# Safety Manual

## Supplement 2.
## **Safe Handling of Life Science Experiment**

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Faculty and Graduate School of Science, The University of Tokyo

## Supplement 2-1. Safe Handling of Life Science Experiment

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### 1. Prevention of Infection

Experimenters need to pay attention to the following in general to prevent infection caused by laboratory microbes and their diffusion to outside areas from laboratories.

(1) Handling of Bacteria

Experimenters should pay full attention when conducting experiments in laboratories, always keeping in mind the fact that they are handling bacteria that are ‘invisible’ but ‘possible to be dangerous.’ It is also important to work for the prevention of infection by carrying out appropriate ‘sterilization,’ ‘disinfection,’ and ‘antisepsis.’

a. Sterilization the microbes inside or around a body. Use an autoclave to subject the area to be sterilized with high pressure saturated steam. With this treatment, spores that have high resistibility against sterilization treatment can be destroyed.
b. Disinfection kills or gets rid of the microbes in a material. The disinfecting method is as follows: ‘application of heat,’ ‘use of chemical substances (alcohol, phenol, heavy metal, ethylene oxide gas, etc),’, ‘filtration,’ ‘exposure to ultraviolet light,’ ‘exposure to radiation,’ etc. Select the method that matches the purpose and is the most reliable treatment.
c. Antisepsis inhibits the growth and the life activity of microbes in a material. Antisepsis is commonly administered in drugs, but it must be noted that there are a lot of antiseptic drugs that have toxic consequences on people.

(2) Handling of Viruses

a. Unlike common bacteria, viruses have high resistibility against antisepsis treatment and cannot be removed by filtration. Therefore, contamination tends to occur when handling viruses. Each treatment must be conducted with full attention, and appropriate after-use care of experimental devices is essential.
b. When infectious virus is used on animals of higher orders, adequate attention needs to be given.

### 2. Experiments with Import-prohibited Animals, Plants, and Microbes

a. If imported animals, plants, and microbes fall under the category of the ‘Murrain Prevention Law’ & ‘Plant Protection Law,’ the permission from the Minister of Agriculture, Forestry and Fisheries is required.
b. In case of handling microbes that fall under the category of the ‘Plant Protection Law,’ annual inspection by the Plant Protection Office and submission of a report to the Office are required.

**3. Handling of Laboratory Animals**

1. Basic Policy
1) Disaster prevention measures for animal experiments / animal room
2) Disaster prevention measures for breeding cages, breeding shelves, etc
3) Other
2. Disaster prevention measures
A. Measures at normal times
(1) Animal experiments / animal room (*Note)
① Place animal rooms inside earthquake-proof buildings.
② Place a safe and strong door in the doorway so that the animals will not be able to escape at a time of disaster.
③ Windows should be either shut or blocked by blinds or wire mesh.
④ Place net or wire mesh over drainage and sewage lines.
⑤ Depending on the kinds of laboratory animals and the contents of the animal experiments, indicate with labels such as ‘Rearing genetically-modified animals.’
⑥ Do maintenance and regular checkups of the facilities and keep them clean and tidy.
(2) Breeding cages and breeding shelves
① Use breeding cages made of strong material and structure.
② For middle-sized and large-sized animals, use breeding cages that do not open by sudden force.
③ When breeding cages are placed on breeding shelves, safety catchers should be used so that the cages will not fall off the shelves.
④ Depending on the kinds of laboratory animals and the contents of the animal experiment, use cabinet-shaped shelves with doors and high shelter density.
⑤ Fix shelves on the walls or on the ceiling. Also, shelves could be fixed onto one another in aT-shaped or L-shaped structure.
(3) Other
① Be always aware of the present conditions such as kinds of breeding animals, number of breeding animals, and contents of animal experiments.

② In order to prevent safety violations, conduct breeding control on the animals that require precautions (animal experiments with genetically-modified animals, specific adventives, and particular animals (poisonous animals)) by taking strict containment measures and nonproliferation measures.

③ Establish a preliminarily contact system for laboratory workers, indication flow & work responsibility, and handling of the animals.

④ If laboratory animals [mammals, birds, crawlers, finned, amphibian, insects] escape from the cages, not only they can harm both people and other animals, but they can destroy the natural environment and ecological system. Therefore, prepare measures for possible escapes.

