Biorisk Management of the Handling of Microorganisms in Microbial Tests

（微生物試験における微生物の取扱いのバイオリスク管理）

This general information describes basic requirements in the safe handling of microorganisms to be considered in performing the microbial tests in the General Tests (4.02 Microbial Assay for Antibiotics, 4.05 Microbiological Examination of Non-sterile Products, 4.06 Sterility Test), the Test for Crude Drugs (5.02 Microbial Limit Test for Crude Drugs and Preparations containing Crude Drugs and Preparations containing Crude Drugs as Main Ingredient), the tests in the General Information G3 Biotechnological/Biological products (Basic Requirements for Viral Safety of Biotechnological/Biological Products listed in Japanese Pharmacopoeia and Mycoplasma Testing for Cell Substrates used for the Production of Biotechnological/Biological Products) and the tests in General Information G4 Microorganisms (Rapid Identification of Microorganisms Based on Molecular Biological Method, Rapid Counting of Microbes using Fluorescent Staining, Disinfection and Decontamination Methods, Rapid Microbial Methods and Preservatives-Effectiveness Tests).

In the work of handling microorganisms, it is required to manage accurately biorisk generated by performing tests. Since risks while handling microorganisms vary depending on the characteristics of microorganisms and the contents of work handled, it is necessary to perform risk assessment for each work to identify, analyze and evaluate the risks, to protect workers handling microorganisms and also to reduce biosecurity risk. In the practice, a responsible person and a person in charge related to biorisk management should be assigned in the organization, and rules and plans for the operation should be established by them. In order to reduce risk, measures for the biosafety of laboratories should be performed by combining four elements: safety control, personal protective equipment, safety equipment, and physical containment facilities/equipment. The established risk management method should be updated through continuous risk reviews[1)].

The basic concept necessary for biorisk management in the handling of microorganisms is shown below.

1. Application

This general information applies to laboratories (including ancillary equipment if necessary) that conduct microbial related tests. It does not cover pharmaceutical manufacturing processes.

2. Explanation of terms

The definitions of terms used in this general information are as follows.

2.1. Laboratory: Facilities/equipment that handle microorganisms for the purpose of performing experiments for inspection, testing, studies, etc.

2.2. Biohazard: A disaster caused by organisms and biological products.

2.3. Classification of microbiological risk level: A classification of the risk of microorganisms to workers handling microorganisms and related persons.

2.4. Laboratory Biosafety: Risk mitigation measures according to biohazard risks are called biosafety. The purpose is to prevent the unintentional exposure, spread and accidental leaks of pathogens or toxins. Among them, laboratory biosafety should be performed by combining four elements, safety control, personal protective equipment, safety equipment and physical containment facilities/equipment.

2.5. Laboratory Biosafety Level (BSL): BSLs are classified from BSL1 to BSL4 by combining the four elements that practice laboratory biosafety, and risk mitigation measures according to each BSL should be constructed.

2.6. Biosecurity: Biosecurity means prevention and control in laboratories in order to protect unauthorized access, loss, theft, overuse, misuse, diversion and intentional release of valuable biological materials which need protection/monitoring.

2.7. Biorisk: Biorisk is the merged risk of laboratory biosafety and biosecurity, and includes all probability and chance where harmful events (accidental infection, unauthorized access, loss, theft, overuse, misuse, diversion or intentional release) will occur.

2.8. Biorisk Management: Biorisk management consists of three elements: risk assessment, risk mitigation and performance.

2.9. Worker handling microorganisms: A person who handles microorganisms directly in a laboratory and a person who enters a laboratory to maintain laboratory facilities.

2.10. Related person: A person who has a possibility of infection, such as a laboratory user who contact with a worker handling microorganisms, a colleague or a housemate of a worker handling microorganisms.

2.11. Good Microbiological Technique (GMT): Standard techniques for the safe handling of microorganisms. It includes the preparation of educational programs for acquiring technology, standard work procedures and rules.

2.12. Personal Protective Equipment (PPE): A set of tools worn by individuals to protect workers handling microorganisms against exposure of biohazardous substances. For example, masks, respiratory protection tools, goggles, gloves, protective clothing, shoe covers, etc.

2.13. Safety Equipment: A set of apparatus, instruments, and devices that protects workers handling microorganisms from exposure to biohazardous substances. For example, electric pipettes, sealed containers, biological safety cabinets,

etc. A biological safety cabinet is to protect workers, laboratory environment and work materials from exposure to infectious aerosols and sprashes. There are mainly two types, an opened type which isolates the inside and outside by air barrier and a sealed glove box type.

