.1 Introduction to biosafety levels and general precautions in bioscience laboratories

1.1.1 Levels of bioscience laboratories

Biosafety levels are the set of biocontainment precautions in bioscience laboratories facilities. Based on *Laboratory Biosafety Manuals* from WHO, bioscience laboratories are classified as biosafety level (BSL) of BSL1, BSL2, BSL3, and BSL4, sometimes given as P1, P2, P3 and P4 (for pathogen or protection level). BSL1 laboratories are available for conducting basic experiments for student training; BSL4 laboratories are only available for research activities with the highest biosafety level.

(1) BSL1 laboratories have biosafety level 1 and are used for general teaching and researching activities. It is suitable for operations that are not pathogenic to human and animal bodies such as operations with non-pathogenic *escherichia coli*, *bacillus subtilis*, and *saccharomyces cerevisiae*. Regulations or rules for microbial operations should be simultaneously obeyed although the users and experimental animals are unable to be infected. This is a safe laboratory that can be used as an open experiment bench.

(2) BSL2 laboratories have biosafety level 2. It is used for simple hygiene assessment, service, and research. It is used for related experiments with potential pathogens to human or the environment and warning signs of biological dangers should be clearly tagged. Experiments with pathogenic *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*, *Plasmodium falciparum*, *Hepatitis A, B, and C* viruses, and human immunodeficiency virus (HIV). Researchers should obey operation manuals and wear protective suits. Effective precautions and emergency treatment should be well prepared in the laboratory. Importantly, infectious experiments should be limited in P2 laboratories; they should not be hazardous to operators or the experimental animals.

(3) BSL3 laboratories are biosafety level 3 and are suitable for specific diagnosis and research of pathogens that generally severely infect human or animals. These biomaterials include *Mycobacterium tuberculosis*, *Francisella tularensis*, SARS coronavirus, and yellow fever virus. There should be limited danger of infection in P3 laboratories, and only permitted entrance should be given to the laboratory while conducting related experiments.

(4) BSL4 laboratories are biosafety level 4 laboratories and have the highest biosafety. Hazardous pathogenic researches with strong infectious pathogen causing severe illness to human and animals are conducted. These pathogens include Ebola virus, and Variola virus. No effective precautions and treatment can be prepared in the laboratory. Only limited entry is given to the laboratory

and the laboratory is placed in clearly separated regions in isolated buildings. Wuhan Institute of Virology of the Chinese Academy of Sciences, Institute of Preventive Medicine are BSL4 laboratories.

1.1.2 General precautions in bioscience laboratories

Fundamental operating methods and safety facts should be studied whilst bioscience education and experiments are to guarantee the safety of human and the experimental animals.

(1) Clarify the working criteria: guarantee human safety, emphasize prevention, and education beforehand.

(2) Conduct experiment matching laboratory safety level, do not exceed the safety levels.

(3) Administration area and experiment area should be separated. Experimental material with potential infectiousness is not allowed to be stored in the laboratory.

(4) No public entry.

(5) Equip experiment suit and gloves during experiments. Take off when leaving the laboratory. Do not store or mix-wash experiment suit and gloves with other clothes.

(6) Clearly label and store experiment materials.

(7) Strictly store experimental microbes according to regulation.

(8) Do not take out reagents or equipment from the laboratory.

(9) No dining in the laboratory. No food and beverage storage in laboratory fridges(Figure 1.1).

(10) Preserve laboratory hygiene by periodically cleaning.

(11) Place fire control unit in handy positions only for necessary use.

(12) Keep safety exit cleared.



Figure 1.1 Precautions for safe use of the refrigerator in the laboratory

Note: It is forbidden to use the laboratory refrigerator to store food, beverages and other edible items.

1.2 Safety guidelines on water usage in bioscience laboratories

1.2.1 Classification of water in bioscience laboratories

In bioscience laboratories, several kinds of water are generally used for mixing solution, providing water to machines, cleaning experimental vessels etc. According to the purity of water from low to high, laboratory water can be classified as distilled water, double distilled water, deionized water, and ultrapure water. Water is selected according to experiment content and requirements. Water purity is standardized by Standardization Administration of the People's Republic of China(GB/T 6682—2008). This is to guarantee the accuracy of experiment results and the safety of machines.

