2024

Safety Guides for Experiments and Practical Trainings

JOINT FACULTY OF VETERINARY MEDICINE
KAGOSHIMA UNIVERSITY

CONTENTS

EMERGENCY MEASURES

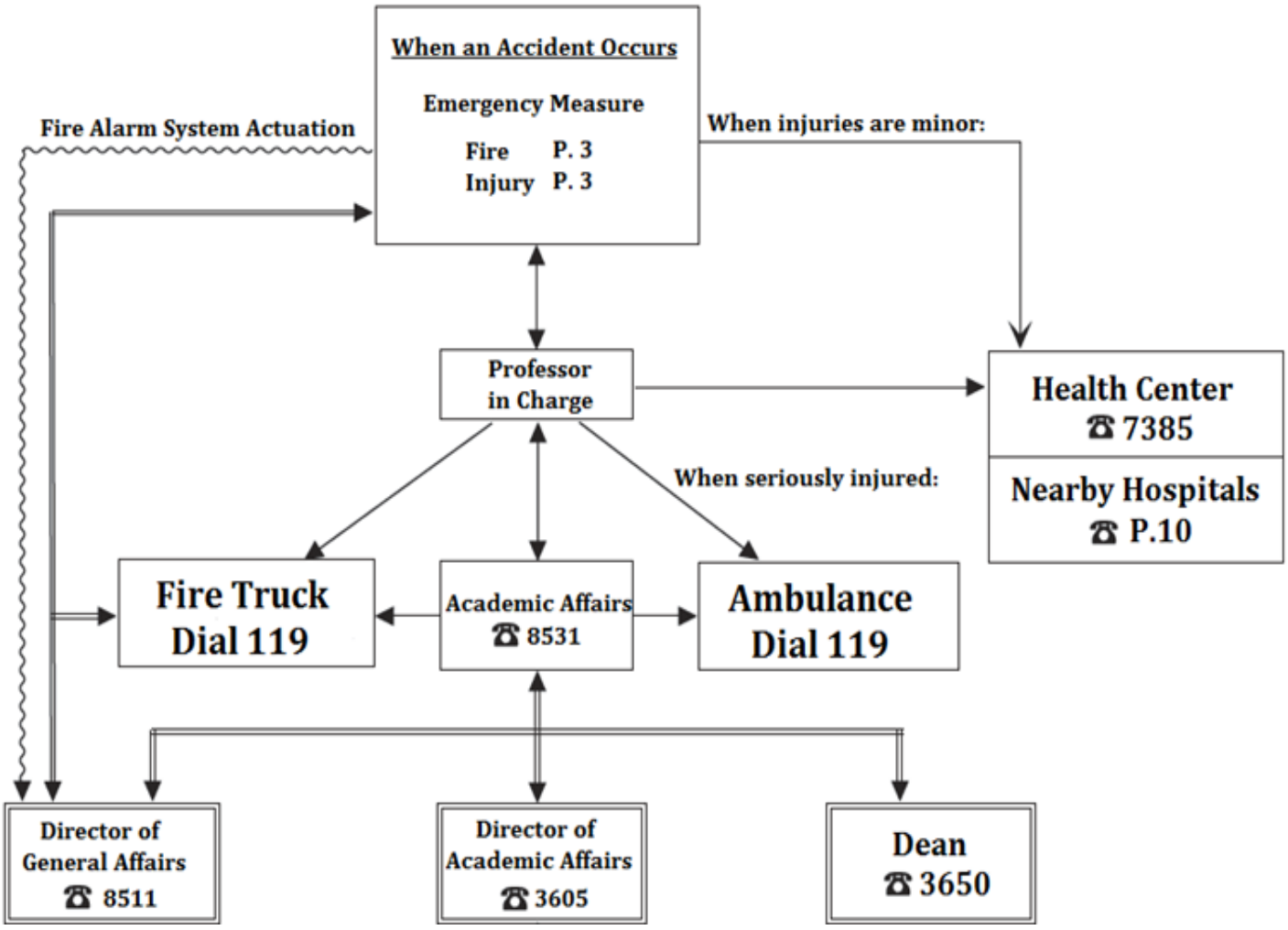
I . EMERGENCY MEASURES AND CONTACTS

When an unexpected accident occurs, in order to minimize damages and injuries, the students involved, along with all other personnel at the scene should cooperate with each other to understand the situation in a calm manner and report to the appropriate departments in line with the following flow charts for emergency response.

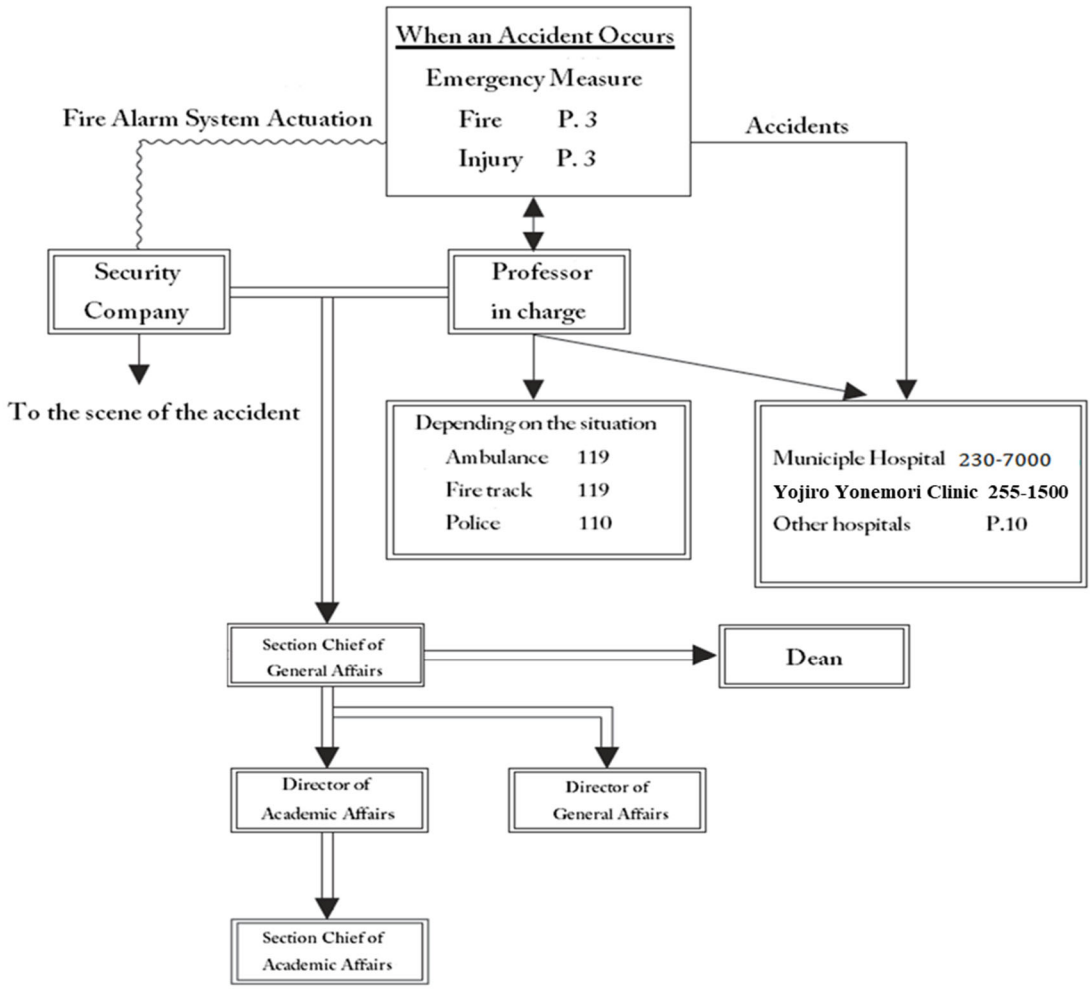
Accidents during other activities besides experiments and practices should be reported accordingly (Earthquake/Storm and Flood: Dial 119, Robbery/Traffic Accident: Dial 110).

Emergency Contacts and Measures

During Office Hours (Weekdays 8:30 – 17:15)



All the experiments after office hours (Weekdays 17:15 – 8:30) and during weekends and holidays have to be approved by the professor in charge.