⑤ Have autoclaves, ethylene oxide gas sterilizers, and liquid nitrogen tanks properly equipped.

⑥ Prepare tools for capturing escaped animals (mesh trap, mouse trap, etc).

⑦ Prepare private electric generators if needed.

⑧ Breed precious animals and phylesis either on a diversified basis in separate places, or keep their gamete or zygote if the animal species' reproduction technology has been established.

⑨ Establish measures to minimize the interruption of long-term research and education.

B. Response to an Emergency Announcement

① Double check [A. Measures at normal times].

② After confirming that there are no individuals in animal experiment rooms and breeding rooms, close the doors and lock them.

C. Response to an Emergency (a natural disaster)

① Figure out the situations in animal experiment rooms and breeding rooms as clearly as possible, and take appropriate measures immediately.

② Check if there are any escaped animals around the building of animal experiment rooms and breeding rooms.

③ To prevent from safety violations, blockade the animal experiment rooms and breeding rooms in which animal experiments that require extra attention have been conducted, and make the areas restricted.

D. Measures after a natural disaster

(1) Investigation / report of the damage of animal experiment rooms and breeding rooms

① In principal, the person in charge of the animal experiment rooms or of the facility will take responsibility.

② If the laboratory animals have already escaped, try to capture them.

③ If middle-sized and large-sized animals, infected animals, or poisonous animals have escaped, report it to the 'Faculty, Center and Facility Disaster Headquarters' immediately

and capture the escaped animals without fail. Also, the ‘Faculty, Center and Facility Disaster Headquarters’ should contact the ‘Disaster Headquarters’ under the confirmation of the ‘Head of Faculty, Center and Facility Disaster Headquarters’ (Head of Faculty, Center and Facility).

④ Pay attention to the following to prevent animal escaping when entering rooms.

- Close the door and keep it locked with the sign of [CLOSED (with the name of the person who closed the door and the time & date of closing)] until possible escape prevention measures are prepared.
- Figure out the situation in a room after opening the door with a group of people paying full attention in order to prevent animals escaping.
- Lock up the animals in the cages from which they escaped if their identifications are clear.
- Lock up the unidentified animals in separate cages or put them down.

⑤ The person in charge (person responsible for either the animal experiment or the facility) investigates the extent of the damage (number of animals that escape out of laboratory, number of surviving animals, number of animals put down, damage to equipment and devices), and reports the damage to the chair of the animal experiment committee along with further responses (put down, breeding or move of surviving animals, etc). The chair gathers all the information together and reports it to the ‘Faculty, Center and Facility Disaster Headquarters’. Also, the ‘Faculty, Center and Facility Disaster Headquarters’ should contact the ‘Disaster Headquarters’ under the confirmation of the ‘Head of Faculty, Center and Facility Disaster Headquarters’ (Head of Faculty, Center and Facility).

(2) Handling of laboratory animals

① Considering the lifeline recovery condition, phylesis sustention of laboratory animals, and proceedings of animal experiment, make a decision whether or not to continue to breed the animals, or to stop the experiment and move the animals to another place, or to euthanize them.

② If a continue-to-breed decision is taken, check the drinking water, food, and floor cloth and ensure the necessary amount of water, food, and floor cloth. Also, take strict prevention measures in order to keep the animals away from wild animals and sanitary insects.

③ Consider the possibility of moving precious animal species and phylesis sustention animals to another place.

④ If a put-to-death treatment is needed, conduct the most appropriate treatment for the animal species. Pay extra attention to handling of infected animals, and conduct antisepsis treatment if necessary.

⑤ Think about the disposal method of the dead animals from each Faculty.

⑥ After the proper treatment, submit a report to the chair of each Faculty, Center and Facility

Animal Experiment Committee with the indication [Treatment done], [Name of the responsible person], etc. The chair gathers all the information together and reports it to 'Faculty, Center and Facility Disaster Headquarters'. Also, 'Faculty, Center and Facility Disaster Headquarters' contact the 'Disaster Headquarters' under the confirmation of the 'Head of Faculty, Center and Facility Disaster Headquarters' (Head of Faculty, Center and Facility).