2.14. Physical containment facilities/equipment: Physical containment facilities/equipment are classified into 4, physical containment level 1, 2, 3, and 4. The object of the facilities/equipment is to provide workers with safe handling of hazardous substance according to the classification of the microbiological risk level.

2.15. Controlled area: An area where biorisk management is required. The area includes not only laboratories for handling microorganisms, but also waste treatment facilities/equipment, wastewater treatment facilities/equipment, air conditioning machine rooms, etc. that have biohazard risk.

3. Risk assessment in handling microorganisms

The following risks accompanying the handling of microorganisms in each execution plan of tests should be evaluated.

3.1. Risks concerning with laboratory biosafety

3.1.1. Risks due to the characteristics of microorganisms

(i) Risks based on the classification of microbiological risk level

Microorganisms have a different extent of harm to humans depending on their species or strain. Considering the symptoms of workers handling microorganisms when infected with microorganisms and impact on related persons, microorganisms are classified to the microbiological risk levels 1 to 4 (Table 1) in descending order of the risk. The classification of individual microbiological risk level differs according to country/region, target (human or livestock), presence of therapeutics or prophylaxis, minimum infective dose, route of infection, amount used, work contents, etc. Microorganisms that do not exist in Japan are often classified as high risk levels.

(ii) Risks due to the routes of microbial infection and exposure

Consider both the infectious route of microorganisms used and the supposed exposure to workers according to handling methods. In natural infection, oral cavity, nasal cavity and ocular mucosa are likely to be routes of infection, and contact with mucosa, oral infection, droplet infection, aerial infection, presence of insect vectors, etc. should be considered. In laboratory infection, attention should be paid to needle-stick infection, infection from skin wounds, and infection caused by contact with contaminants such as tools.

(iii) Risk due to host sensitivity

The different risks of susceptibility of workers handling microorganisms to microorganisms used should be considered. As a preventive measure for infection, vaccination can provide resistance to workers handling microorganisms and reduce the risk of infection.

(iv) Risks by using microorganisms specified in relevant laws and regulations

When using, possessing, storing and transporting microbial species, strains and toxins specified by the laws [2-5], comply with the relevant laws. For general matters, refer to the laws, notices and administrative communications that describe them in detail.

3.1.2. Risks from handling operations

(i) Risks due to the form and amount of microorganisms handled

Pipetting and other operations often generate droplets and aerosol, and aerosol containing microorganisms have a high risk of being diffused extensively by air currents. Consider the form and the amount of microbial species, strains and toxins. Take into account that as the amount of microorganisms and toxins handled increases, the associated risks increase.

(ii) Risks due to the skill of workers handling microorganisms

Consider that work by persons who do not have sufficient knowledge about microorganisms to be handled or who have not received sufficient education and training on how to handle microorganisms appropriately becomes to be high risk.

(iii) Risk due to the shape of tools handled

Considering that using glassware for work increases not only the risk of contamination due to breakage but also the risk of infection through wounds caused by breakages, examine the risk when using glassware.

(iv) Risks accompanying work contents

Consider that opening containers containing liquid or a powder, handling liquid using a pipette or pipetter, stirring liquid with a vortex mixer or transporting a supernatant after centrifugation to another container may increase the risk of generating aerosol.

(v) Risks in each work process

If there are multiple work processes, consider that the risk varies depending on the work contents of each process.

(vi) Risks on accepting/dispensing microorganisms

Consider new risks arising from the acceptance and dispensation of microorganisms, strains and toxins.

(vii) Risks on transporting microorganisms

When transporting samples containing microorganisms, consider that the risk (impact on external) differs between transporting within controlled areas and transporting to the outside of controlled areas.

(viii) Risk of infectious wastes

All tools and samples contaminated with microorganisms during work should be handled as infectious waste having the risk of infection until they are decontaminated (disinfection or sterilization) to inactivate microorganisms.

(ix) Risks in case of emergency

Consider emergency response when workers handling microorganisms are exposed to microorganisms, contamination of facilities/equipment, leakage of microorganisms to the outside of facilities, etc.

3.2. Risks concerning with biosecurity

When entrance management to microorganism handling facilities and a storage management method of microorganisms are not properly carried out, illegal access to microorganisms, loss, theft, overuse, misuse, diversion, intentional release, etc. become risks in biosecurity.

4. Risk mitigation measures in handling microorganisms

For each risk clarified by evaluation, necessary measures should be taken to reduce the risk to workers handling microorganisms and related persons. The following contents are necessary.

4.1. Establishment of biorisk management system

Institutions that possess and handle microorganisms are required to establish a management organization for biorisk management regardless of the number of workers handling microorganisms[6-8].