Departments	During Office Hours Phone Number		After Office Hours
	Direct	Extension	
Professor in Charge*	All students should have contact info for the professor in charge.		
Dean	285-3650	3650	
Director of General Affairs	285-8511	8511	
Director of Academic Affairs	285-3605	3605	
Section Chief of General Affairs	285-8515	8515,8516,3596,3598	
Section Chief of Academic Affairs	285-8550	8550,8531,7739,3548	
Health Center	285-7385	7385	
Central Police Station Arata Police Box	099-222-0110 099-251-4554		

Ⅱ. EMERGENCY MEASURES AT THE SITE OF ACCIDENT

When an accident happens, assess the situation, ask bystanders for help, contact the professor in charge and the appropriate office and/or authorities according to the flow charts shown in the last two pages, and take the following emergency measures. A first-aid kit is located at each laboratory and the Office of Academic Affairs.

Early Fire Extinguishing

In Case of Fire	・ Immediately turn off the gas and power at the source of the fire. ・ Move any burnable materials and dangerous objects to a secure location. ・ Extinguish the fire. When there is only small amount of material on fire, wait carefully until it burns out. When the flames spread, aim the nozzle of fire extinguisher to the base of the flames. Splash water around the source of the fire to prevent from spreading the flames. Dredge dry sand over water-reactive substances. ・ When the fire grows too large to be extinguished by those above procedures or the environment becomes dangerous, evacuate outside, rescuing injured only if you can do so safely.
When Clothes Catch on Fire	・ Do not panic and move around to avoid fanning the flames. Be careful not to breathe in the blaze (it might damage the respiratory tract and might lead to death). ・ Roll on a floor. Smother a fire with wet towels. Pour water.

Surgical First-Aid Treatment

Burns	・ Cool the burn immediately with running water or iced water (for 10 – 30 minutes). Cover the wound with a clean towel/gauze (not fluffy cotton)-wrapped ice bag or cold pack, and receive medical treatment from a doctor. Dried towels/gauze might stick to skin so use wet ones to avoid effecting on the treatment afterwards. ・ Determine the degree of burn from the burn area and the depth. When the burn is severe, the person might be in shock and the hands and feet may be cold, they may turn pale, or vomit, and this can be fatal. ・ The burned area should be covered with clean sheets or towels and the person should be immediately transported to a hospital. ・ Give water or salted water when needed.
Cuts, Scrapes, Punctures, and Sharps Injuries	・ Wash the wounds well, and remove any bits of glass and foreign substances. ・ Pour hydrogen peroxide into the wounds and wash any foreign substances off with disinfectant soaps or foams. ・ Stop the bleeding by compressing the wound with sterile gauze. Apply some povidone iodine, chlorhexidine or ethanol, protect the wound with clean gauze, and apply a bandage.

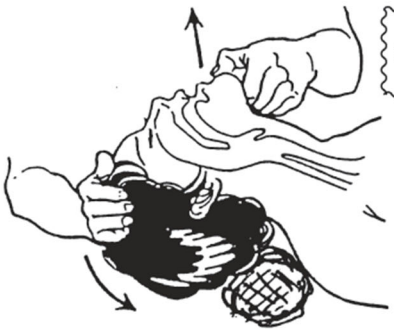
	· When bleeding severely, cover the wound with a clean towel/gauze bandage to stop bleeding, and go to a hospital to receive treatment. · Especially when the wound is contaminated with mud, it might lead to tetanus or a gas bacillus infection, thus clean the wound well and seek instructions from a medical specialist.
Bruise Sprain Fracture	· Cool the affected area, splint and secure it with elastic bandage wrapping, and see a doctor. · For a fracture, immobilize the joint above and below the broken area in a splint and see a doctor.

Emergency Measures for Drug Misuse

Example	Medicine	Emergency Procedures
Damaging of clothes	Acid Alkali	· Wash it with ammonium carbonate or diluted ammonia water and rinse it well. · After washing it with boracic acid or dilute acetic acid solutions, rinse it well.
Sticking to a skin	In general	· Take off the contaminated clothes and wash the skin with running water. If the skin is chemically burned, do not rush to take off the contaminated clothes, flush the burns for a while to see a condition of skin to avoid skin peeling.
	Strong Acid Strong Alkali Phenol	· After rinsing the skin with water, wash it with sodium hydrogen carbonate-saturated water. · After rinsing the skin with water, wash it with 2% acetic acid solution. N.B.: Exercise the utmost caution for those above procedures, because heat of neutralization for an acid-alkali reaction or the products of neutralization may cause irritation or a burning sensation of skin. · Rub it carefully with alcohol to remove it.
When to eye(s)	In general	· Flush eyes (for 5 – 15 minutes). Either portable or faucet-mount eye flush device/station should be available in easily accessible locations. · Do not use acid or alkali solutions to wash the eye(s). · Do not touch eyes. · Even when experiencing no eye pain after flushing the eyes, go see a doctor.
When medicine has been ingested	In general	· Contact a doctor immediately, and inform the type of medicine/chemicals, amount and the time that happened to seek care instructions. When unconscious/convulsive, it is best not to do anything other than keeping him/her breathing. · Induce vomiting (except when the person had swallowed corrosive chemicals containing acid or alkali, or liquid hydrocarbon; it might burn holes in the stomach or vomit might go into the respiratory tract). · Give an aqueous suspension, such as milk, beaten egg, water, tea, flour or starch.
	Strong Acid	· Give an aqueous suspension, such as milk, magnesium oxide, or aluminium hydroxide.

	Strong Alkali Mercury Silver Nitrate Methanol	・ Give dilute (1 – 2%) acetic acid solution or some lemon juice. ・ Give egg whites mixed with water or skim milk. ・ Give some saline. ・ Give 1 – 2% sodium hydrogen carbonate water to wash out/clean the stomach.
When breathing in gas	In general	・ Move to a well-ventilated area to get the fresh air, rest and keep warm. ・ Depending on the situation, perform artificial respiration.
	Cyanogen Chlorine Bromine Ammonia	・ Immediately sniff amyl nitrite. ・ Sniff the alcohol. ・ Sniff dilute ammonia water. ・ Administer oxygen inhalation/give oxygen as tolerated.
※AED is located at the entrance of Joint-Use Building. ※Joint Faculty of Veterinary Medicine Building A is equipped with emergency shower in the hallways on each floor, and each laboratory at the Joint-Use Building is equipped with emergency eye washer.		

First Aid for Unconsciousness
CPR (Cardiopulmonary Resuscitation)



an unconscious person

Establish an airway

Wipe fluid or foreign objects away. Turn the face up. Tilt head.

(It will be more effective if you lift up the chin)

Check for breathing.

No breathing

Breathing

Artificial respiration *

Keep cheking for breathing

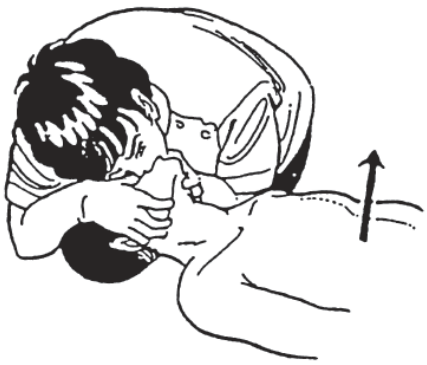
Check the pulse

No pulse

Yes

Cardiac Compression and ** Artificial respiration

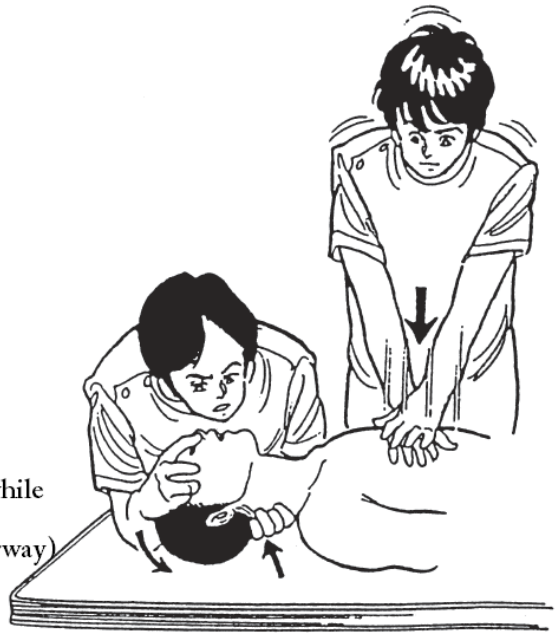
Artificial * respiration



[mouth-to-mouth resuscitation]

Lift up the chin gently with one hand while pushing down on the forehead with the other to tilt the head back. (open the airway)

Give 2 full breaths. Start compressions using both hands - 15 times.