(1) Distilled water is made from distilling equipment that isolates water from

solutions of solids or other materials. Distilled water does not contain the electrolyte, free ion, or inclusion. The main usage is preparing reagents, cleaning experiment vessels, and the operation and maintenance of machines.

(2) Double distilled water is obtained by distilling twice. Inorganic salt, organic matter, microbes, dissolvable gas, and volatile impurities are rather low in content. It is generally used for preparing bio-buffer, cleaning experiment vessels, and the cleaning and maintenance of water consuming equipment.

(3) Deionized water is purified with an anion-cation exchange column. It is also called ion-exchanged water. Its general uses are preparing buffer, cleaning specially required experiment vessels, and cleaning and maintenance of delicate water consuming equipment.

(4) Ultrapure water is pretreated, deionized, reverse osmosed, and ultra-purified and goes through many other purification processes until the electric resistivity is around $18.18 \text{ M}\Omega \cdot \text{cm}(25 \text{ }^\circ\text{C})$, the salt density is less than 0.1 mg/L (GB/T 6682—2008). There is almost no electrolyte, gas, colloid, organic matter, bacteria, or virus etc. Theoretically, hydrogen ions and hydroxide ions are the only existing ions. It is generally used for preparing solution without electrolyte, conducting a delicate experiment, and maintaining delicate water consuming equipment etc.

1.2.2 General precautions on using water

(1) Bioscience laboratory water is not drinkable.

(2) Water source, storage, and usage should be kept far from electricity.

(3) Bioscience laboratory water should be stored in a specific container to avoid water pollution.

(4) The operator should not leave the scene during the process of producing experiment water to avoid leakage and liquid flooding.

(5) Periodically inspect water consuming equipment for any risk of leakage.

Do not transport water supply equipment to avoid damage.

(6) Avoid operation of water consuming machines with wet gloves.

(7) In cases of water-cut, tighten taps to avoid flooding when water comes back.

(8) Tighten all taps before leaving the laboratory. If the laboratory will be empty for a long time, close taps and machines.

(9) Common equipment consuming water and precautions:

①Distillation equipment: be cautious of water shortage and water leakage.

②Pure water filter: be cautious of water shortage, water leakage, and remember to close the water outfall tap.

③Ice machines: be cautious of water shortage, water leakage, and chilled water leakage due to dysfunction of the temperature monitor.

④Laboratory water bath: be cautious of water shortage, water leakage, and dry boiling.

⑤Ultrasonic cleaners: be cautious of water shortage or water overfills.

⑥Autoclave: be cautious of water shortage, dry boiling, water leakage, water overfills, and suck-back which occurs if the exhaust port sank in water.

⑦Electrophoresis apparatus: be cautious of liquid leakage, electricity leak.

1.3 Safety guidelines on electricity usage in bioscience laboratories

1.3.1 Classification of electricity in bioscience laboratories

Two common types of electricity generally used in bioscience laboratories are direct current(DV) and alternating current(AV). Common source of DV include aneroid battery, storage battery, and alternating AV through converter, rectifier

(by hinder current going backward), and filter (by erasing the fluctuations in current after going through a rectifier). General equipment including computer hard wares, multi-meter, portable UV analyzer need DV to operate. Three phase electricity consists of three phase wires, and the voltage between every two wires is 380 V. It is usually used on equipment with special requirement for three phase electrical power such as three phase motors, and ultra-low temperature freezers, such as $-80\text{ }^{\circ}\text{C}$ freezer etc. Two phase electricity has two phase wires of which the voltage in between is also 380 V. It is usually used in alternate electric welders. Single phase electricity is provided by a live wire and a null wire. A live wire is the wire providing power to circuits, and a null wire is usually applied to active circuits, grounding from voltage transformer to activate a main working circuit under 220 V. Common applications are illumination and home appliances. Common laboratory illumination and equipment are all operated under single phase electricity.