- Clarify roles, authorities, and responsibilities in the management organization.
- Assign a responsible person for biorisk management.
- Assign a person in charge of biorisk management.
- Establish rules and plans for biorisk management.

The contents to be performed include the following.

- Reduce risks in laboratory biosafety.
- Reduce risks of biosecurity.
- Carry out education and training for biorisk.
- Establish and carry out a maintenance plan for facilities/equipment in controlled areas.
- Comply with relevant laws and regulations.

4.2. Mitigation of risks concerning with laboratory biosafety

There are four main elements in risk mitigation measures concerning handling microorganisms: safety control, personal protective equipment, safety instruments/devices and physical containment facilities/equipment. Reduce risks by performing laboratory biosafety measures (Table 2) that combine the four elements according to biorisk[9].

(i) Safety Management

Safety management includes all related matters and requires the following:

- Establish rules for various items necessary for the safe handling of microorganisms.
- Prepare standard operation procedures based on good microbiological technique (GMT).
- Continue education and training in order to learn good microbiological technique (GMT).
- If there are effective preventive measures such as vaccines against microorganisms to be used for the health management of workers handling microorganisms, the vaccination history of workers handling microorganisms should be managed.
- Establish emergency measures.
- Carry out education and training for biorisk.

(ii) Personal protective equipment

Use appropriate personal protective equipment (PPE) during work to reduce the risk of microbial exposure. Select the appropriate personal protective equipment (PPE) according to the characteristics and infectious routes of microorganisms to be handled, and work contents.

(iii) Safety equipment

Use an electric pipette, etc., so that workers handling microorganisms do not contact with microorganisms directly. Use non-leaking tools/devices made of materials that are not easily damaged. When disposing sharp tools such as injection needles, dispose them in containers (such as needle collection containers) that do not allow them to penetrate.

Microorganisms should be handled in biological safety cabinets, etc. to reduce the risks of exposure to microorganisms contained in aerosol generated and their spread to workplaces. For samples with high risk of aerosol infection, use a centrifuge applied with measures to confine aerosol. Safety equipments used in a biological safety cabinet should be taken out after decontamination in the biological safety cabinet.

Microorganisms (including bacterial spores and fungal spores) should not be handled in a clean-bench etc. where containment capability is not guaranteed.

(iv) Physical containment facilities/equipment

Define a risk level by risk assessment based on the characteristics of microorganisms and work contents, and use necessary physical containment facilities/equipment. For facilities/equipment there are requirements specified for each containment level[10,11], and facilities/equipment being physical containment level 3 or higher are required to take effective measures to prevent workers handling microorganisms from exposure by aerosol containing microorganisms generated during work and to prevent leakage to surrounding areas.

(v) Risk mitigation at the time of accepting/dispensing microorganisms

Comply with the relevant laws[2-5] when accepting and dispensing microorganisms. When accepting new microorganisms in an institution, the institution should define a laboratory biosafety level (BSL) by assessing the microbiological risks, and determine in advance necessary items such as measures for emergency and exposure. Before dispensing, check the laboratory biosafety of the recipient. For general matters, refer to the laws, notices, and administrative communications, etc. that describe them in detail.

(vi) Risk mitigation on transporting microorganisms

When transporting microbial samples, take appropriate measures to prevent leakage even when transporting within controlled areas. When transporting outside the controlled areas, it is fundamental to apply triple packaging that prevents the sample from leaking[12]. Comply with the laws[2-5] when transporting outside facilities.

(vii) Risk mitigation of infectious wastes

Inactivate infectious wastes absolutely by agents suitable for target microorganisms or by autoclaving. Inactivation treatment should be completed within a controlled area.

(viii) Risk mitigation in case of emergency

Document appropriate measures in preparation for emergency such as microbial exposure or leakage. The measures should include communication methods, maintenance of a communication network, specific measures, stockpiling of necessary devices/tools, and education/training for them. Establish an organization framework to implement them.

4.3. Mitigation of risks concerning with biosecurity

Mitigation of the risk in biosecurity should include the following[13]:

(i) Access controls for workers handling microorganisms

- ID management
- Registration management of workers handling microorganisms
- locking
- Entry/exit management

(ii) Control of microorganisms

- Storage and acceptance/dispense management of microorganisms

4.4. Education and training for biorisk

To improve the skills of workers handling microorganisms, perform educational training for understanding risks in handling microorganism and measures for them. The characteristics of microorganisms, risks due to work, acquisition and training of good microbiological technique (GMT), emergency response, etc. are important. The education/training should be repeated.