[cardiac compressions]

Bring your shoulders directly over the person's sternum. Press downward, keeping your arms straight. For an adult, depress the sternum about 3 - 5cm the depth of the chest - about 80 times per a minute.

AED Procedures

⚠ WARNING	Do not use on children below 8 years or 25 kg. ⊘
	Do not use in an explosive environment, eg oxygen enriched, gaseous or fume environment ⊘
	When the victim is laying under the water, move the body to the dry surface and perform an AED. ❗

Guidance

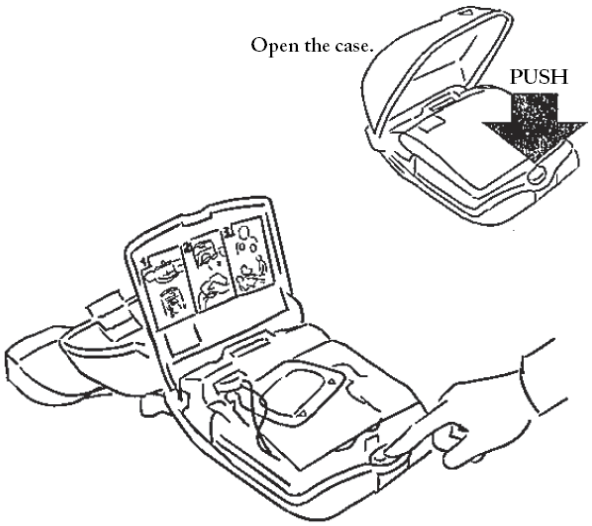
Follow the visual & voice prompts of the AED

1 Push the button to open the case

Open the case. It will automatically turn on.

Please call the ambulance immediately.

- Dial 119 if the call has not been made yet.

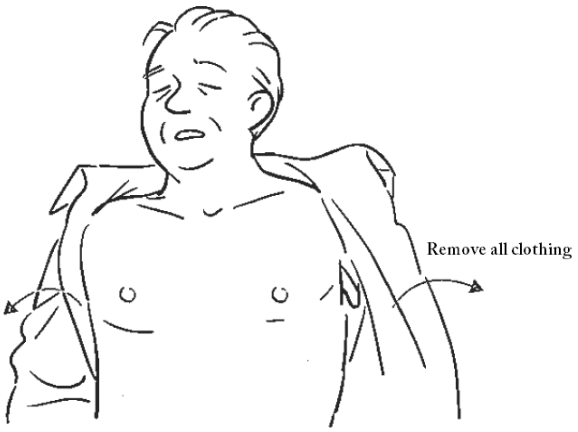


2 Remove all clothing from the chest

The person's chest must be bare and dry.

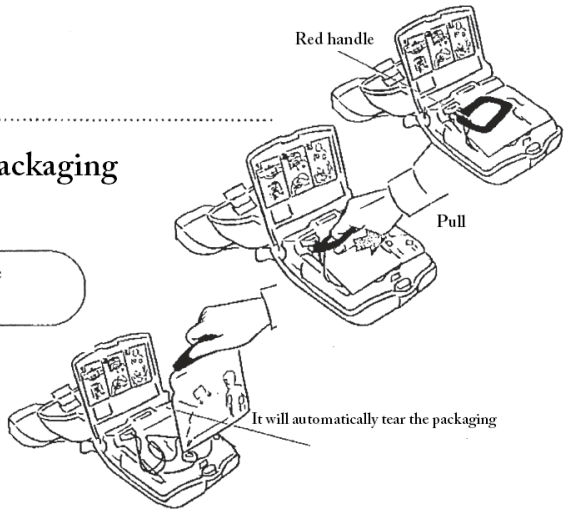
Please remove all clothing from the chest.

- Use the scissors in the box to cut clothes if needed.
- Use a cloth to dry the skin if it is wet.
- Use a shaver to shave hair on the chest if needed.



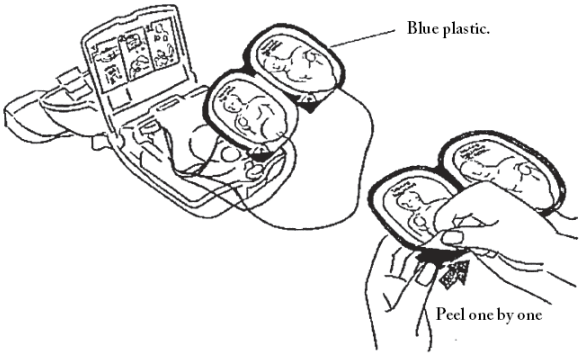
3 Pull the red handle and tear the packaging

Please pull the red handle and open the packaging.



4 Take the pads out

Peel each pad from blue plastic.

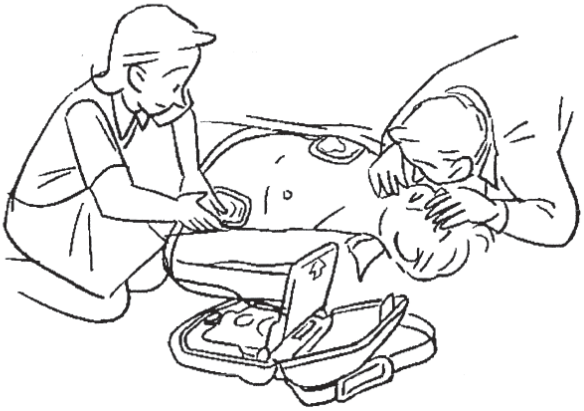


5 Place the pads.

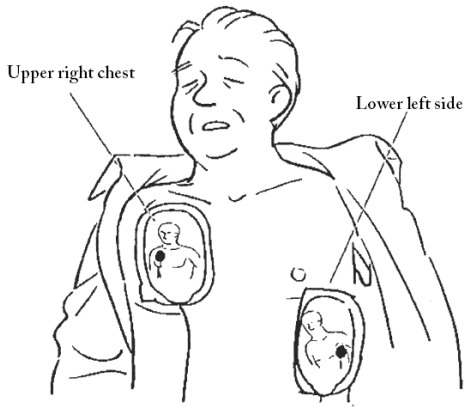
Place the pads on the chest as shown on the pads surface.

Please place the pads on the bare chest as shown

◆ Dot remove the pads once placed.



⚠ WARNING
Do not place the pads on the top of the implanted pacemaker or implantable cardioverter defibrillator. ⊘
Do not place the pads on the medicine pasting sheet. ⊘
Do not place the pads on top of each other. ❗
Make sure not to have any air bubbles between the pads and the skin. ⊘



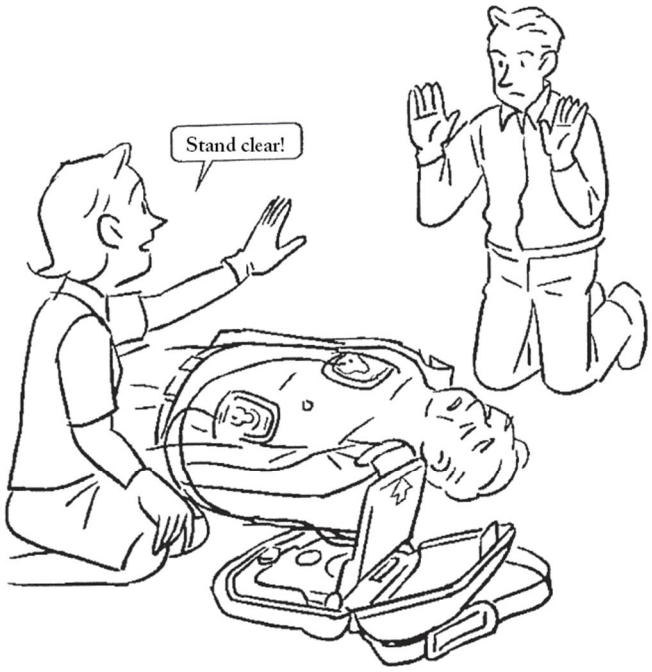
6 Do not touch the patient. Stand Clear.

Once the pads are applied and the cables plugged in, the AED should automatically start analyzing the heart rhythm.