(3) Restart of Animal Experiments

① Do not resume animal experiments until safety is completely confirmed, proper treatments for animal experiments are conducted and measures of recovery are made to a certain extent.

② Make sure animal breeding and animal experiments comply with the laws and regulations before the resumption.

Note) [Animal experiment / Breeding room] is a facility with equipment in which animal experiments are conducted and properly-treated laboratory animals are fed and kept (including the feeding, keeping and breeding of laboratory animals before animal experiments).

Note) Conduct the handling of laboratory animals, treatment or disposal method based on [Regulations for Animal Experiment Operation by the University of Tokyo], [Manual for Animal Experiment Operation by the University of Tokyo] and related laws and regulations of the University of Tokyo.

<Reference> Research Support for Bioscience at the University of Tokyo

http://www.adm.u-tokyo.ac.jp/gakunai/res/res1/kenkyoweb/bioscience/

## 4. Handling of Laboratory Microbes

1. Basic Policy

1) Disaster prevention measures for laboratories and storage rooms

2) Disaster prevention measures for experimental appliances and storage equipment

3) Disaster prevention measures for equipment and devices

4) Other

2. Disaster Prevention Measures

A. Measures at normal times

(1) Laboratories / Storage rooms

① Take containment measures or diffusion prevention measures for Laboratory Microbes (*Note) depending on the degree of risk.

② Take strict measures for the pathogenic microbes that are classified to high degree of risk, BSL 3 (Bio-safety Level 3) and the genetically-modified microbes that require P3 diffusion prevention

measures.

③ Laboratories / Storage rooms must be equipped with lockable doors and placed inside earthquake-resistant buildings.

④ Put up signs such as [In BSL 3 (P 3 Level) Experiment], [No Visitors Allowed], etc.

(2) Experimental Appliances / Storage Equipment

① Choose experimental appliances and storage equipment in view of the microbes' degree of risk and storage period.

② Keep fragile experimental appliances inside safe and stable storage equipment.

③ Place shock-absorbing materials in between experimental appliances inside storage equipment, or place water-absorbing sheet to prevent the liquid inside the appliances from splitting in case of leakage.

④ Be careful not to break the experimental appliances when storing frozen liquid samples in them.

⑤ Do not forget to indicate the contents of storage equipment.

(3) Equipment / Devices

① Fix equipment and devices on the floor or on the wall, or use a stopper so that they will not move.

② Always make sure to lock cabinets (refrigerator, chest freezer, etc.) so that the devices inside the cabinets will not fall out when the doors open by vibration.

(4) Other

① Take preventive measures, even at normal times, since the diffusion of laboratory microbes could cause serious damage such as outbreak of infection, contamination of the natural environment, or destruction of the natural ecology.

② Always familiarize yourself with laboratory microbes' names, kinds [storage sample, cultivated sample, testing sample, biologic sample (human or animal blood, tissue, etc)], morphology (tube, agar plate, incubation, etc), storage areas & cabinets, and areas of intended use.

③ Prepare data per each laboratory microbe with details of nature, degree of risk, storing instructions (storage temperature, etc), method of antisepsis or inactivation, method of disposal, etc.

④ Have always antiseptic substances ready that sterilize or inactivate laboratory microbes.

⑤ Preliminarily establish a contact system for laboratory workers, with indication flow & work responsibility.

⑥ Prepare a private electric generator and decide a place to obtain freezing medium (ice, dry ice, liquid nitrogen) for disasters, as microbes kept at low temperature become extinct by the increase of temperature.

⑦ Establish measures to minimize the interruption of a long-term research and education. Also, take appropriate measures to prevent the loss of precious microorganism culture and research

samples.

B. Response to an Emergency Announcement

① Double check [A. Measures at Normal Times].

② Confirm the storage preferential situation of the laboratory microbes with high degree of risk.

C. Response to an Emergency (a natural disaster)

① Figure out the affected situation of the laboratory microbes as clearly as possible and take appropriate measures immediately.

② Blockade the area in which the laboratory microbes were used, and restrict the area immediately.

D. Measures after a natural disaster

(1) Investigation & report of disaster damage

① Supervisor of a laboratory / storage room or microbe-handling-manager will be the person in charge.