4.5. Compliance with relevant laws and regulations

Regarding the handling of specified microorganisms, etc. in the laws[2-5], comply with the relevant laws regarding possession, management and transport of microorganisms and toxins. For general matters, refer to the laws, notices, and administrative communications, etc. that describe them in detail.

5. Review and update of biorisk management

The risk management should be carried out by the Plan-Do- Check-Action (PDCA cycle).

6. References

1) General information “Basic Concept of Quality Risk Management”

2) October 2, 1998, “Act on the Prevention of Infectious Diseases and Medical Care for Patients with Infectious Diseases”.

3) 1951, “Act on Domestic Animal Infectious Disease Control”.

4) May 4, 1950, “Plant Protection Act”.

5) 2003, “Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms”.

6) CEN (European Committee for Standardization). CWA (CEN Workshop Agreement) 15793” Laboratory biorisk management”, Sep. 2011.

7) ISO 35001: 2019, Biorisk management for laboratories and other related organisations.

8) CEN (European Committee for Standardization). CWA (CEN Workshop Agreement) 16393 “Laboratory biorisk management-Guideline for the implementation of CWA 15793: 2008”, Jan. 2012.

9) WHO, Laboratory biosafety manual Third Edition, 2004. ISBN 92-4-154650-6.

10) February 1, 1963, Ministry of Health, Labour and Welfare Ordinance No. 2 “Regulations for Buildings and Facilities for Pharmacies”, Article 8 “Buildings and Facilities of Manufacturing Sites for Manufacturers of Specified Biological Drugs”

11) December 24, 2004, Ministry of Health, Labour and Welfare Ordinance No. 179 “Ministerial Ordinance on Standards for Manufacturing Control and Quality Control of Pharmaceuticals and Quasi-drugs” Chapter 2 Section 4 “Manufacturing Management and Quality Control of Biological Drugs”.

12) WHO, Guidance on regulations for the Transport of Infectious Substances 2013-2014.

13) WHO, Biorisk management: Laboratory biosecurity guidance, 2006.

Table 1. Classification of microbiological risk level

Microbiological risk level	criteria
1	There is no or low risk for workers handling microorganisms and related persons. A microorganism that is unlikely to cause human and animal disease (those that do not cause disease to healthy humans).
2	Moderate risk for workers handling microorganisms and low risk for related persons. It can cause disease if it infects humans or animals, but it is unlikely to cause serious health damage to workers handling microorganisms and related persons. There are effective treatments and preventive measures, and low risk of spread to related persons. Many people already have acquired immunity to it and the infection can be easily prevented.
3	High risk for workers handling microorganisms, low risk for related persons. Infection with humans or animals causes serious disease, but is usually less likely to be transmitted from infected persons to related persons. There are effective treatments and preventive measures.
4	High risk to workers handling microorganisms and related persons. Infecting humans or animals can cause serious disease, and transmission from infected persons to related persons can occur directly or indirectly. Usually there is no effective treatment or preventive measures.

Table 2. Classification of laboratory biosafety Level (BSL) and measures

BSL classification	Safety management	Personal protective equipment	Safety equipment	Facility/ equipment (physical containment level)
BSL1	GMT and management system (management organization, handling procedures, education/training)	Personal protective equipment	Safety equipment	Level 1 (Basic laboratory)
BSL2	Standard microbial handling procedures corresponding to the microbiological risk level 2, in addition to the requirements of BSL1	In addition to the requirements of BSL1, personal protective equipment complying with the microbiological risk level 2	Safety equipment complying with the microbiological risk level 2, in addition to the requirements of BSL1	Level 2 (Basic laboratory complying with the microbiological risk level 2)
BSL3	Dedicated standard microbial handling procedures corresponding to the microbiological risk level 3, in addition to BSL2 requirements	In addition to the requirements of BSL2, dedicated personal protective equipment complying with the microbiological risk level 3	Dedicated safety equipment complying with the microbiological risk level 3, In addition to BSL2 requirements	Level 3 (physical containment laboratory)
BSL4	Dedicated standard microbial handling procedures corresponding to the microbiological risk level 4, in addition to the requirements of BSL3	In addition to the requirements of BSL3, dedicated personal protective equipment complying with the microbiological risk level 4	Dedicated safety equipment complying with the microbiological risk level 4, in addition to the requirements of BSL3	Level 4 (high grade physical containment laboratory)

Comprehensive risk management methods are classified from BSL1 to BSL4 according to each microbiological risk level, and measures are added and strengthened in order according to newly generated and concerned risks as the BSL value increases. In particular, BSL3 and BSL4 require the use of dedicated good microbiological technique, personal protective equipment and safety equipment.