Do not touch the patient. Analyzing heart rhythm.

- After analyzing, when the electronic shock is needed, the AED will automatically prepare for the shock.
- The AED will warn when the patient body is moved. Do no touch the patient while analyzing.

Shock advised. Charging. Stay clear of patient.

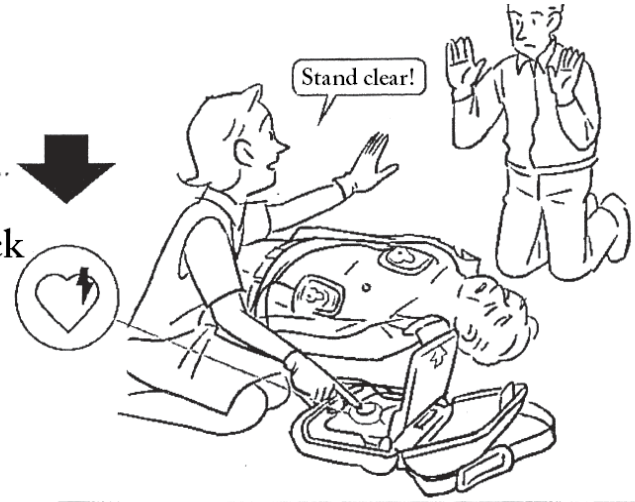


7 Press the button to deveriver the shock

Make sure no one is touching the patient, and push the button to deliver the shock.

Everyone stand clear! Press the button now.

- AED button will turn red.
- The shock will be canceled if the button is not pressed within 15 seconds. In that case, please follow the guidance.



⚠WARNING

Do not touch the patient nor the electric conduction area.

8 Stand clear and wait for the analyzed result

After the shock is delivered, the AED will automatically analyze the heart rhythm.

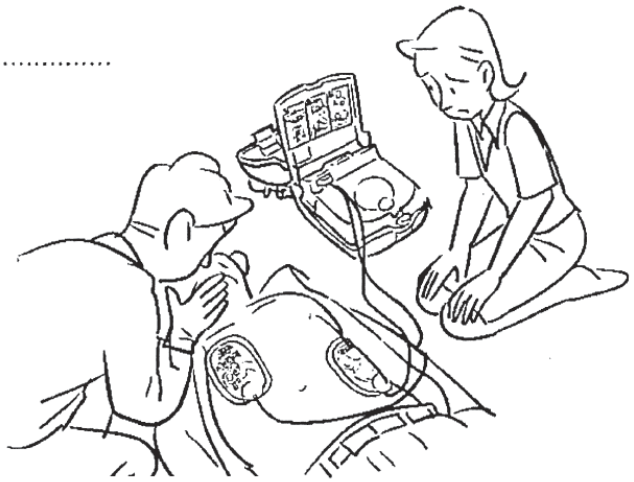
The shock delivered. Do not touch the patient. Checking the heart rhythm.

There is a case when another shock is needed after analyzing.

Please follow the audio guidance.

9 Check for a sign of life

Monitor the patient's condition, such as
1) normal breathing, 2) cough, 3) body movement.



No need to deliver the shock. Check for a sign of life. If there is no sign of life, please perform a CPR.

Sign of Life

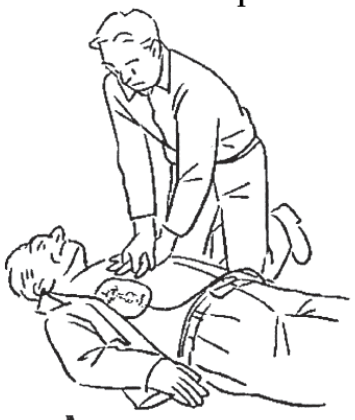
No Sign of Life

Perform CPR for a minute

Follow the audio guidance.

Resuscitation

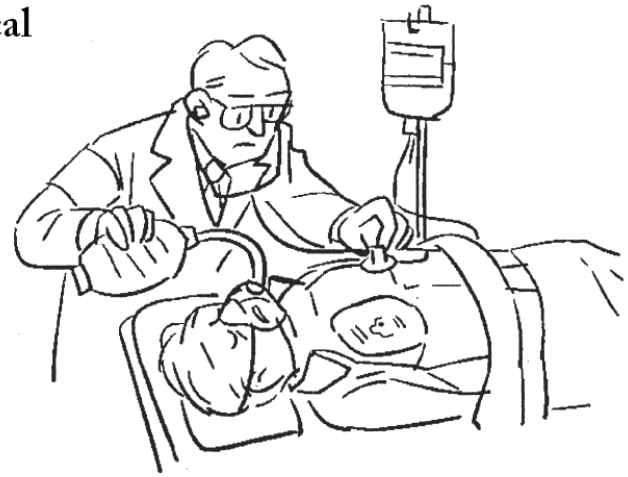
Cardiac Compression



10 Keep the pads until Emergency Medical Services personnel are on the scene

Do not turn off the AED and keep the pads on until an ambulance arrives.

- AED regularly checks for heart rhythm. Please follow the audio guidance until Medical Services personnel are on the scene.



Ⅲ. MEDICAL INSTITUTIONS

Near Kagoshima University

Medical Field	Names of Hospital	Telephone	Address
General	Municipal Hospital	099-230-7000	37-1 Uearata
	Yojiro Yonemori Clinic	099-255-1500	1-7-1 Yojiro
Internal	Shinsei Hospital (Former Seijin Hospital)	099-254-3332	16-30 Uearata
Surgery	Odashiro Hospital	099-253-8111	1-25-6 Arata
Orthopedics	Masuda Orthopedics	099-257-8100	1-1-1 Korimoto
Ophthalmic	Kawabata Eye Clinic	099-822-1110	3-2-1 Korimoto
Dermatology	Arata Dermatological Clinic	099-821-1112	2-39-16 Arata

Near the Farm

Site Name	Name of Hospital	Telephone	Address
Iriki Farm	Ichihino Kinen Hospital	0996-38-1200	3079 Ichihino, Hiwaki-cho, Satsumasendai City
Osumi Large Animal Clinical Center	Harubyu Clinic	099-478-2153	6045-1 Nogata, Osaki-cho, Soo-gun

Korimoto Campus – Health Center

Extention: 7385　E-mail: hoken@kuas.kagoshima-u.ac.jp

There are specialized doctors (internal medicine, psychology), occupational health physicians, counselors, and public health nurses at the center.

When contacting the center, make sure to clearly inform the condition [***When, Where, Who, How, Where***] and seek instructions.

When contacting a medical institution or calling for an ambulance, do the same; make sure to clearly inform the condition. At reception, the patient or an attendant should show his/her ID and explain the situation.

Ⅳ. PERSONAL ACCIDENT INSURANCE FOR STUDENTS PURSUING EDUCATION AND RESEARCH

Veterinary medicine is a practical science (practice-oriented and competency/skill-based learning/training rather than theory-based learning), and the ratio of experiment and practical training in the curriculum is accordingly higher compare to the other science faculties.

During experimental practices, hazardous drugs are often used involving substantial risks by mishandling or misoperation. Furthermore, there may be risks to destroy the extramural facilities or to injure others by accident in both intramural and extramural clinical practice.

So in our faculty, all students must take out the Personal Accident Insurance for Students Pursuing Education and Research (PAS) and Liability of Insurance coupled with "PAS". For further information, please refer to the brochure sent at the University enrollment.

FIRE AND EARTHQUAKE DISASTER

Ⅰ. FIRE

1. Fire Emergency Procedure

(1) Scream "Fire! Fire!" without any hesitations to inform others around.

(2) If possible, conduct an initial fire extinguishing. Keep the following three steps in mind.

① Turn off all devices, and use a fire extinguisher to stop the fire.

② When the clothing is on fire, pour water over immediately or roll on the floor.

③ Remove any burnable materials away from the fire.

(3) When it is hard to extinguish the fire, contact the appropriate office in accordance with the emergency measures described in the foregoing chapter.

2. Fire Prevention

(1) Keep water nearby in the areas where the fire might occur.

(2) Regularly check and know where the fire extinguishers are kept and how to use them.

(3) Do not place anything burnable/flammable near fire.

(4) Regularly inspect any electronic/gas appliances and know how to use them correctly.

(5) Do not leave a room while a heater or gas burner is on. Make sure to turn them off and close the main gas tap before leaving the room.