② Inspect any damage or destruction of the devices and equipment that contained microbes.

③ Damage of devices, diffusion and littering of the microbes inside cabinets and refrigerators can possibly happen. In that case, open the doors of cabinets and refrigerators after preparing the handling measures, and conduct antisepsis and disinfection as well as disaster damage investigation at the same time.

④ Conduct complete infection prevention and anti-contamination measures and then conduct investigation and treatment before entering the areas in which microbes with a high degree of risk have been used.

⑤ If any contamination of microbes is found, blockade and restrict the contaminated area. If the microbes have a high degree of risk, report the situation to the related people and ‘Faculty, Center and Facility Disaster Headquarters.’ Also, ‘Faculty, Center and Facility Disaster Headquarters’ contact the ‘Disaster Headquarters’ under the confirmation of the ‘Head of Faculty, Center and Facility Disaster Headquarters’ (Head of Faculty, Center and Facility).

⑥ Summarize the disaster damage (kinds and quantity of dispersed pathogenic microbes, damage to equipments and devices, etc.) and measures to be taken in the future (treatment method, storing method for surviving microbes and samples, etc.) per facility and report it to the chair of Laboratory Microbes Safety Control Committee of each department. The chair gathers all the information together and reports it to ‘Faculty, Center and Facility Disaster Headquarters’. Also, ‘Faculty, Center and Facility Disaster Headquarters’ contact the ‘Disaster Headquarters’ under the confirmation of the ‘Head of Faculty, Center and Facility Disaster Headquarters’ (Head of Faculty, Center and Facility).

(2) Treatment of Laboratory Microbes

① Carry out complete disinfection and antisepsis in order to stop the spread of contamination.

② Discard the waste after sterilizing it in a sterilization bag.

③ Be careful not to get hurt by broken glass and devices when conducting the sterilization.

④ Report to the chair of the committee after the treatment and indicate as [Laboratory microbes have been treated].

⑤ Pay attention to your health condition and see a doctor immediately if you have any health problems.

(3) Restart of Laboratory Microbe Use

① Do not resume experiments and inspections until treatment of contamination caused by microbes is completed, measures of recovery are made to a certain extent, and safety is completely confirmed.

② Do not start using laboratory microbes with a high degree of risk until the containment equipment (e.g. safety cabinets, etc.) and antisepsis devices (e.g. autoclaves, etc.) are completely restored.

Note) In addition to pathogenic microbes that cause an infection in people and animals, all the microbes used for experiments including microbes and genetically-modified microbes that produce injurious effect on other organism (including plants) fall under the category of Laboratory Microbes (bacteria, fungi, viruses (including prion) and parasites).

Note) Conduct the handling and safety control of laboratory microbes based on [Safety Control Regulations for Laboratory Microbes by the University of Tokyo], [Safety Control Manual for Laboratory Microbes by the University of Tokyo] and related laws and regulations of the University of Tokyo.

<Reference> Research Support for Bioscience at the University of Tokyo

http://www.adm.u-tokyo.ac.jp/gakunai/res/res1/kenkyoweb/bioscience/

## 5. Safe Handling of Mutant Agents / Cancer-causing Agents

Refer to MSDS for the handling and treatment method of mutant agents and cancer-causing agents such as ethidium bromide and nitrosoguanidine.

## 6. Bio-related Committees

As for genetic recombination experiments, registration of the experiment facility and submission of experiment proposal are required. The ‘Recombinant DNA Experiment Safety Control Committee’ which will be reorganized into the ‘Genetic Recombination Experiment Safety Control Committee’ conducts inspections on applied facilities and deliberations regarding applied proposals. Experimenters are required to participate in safety training sessions.

Regarding the experiments on animals (mammals, amphibian, etc), submission of the experiment

proposal is required. The Animal Experiment Committee conducts deliberations regarding safety control. In order to use the Life Science Animal Resources Center, registration to the center and participation in training sessions are required. When conducting experiments with laboratory microbes (bacteria, fungi, viruses, protozoa and parasites) that have pathogenicity or storing the microbes, submission of notifications is required. The Biosafety Committee conducts deliberations regarding safety control of laboratory microbes.