1.3.2 General precautions on electricity usages in bioscience laboratories

1. Home appliance electricity voltage in PRC is 220 V. Electric shock occurs when the voltage in between human body is above 36 V, and the current exceeds 10 mA.

2. Common sockets in laboratories include single phase two-hole sockets, single phase three-hole sockets, three phase four-hole sockets. Three and four hole sockets have specific grounding hole for protection reasons, and this grounding hole should be connected with the null wires and the grounding wire of the laboratory. In three-hole sockets, the upper hole connects with the grounding wire; the left hole connects the null wire; and the right hole connects the live wire. For two-hole sockets, the left hole is the null wire and the right side is the live wire. On a standard socket in PRC, a red cable indicates the live wire (live, L); a blue cable indicates the null wire (null, N); the cable in yellow and green

indicate the grounding wire (earth, E), also called the flexible cord. Surface mounted socket should be installed no lower than 1.3 meters above the ground. Flush mounted sockets are generally installed between 0.2 to 0.5 meters over ground. Sockets must be installed strictly according to national standard to prevent safety disasters.

3. Before connecting to power, the power of the application should be considered with the power of the socket and should not exceed the rated power of the socket. If the rated power is exceeded, sockets will be burnt from overload. In serious occasions, fire accidents happen.

4. Switches and appliances must be installed with appropriate standard fuses. Never replace fuses with other metal wires to avoid damaging equipment and causing fire.

5. When electricity cut or voltage fluctuation occurs, high power appliances should be switched off, and turned on after the electricity recovers. For example, if electricity turns on again within 3 to 5 minutes after turning off the -80°C freezer, the compressor is under risk of being burnt due to high starting voltage that is much higher than the operating voltage.

6. Before using laboratory appliances, plug in the socket first, then turn on the power switch; when shutting down, turn off the power, then pull out the socket.

7. While preparing liquid materials in laboratories, stay away from electricity sources to avoid circuit shortcuts (Figure 1.2).

8. Do not pull or wrongly connect cables. Turn off the power and change a cable whenever the cable is worn.

9. Do not move appliances with power on. If any necessary movement is needed, turn off the power to avoid electric shock.

10. Do not touch switches, plug or pull cables, or touch equipment with power on. Do not change light bulbs or electrical units with wet hands or wipe electrical machines with wet towels.

11. Cut off the power while inspecting or fixing electronics. Any damage of electronics should be sent to the factory or given to professionals to fix. Amateurs are not allowed to fix electrical devices connected to power.



Figure 1.2 Precautions for electrical safety during preparing reagents

Note: Liquid reagents should be prevented from overflowing and kept away from the power supply. Otherwise, it may cause short circuit and fire.

1.3.3 First-aids for electricity accidents

(1) Do not have any physical contact with people in electric shock (Figure 1.3). Turn off the central switch, use a dry wooden stick to separate human body and the cable. Then call 120 for help and do the following steps simultaneously:

- ① Take off tight clothes of the victim that hinders breathing.
- ② Inspect the victim's mouth, clean any oral mucus.
- ③ If breath terminates, conduct artificial respiration. If heart beat terminates or has irregular vibration, conduct artificial chest compression. Do not give up rescuing.

(2) In case of fire, try to cut off power within fire range.

(3) Cover soil, sand or use a fire extinguisher after an electrical fire, but do

not use foam extinguishers, as foam extinguishers are conductive.



Figure 1.3 Precautions for handling electric shock in laboratories

Note: If the electric shock occurs, the power should be cut off immediately. Do not touch the electric shock body by hand when the power is not cut off.

1.4 Safety guidelines on gas usage in bioscience laboratories

1.4.1 Common gases in bioscience laboratories

Commonly used gases in bioscience laboratories include carbon dioxide, oxygen, nitrogen, nitric oxide, hydrogen, natural gas, compressed air. Some of these gases are combustion supportive, combustible, and toxic. Hence, it is necessary to be familiar with common gases in the laboratory (Table 1.1) to avoid accidents. Gas tank (gas bomb, also gas cylinder) has standard coloring and labeling in Coloured cylinder mark for gases (GB/T 7144—2016).