(6) Regularly clean and organize experimental rooms and buildings, and secure evacuation routes.

(7) Smoking is prohibited as well on University premises as in any and all university-owned buildings and facilities.

Ⅱ. EARTHQUAKE

1. Earthquake Emergency Procedures

(1) Turn off all electrical equipment. Open doors to secure evacuation routes.

(2) Duck under or drop to the floor taking cover by getting under a desk or sturdy table. Stay away from objects that could topple or fall and watch out for falling objects.
(3) When fire breaks out, inform others around and try to put out the fire.
(4) Help/rescue any injured people, as far as you can do so safely.
(5) Do not rush outside incautiously. Check your surroundings. Do not use the elevators.

2. Earthquake Prevention

(1) Any dangerous materials/items should be secured not to fall, drop or shake.
(2) Heavy devices and bookshelves should be fixed to the floor, wall, or pole.
(3) Check for where the fire extinguishers, fire hydrants, and fire alarms are located and know how to use them.
(4) Regularly clean and organize experimental rooms and buildings, and secure evacuation routes.

Ⅲ. GAS DISASTER

1. Gas Emergency Procedure

(1) Turn off all electrical equipment to prevent fires.
(2) Close the main gas supply tap.
(3) Ventilate. Do not turn on the ventilation fan.
(4) Get out of the area immediately, if the situation is out of control.
(5) Report to the appropriate authorities.

2. Gas Leak Prevention

(1) Regularly inspect the gas pipes and know how to use them correctly.
(2) When moving devices, be careful not to damage/break gas knobs and gas pipes.
(3) Check for the location of the gas shutoff valve and know how to operate.

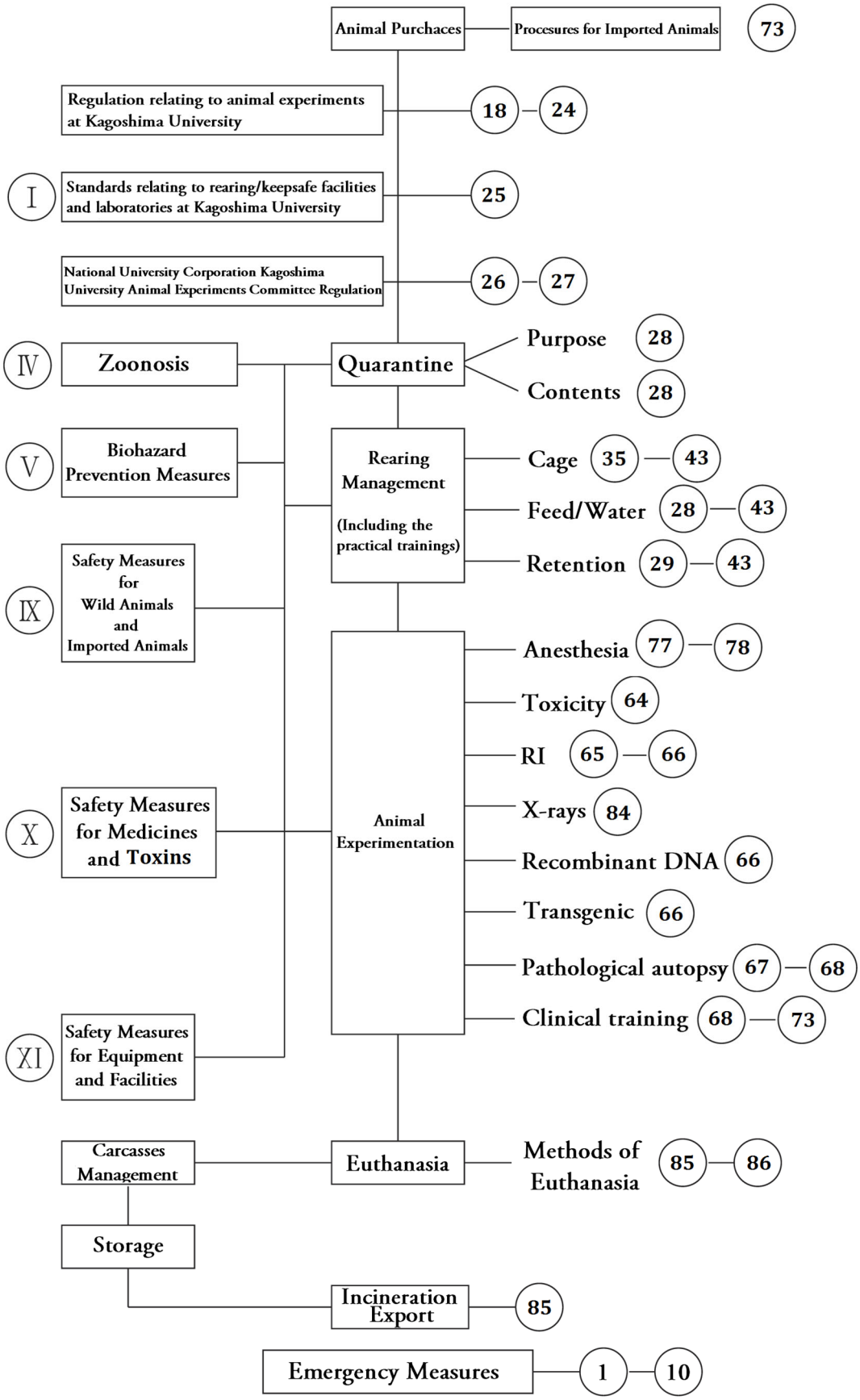
Ⅳ. EVACUATION

The standard fire and earthquake precautions to be taken when evacuating is set forth below.

(1) Evacuate immediately when the situation cannot be controlled.
(2) There is a limitation to how much fire extinguishers can put out a fire depending on characteristics of fires: in general, when interior finishes of the walls are on fire. Do not fight the fire when the fire reaches the ceiling.
(3) Before evacuating, shut off gas and electricity, and take appropriate measures for dangerous materials. Close the door after making sure that no one is left behind.
(4) When in a hallway, if there is no announcement, watch the movement of the smoke and evacuate to windward. The speed of smoke dispersion indoors is vertically 3~4 m/s, and horizontally 0.5~0.8 m/s.
(5) Do not use the elevators when evacuating, because they could be out of service due to power outage.
(6) Stairwells can be dangerous since they are smoke passage. Think of evacuation routes and know the building structure and where the emergency exits are.
(7) Cover your mouth, stay low and evacuate when there is a lot of smoke.
(8) When emergency stairs and ladders cannot be used, escape through windows to the terrace and get down with caution (There are no handrails on the terrace.).
(9) Rooftop evacuation is only for emergencies, because some buildings are installed with roof vents.
(10) Close the fireproof doors after checking that no one is inside. They can be opened again by pushing or pulling hard. Leave the fireproof shutters slightly open. When there is a need to open the shutter, use the crank handle kept in the fire hydrant box.

ANIMAL EXPERIMENTS AND PRACTICES

FLOWCHART OF SAFETY MANAGEMENT OF ANIMAL EXPERIMENTS



Introduction

Animal experiments, unlike chemical experiments, require the handling of live animals for experiments as research models/materials in animal testing. Therefore, students should be aware of the risks that animals can cause harm to humans and should try to prevent them. Risk prevention measures benefit not only humans, but also animals to avoid unnecessary pains.

The term ‘Biohazard’ has been used for laboratory infections by pathogenic microorganisms such as viruses, organisms, fungus, and protozoans. Various types of biohazards have been affecting humans for ages, and prevention and control measures have been implemented.

In animal experiments, biohazard countermeasures must be prepared not only for pathogens that transmit from animals to humans, but also for the ones that spread only among animals. Also, prevention measures have to be taken for various risks in the course of animal experiments as well as for pathogens.

This handbook describes theories and practices of each safety measure in animal experiments according to the flowchart in the previous page. Starting with quarantine, it explains basic safety measures, rearing and retention methods of each animal species, zoonosis, and biohazard prevention measures in series. Furthermore, it describes experiments that require the use of toxic chemical substances, radioisotopes, recombinant DNAs and transgenic animals. It also refers to the safety measures of pathological autopsy, animal clinical training, wild animals and imported animals, and medicine and toxicity. Safe and unsafe handling of various tools and equipment used for animal experiments are described as well as the significance and specific methods of euthanasia of animals.

This handbook was drawn up with the expectations that students will be able to conduct daily experiments and practical trainings safely. Students should read this handbook carefully before conducting any animal experiments and keep these instructions in mind.

Ⅰ. BEFORE CONDUCTING ANIMAL EXPERIMENTS

1. Proper Animal Experiments

Animal experiments are essential for life science researches in studies of infectious disease prevention, overcoming incurable diseases and livestock improvements and so forth. In these experiments, the research protocol has to be clear, experimental procedures have to be scientific, and research results have to be reproducible as well as universal and effective. Therefore, genetic predisposition of animals needs to be clear, and the environmental factors surrounding animals have to be strictly regulated.

On the other hand, animals have long been companions of humans, as well as food supplies to humans to support our lives. Animals have been sacrificing their lives to contribute to human health and welfare. With this in mind, it is obvious that humans need to be more grateful, have higher ethics, and conduct experiments with as less pain as possible taking full account of the animal welfare. Knowledge gained from these animal experiments has to be used not only to promote human health and welfare, but also to save animals from diseases, preserve wild animal habitats, and promote the prosperous coexistence between animals and humans.

2. Animal Experiment Regulations

In Japan, "*Act on Welfare and Management of Animals (Act No. 105)*" was proclaimed in 1973, and it established basic concepts of animal management and conservation. Based on the Article 11, "*Standards Relating to the Care and Management of Experimental Animals (Notification No.6, of the Prime Minister's Office)*" was implemented in 1980 to provide ethical standards when dealing with laboratory animals.

Recently, based on the aforementioned Act and Standards, "*Act on Welfare and Management of Animals (No. 79, of the Ministry of the Environment)*" and "*Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain (Notification No. 88, of the Ministry of the Environment)*" were newly enacted in September 2012 and in April 2006 respectively. Furthermore, "*Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions (Notification No. 71, of MEXT)*" was notified in June 2012. The Guide for "*Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain"* was published in October 2017: it gives a clear exposition of the prevention of health and safety hazards in animal experimentation as well as standards relating to the care and management of laboratory animals and animal testing.

At Kagoshima University, "*National University Corporation Kagoshima University Animal Experiment Committee Regulation*" (April 2004), "*Standards Relating to the Care and Management of Laboratory Animals at Kagoshima University*" (March 2008), and "*Regulations Relating to Animal Experiments at Kagoshima University*" (April 2008) were established. Under the Kagoshima University Animal Experiment Committee, which was appointed by the university president and vice presidents, the guidelines are properly implemented. Besides, Kagoshima University Animal Experiment Committee issued *"Arrangements Relating to Animal Experiments at Kagoshima University"* (August 2017) and *"Guidelines for Animal Experiments with the Use of Hazardous or Carcinogenic Substances"* (January 2018), and strive to prevent any risks and hazards associated with animal experiments.

Regulations Relating to Animal Experiments at Kagoshima University

March 26, 2008

Regulations No. 23

(Purpose)

Article 1. These regulations prescribe the responsibilities of the President, the establishment of the Animal Experiment Committee, the approval process for animal experiment plans, the care and management of laboratory animals, and other necessary matters in order to properly conduct animal experiments, and the care and management of laboratory animals at Kagoshima University (hereafter called "the University").

(Basic Principle)

Article 2. As for animal experiments, "*Act on Welfare and Management of Animals (Act No. 105)*" (hereafter called "Act"), "*Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain (Notification No. 88, of the Ministry of the Environment)*" (hereafter called "Care and Management Standards"), "*Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions (Notification No. 71, of MEXT)*" (hereafter called "Fundamental Guidelines)", "*Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan)*" (hereafter called "Guidelines"), and "*Guidelines for Animal Disposal Methods (Notification of the Prime Minister's Office)*" shall be used as basic principles.

2 When conducting animal experiments, the experiments shall be conducted in accordance with relevant laws, and the *Act and Care and Management Standards* based on 3Rs (Replacement: find alternatives to using animals as much as possible, Reduction: reduce the numbers of animals to be used as little as possible, Refinement: use the methods that relief pains of animals as much as possible).

3 Care and management of laboratory animals shall be conducted in consideration of the Five Freedoms of animal welfare, which are internationally accepted standards: Freedom from hunger and thirst; Freedom from discomfort; Freedom from pain, injury, or disease; Freedom to express normal behavior; Freedom from fear and distress, to the extent that the purpose of scientific use of laboratory animals can be satisfied.

(Definition)

Article 3. In these regulations, the meanings of the terms listed below shall conform to the following definitions:

(1) "Animal Experiment": use of a laboratory animal (*5) for educational purpose, experimental research or manufacture of biotics or other scientific applications

(2) "Care and Management Facilities": facilities that undertake care management of laboratory animals, or facilities to conduct animal experiments

(3) "Laboratory": an animal experiment room where experimental operations (including less than 48 hours of temporary keeping) are carried out on laboratory animals

(4) "Facilities. etc": care and management facilities or laboratories

(5) "Laboratory Animal": an animal belonging to a mammal, birds, or reptiles reared or managed at a facility or other animals (hereafter called "other animals") that are used in experiments, including animals in transit to the facility

(6) “Animal Experiment Protocol”: a plan to carry out an animal experiment
(7) “Animal Experimenter”: a person who conducts an animal experiment
(8) “A Person Responsible for Animal Experiment”: an animal experimenter who governs all the work relating to animal experiment
(9) “Manager”: a person in charge of overall managements for laboratory animals and its facilities, under the appointment of the president, such as Dean of Joint Faculty of Veterinary Medicine, Dean of Joint Graduate school of Veterinary Medicine, Director of respective facilities and Departments
(10) “Manager of Laboratory Animals”: a person who possesses knowledge and experience in laboratory animals, and supports a manager in charge of overall management for laboratory animals and facilities
(11) “Caretaker”: a person who undertakes care management of laboratory animals under a manager of laboratory animals or an animal experimenter
(12) “Managers. etc.”: the President, the manager, the manager of laboratory animals, the animal experimenters, and the caretakers.
(13) “Guidelines, etc.”: basic standards and guidelines on animal experiment adopted and required by the Ministry of Health, Labor and Welfare, and the Ministry of Agriculture, Forestry and Fisheries, to be compliant

(Range of Application)

Article 4. The Guidelines should be applied to all the experiments conducted at the University using mammals, birds, reptiles and other animals as well as outside of the University under the supervision of the University’s Faculty and staff.

(ii) When an animal experiment is to be commissioned to other organizations, a person responsible for the animal experiment should confirm that experiments are appropriately conducted in adherence to the spirit of the guidelines.

(Responsibilities of the President)

Article 4-2 The President is ultimately responsible for conducting animal experiments, and for care and management of laboratory animals.

(Animal Experiment Committee)

Article 5. The President shall be responsible for the approval of animal experiment plans, monitoring of implementation status and the results, improvement measures based on the results, maintenance and approval of care and management facilities, approval of laboratories, educational training, safety management related to animal experiments, self–assessment, evaluation, validation by external experts, information disclosure, and other measures necessary for the proper implementations of animal experiments.

2. The President shall appoint an animal experiment committee (hereafter called “Committee”) as an administrative and advisory body in order to carry out the responsibilities of the preceding paragraph.

3. Necessary matters relating to the Committee shall be set separately.

(Formulating Animal Experiment)

Article 6. A person responsible for animal experiment shall formulate experimental plans based on the following matters, from the perspective of assuring the reliability of data acquired from animal experiments etc., and submit an "Animal Experiment Protocol (new version—Appended Form 1)" to the President for permission.

(1) Research purpose, significance, and necessity.
(2) Proper use of laboratory animals after taking into account of alternative methods.
(3) Selection of laboratory animal species that fit the purpose of research, the numbers of laboratory animals that influence an accuracy of animal experiment results and its reproducibility, reduction of numbers of animals used for animal experiment in consideration of genetic, microbiologic quality, rearing and caring requirements.
(4) Proper practices of animal experiment with relieving pains.
(5) Setting the humane endpoints (the timing of dropping the experiments to relieve an animal from intense pain) while formulating the experiments with high degree of pain (fatal toxic examinations, infection experiments, radiation exposure experiments, etc.).

2. The President shall require the responsible person for animal experiment to submit "Animal Experiment Protocol" to the Committee prior to the start of animal experiments, to determine whether to approve or disapprove its feasibility and appropriateness through the review by the Committee, and inform the result to the appropriate person in charge of the animal experiment.
3. A person responsible for animal experiment shall not start the experiment unless receiving an approval from the President.
4. A person responsible for animal experiment shall submit another "Animal Experiment Protocol (Modification/Addition) (Appended Form 2)", and ask for approval from the President when the following matters have changed/modified.

(1) A change of an animal experimenter
(2) A change of experiment periods
(3) Addition to the numbers of animals
(4) Change of animal species used
(5) Addition of test substances (medicine) or change of administration route
(6) Change of facilities or laboratories

5. A person responsible for animal experiment shall submit "Animal Experiment Application of using hazardous substance (carcinogen. etc) (Appended Form 2)" along with "Animal Experiment Protocol"

(Matters to Be Observed)

Article 7. A person responsible for animal experiment shall observe the following matters as well as laws and regulations, Care and Management Standards, Guidelines related to animal experiments, when conducting an animal experiment.

(1) Animal experiments shall be conducted at a facility properly maintained and managed.
(2) The following matters described in "Animal Experiment Protocol" shall be observed.
    (i) Proper use of anesthetics and analgesics
    (ii) Consideration for experimental ending time (including the humane endpoints)

(iii) Proper postoperative management
(iv) Proper choice of euthanasia

(3) Experiments that require attention to safety management (experiments using physically, chemically dangerous materials, narcotics, psychoactive drugs, pathogen, and genetically modified animals) shall be conducted in accordance with related laws and regulations of the university.
(4) Ensure a proper and safe facility and equipment when conducting the animal experiments that involve physically or chemically dangerous materials or pathogens.
(5) Endeavour to acquire necessary experimental techniques for future experiments.
(6) Major lifesaving surgery that is highly invasive shall be performed under the supervision of an experienced person.

2. The President shall require the manager to report the results of the implementation of the animal experiment plan, including the number of animals used and any changes from the plan upon completion or cancellation of the experiment by submitting an "Animal Experiment Report (Appended Form 3)", and take necessary improvement measures to ensure adequate implementation of animal experiments based on the advice of the Committee as necessary. Until the experiment be completed/cancelled, the implementation status must be reported to the President.

3. Guidelines for handling and management of hazardous substances (carcinogen. etc) shall be decided separately by the Committee.

(Establishing Care and Management Facilities)

Article 8. When establishing or changing a care and management facility, the manager shall submit an "Application for Approval of Care and Management Facility Establishment (Appended Form 4)" and ask for an approval of the President.

2. The President shall have the Committee investigate the facility filed for, accordingly decide based upon its suggestions, and notify the relevant manager of the result.

3. The manager of laboratory animals, animal experimenters, and caretakers shall only be allowed to rear laboratory animals or conduct animal experiments at the approved facility.

4. The President shall require the manager and the manager of laboratory animals to report on the status of the care and management facility of the laboratory animals, and indicate a direction for improvement based on the advice of the Committee as needed.

(Requirements of Care and Management Facilities)

Article 9. Care and Management Facilities shall fulfill the following requirements.

(1) Facilities should have the structure to keep proper temperature, humidity, ventilation and brightness.
(2) Facilities should have the rearing facility according to species, physiology, ecology actions, behaviors and the numbers of animals.
(3) Facilities should have the structure for easily cleanable/sterilizable floors and walls, and sanitary facility to wash and sterilize devices.
(4) Facilities should have the structure and strength that animals will not escape.

(5) Facilities should have a measure to prevent bad influences to surrounding environment such as smells, noises, and wastes.
(6) There should be a person in charge of the facility.

(Establishing Laboratories)

Article 10. When establishing or changing a laboratory other than the care and management facilities, the manager shall submit an "Application for Approval of Laboratory Establishment (Appended Form 5)" and ask for an approval from the President.

2. The President shall have the Committee investigate the laboratory filed for, accordingly decide based upon its suggestions, and notify the relevant manager of the result.

3. The manager of the laboratory animals, animal experimenters and caretakers shall only be allowed to conduct animal experiments (including a temporary keeping of animals less than 48 hours) at the approved laboratory.

(Requirements of Laboratories)

Article 11. Laboratories shall fulfill the following requirements.

(1) Laboratories should have the structure and strength that animals cannot escape, and the environment for escaped animals to be easily captured.
(2) Laboratories should be easily cleaned and sanitized for excretions and blood, etc.
(3) Laboratories should be always kept clean and have a measure to prevent bad influences to surrounding environment such as smells, noises, and wastes.

(A Facility Maintenance/Management or Improvement)

Article 12. The manager shall endeavor to maintain, manage, or improve the facilities to properly manage the laboratory animals and conduct the animal experiments.

2. The manager shall secure the environment to be able to appropriately care and manage laboratory animals, considering species, physiology, ecology actions, behavior of laboratory animals.

(Disuse/Decommissioning of a Facility)

Article 13. The President shall approve the disuse of the facilities after the investigation by the Committee based on a "Notification of Disuse of a Facility" (Appended Form 6) submitted by the manager.

2. The manager shall endeavor to hand over laboratory animals to other facilities as necessary, in cooperation with a person responsible for animal experiment.

(Manual Preparation and Announcement/Observance)

Article 14. The manager or the manager of laboratory animals shall prepare a manual for rearing and caring laboratory animals and ensure that the animal experimenters and caretakers are informed of and comply with the manual.

(Maintaining Health and Safety of Laboratory Animals)

Article 15. The manager of the laboratory animals, animal experimenters and caretakers shall observe the "Care and Management Standards" and endeavor to keep the laboratory animals healthy and safe.

(Introduction of Laboratory Animals)

Article 16. The manager shall introduce laboratory animals from the institutions that are appropriately managed in compliance with related laws and regulations.

2. The manager of laboratory animals shall implement proper quarantine and isolation of newly introduced animals as needed.

3. The manager of laboratory animals shall take necessary measures to acclimatize and adjust animals to the new environment.

(Feeding and Water Supply)

Article 17. The manager of the laboratory animals, animal experimenters and caretakers shall provide proper feeding and watering according to physiology, ecology actions and behaviors of laboratory animals.

(Health Management)

Article 18. The manager of the laboratory animals, animal experimenters and caretakers shall perform necessary health management for laboratory animals to prevent any injuries and diseases caused by factors other than the experimental purposes.

2. The manager of the laboratory animals, animal experimenters and caretakers shall properly treat laboratory animals when they are injured or sick caused by factors other than the experimental purposes.

3. The manager of laboratory animals shall ensure that the number and condition of laboratory animals kept in the facilities are monitored through daily management and maintenance as well as on regular patrols, etc.

(Rearing of Different Species or Multiple Animals)

Article 19. The manager or the laboratory animals, animal experimenters and caretakers shall consider the combination of animals when rearing different species or multiple animals at the same facility.

(Keeping Records and Report)

Article 20. Managers etc. shall organize and keep records on suppliers, breeding histories and disposal methods of carcasses.

2. The manager shall report to the President on the numbers and species of laboratory animals that are kept at the facility every fiscal year.

(Provision of Information When Handing Over)

Article 21. Managers etc. shall provide the recipient with information on characteristics, rearing methods, and any infectious diseases of laboratory animals when handing them over.

(Transfer)

Article 22. Managers etc. shall endeavor to observe the Care and Management Standards, to ensure the health and safety of animals, and to prevent any dangers to humans when transporting laboratory animals.

(Risk Prevention)

Article 23. The manager shall determine the capturing methods of escaped animals beforehand (i.e. Response plan for escaped animals).

2. The manager shall promptly notify the related institutions when laboratory animals that are potentially harmful to humans have escaped from the facility.
3. The manager shall take the necessary procedures to prevent the manager of laboratory animals, animal experimenters, and caretakers from contracting infectious diseases and allergies transmitted by laboratory animals, as well as from animal bite wound infections.
4. When keeping poisonous animals such as poisonous snakes, the manager shall set separate procedures to prevent any dangers to humans.
5. The manager shall make sure that people who are not a part of the experiment animal team have no access to the laboratory animals.
6. The manager of laboratory animals, animal experimenters, and caretakers shall strive to mutually provide the information and data necessary to prevent potential risks of harm caused by laboratory animals.
7. The manager and other supporting staff shall take necessary measures, for people not involved in the care and management of laboratory animals, to avoid the risk of contact with laboratory animals.

(Emergency Procedures)

Article 24. The manager shall create emergency procedures for earthquakes and fire and inform relevant personnel of its plan (such as Emergency Response Plan).

(ii) In the event of an emergency situation, the manager and other supporting staff shall strive to protect laboratory animals, and prevent human harms of escaped laboratory animals and the occurrence of environmental conservation problems.

(Response to Amphixenosis)

Article 24-2. The manager of the laboratory animals, animal experimenters and caretakers shall make efforts acquiring enough knowledge and collecting information of common infectious disease for humans and animals.

(ii) The manager, the manager of the laboratory animals and animal experimenters shall make efforts developing a communication system with public health institutions to be able to take necessary actions promptly in the event of an outbreak of zoonosis.

(Educational Trainings)

Article 25. The President shall require the Manager of Laboratory Animals, Animal Experimenter, and Caretakers to take the educational training courses on the following matters.

(1) Laws, regulations, and guidelines on animal experiments stipulated by the University
(2) Basic matters relating to the animal experiment procedures

(3) Basic matters relating to the care and management of animals
(4) Securing safety and safety management
(5) Matters relating to zoonosis
(6) Other relevant matters for appropriate implementation of animal experiments

2. The President shall create and save the records of date, contents, and names of instructors and participants.
3. The President shall strive to ensure that the necessary trainings are provided according to respective job assignments to the manager of laboratory animals, animal experimenters, caretakers, and caretakers.
4. Other necessary matters for the implementation of trainings not stipulated in the preceding two paragraphs shall be stipulated separately by the Committee.

(Self-Assessment, Evaluation and Verification)

Article 26. The President shall have the Committee conduct an internal review, self- assessment/evaluation on the conformity to general guidelines and the compliance with the *Standards Relating to the Care and Management of Laboratory Animals* and basic guidelines.

2. The Committee shall conduct the self- assessment/ evaluation, and report the results to the President.
3. The Committee may ask the Manager, Animal Experimenter, and Caretakers to turn in any necessary documents for self- assessment/ evaluation.
4. The President shall periodically gain verifications from outside experts of the results on the self-assessment/ evaluation.

(Information Disclosure)

Article 27. The President shall annually disclose the information (guidelines, status of the care and management of laboratory animals, results of self- assessment/evaluation, and verification by outside experts) relating to the animal experiments.

(Application Mutatis Mutandis)

Article 28. Any animal experiments with animals other than "Laboratory Animals" specified in Article 3. (5) shall be conducted in line with the *Standards Relating to the Care and Management of Laboratory Animals*.

(Punitive Provisions)

Article 29. The President may immediately suspend the animal experiment conducted by violators of the regulations stipulated hereby and prohibit the animal experiment for a certain period of time.

2. The President may seek the advice of the Committee regarding the punitive actions.

(Miscellaneous Provisions)

Article 30. In addition to the matters provided for by this regulation, any necessary matters shall be prescribed by the President separately.

Supplementary Provisions

The Regulations shall become effective from April 1, 2008.

Supplementary Provisions
The Regulations shall become effective from November 1, 2012.

Supplementary Provisions
The Regulations shall become effective from April 1, 2015.

Supplementary Provisions
1. The Regulations shall become effective from January 1, 2018.
2. The Regulation of Laboratory Animals (Determined on April 1, 2008, by the Board of Directors at Kagoshima University) shall be abolished.

Supplementary Provisions
The Regulations shall become effective from April 1, 2019.

Supplementary Provisions
The Regulations shall become effective from November 1, 2019.

Supplementary Provisions
The Regulations shall become effective from December 15, 2020.

Supplementary Provisions
The Regulations shall become effective from November 1, 2021.

Supplementary Provisions
The Regulations shall become effective from May 1, 2022.
*Appended forms are omitted.

Standards Relating to the Care and Management Facilities and Laboratories at Kagoshima University

April 1, 2020
Regulations No. 23

Article 1. This regulation shall prescribe necessary matters on care and management facilities and laboratories at Kagoshima University based on the *Regulations Relating to Animal Experiments at Kagoshima University,* Article 30.

Article 2. The care and management facilities shall be signed with its signage, "Care and Management Facility".

Article 3. The care and management facilities shall satisfy the following requirements; provided, however, that this shall not apply to livestock.

(1) Manager of Laboratory Animals
The manager of laboratory animals shall be appointed.
It shall be noted that the manager of laboratory animals is a person who supports the manager and is in charge of managing laboratory animals, and a person who has general knowledge of laboratory animals related to regulations for laboratory animals, infections including zoonosis, breeding and genetics, physiology, ecology actions, behaviors, as well as care management skill and diagnosis.

(2) Structure of Floor and Wall
(i) It shall be easily cleanable and have the structure and strength that animals cannot escape.
(ii) It shall be the structure that is no risk for injury to the animals.

(3) Temperature and Humidity
(i) There shall be air conditionings to control the temperature.
(ii) There shall be the structure to keep proper room temperature according to species.
(iii) There shall be the facilities that monitor temperature and humidity all the time.
(iv) It is desirable that humidity control facilities such as a humidifier shall be installed.

(4) Ventilation
Ventilation facilities shall be installed.

(5) Lighting
(i) There shall be sufficient inside lights.
(ii) It shall be maintained proper illumination. (Illumination target: 150-300 lux, 40-85 cm above a floor)
(iii) It is desirable that light from outside the room should be blocked and devices with a light/dark time control function should be installed.

(6) Rearing of Different Species

(7) In the case of care and management for different species or multiple animals, those animals shall be housed with appropriate combinations without disturbing the experiments, etc. Rearing facility
(i) There shall be racks and cages according to animal species.

(ii) There shall be the structure and strength that animals cannot escape.
(iii) There shall be the structure to be easily cleaned and disinfected.
(iv) There shall be enough space and area for each laboratory animal so that they could do easily their daily activity such as standing up and laying down naturally.
(v) The structure shall be free of sharp edges and protrusions, and the gap between cages is not to be damaged or trapped the animals' bodies or limbs.

(8) Prevention Measures for Escaped Animals

The doorway has a structure that shall be able to lock the doors.
(i) Drainage shall have drain covers so that animals cannot escape.
(ii) Prevention measures according to animal species shall be taken. (e.g., rat guard, anteroom, etc.)
(iii) There shall be capture tools for escaped animals. (e.g., capture net, etc.)
(iv) The contact network in case of escaped animals shall be organized and posted.

(9) Hygiene Control

(i) There shall be cleaning supplies.
(ii) There shall always have disinfectant-ready.
(iii) Odor control measures shall be taken by installing ventilation facilities or air cleaners.
(iv) Noise control measures according to animal species shall be taken.
(v) Waste and animal carcass shall be incinerated or sent to a professional disposal company.
(vi) There shall be a refrigerator for a temporary storage of animal wastes and carcasses.

(10) Care and Management Manual

(i) A standard operation procedure for care and management (manual) shall be prepared and posted.
(ii) The manual shall be disseminated to the persons concerned and must be observed.

(11) Emergency Manual

A Plan for measures in case of emergency such as earthquake, fire etc., shall be established, posted and disseminated.

(12) Records

The following records and documents shall be organized and retained.
(i) Records of animal quarantine (including document inspection) and history of animals carried in and out.
(ii) Daily records of rearing and management (status of access, animals' health, escaping, room temperature and humidity).
(iii) Hygiene control (washing and sterilization for rearing supplies, disposal of wastes and carcasses, cleaning and disinfection).
(iv) Records of maintenance and inspection of facilities and equipment.

(13) Zoonosis Control Measures

(i) Zoonosis Control Measures shall be established, posted and disseminated.

(ii) When animals are kept continuously for more than 6 months, the microorganisms to be tested should be determined for each animal species and monitored periodically.

The monitoring items and frequency for each animal species are shown below.

Animal species	Infection	Required/ Recommended	Quarantine at Introduction (Document Inspection)	Every 6 months	Less than every 1 year
Mouse/ Hamster	Lymphocytic choriomeningitis（Arenavirus）	Recommended	◯	◯	
Rat	Hantavirus	Required	◯	◯	
Monkey	Macacine alphaherpesvirus	Required	◯		◯
	Tuberculosis	Required	◯	◯	
	Dysentery	Required	◯	◯	
Dog	Brucellosis	Required	○	○	
	Rabies	Required	Prevention by vaccination		
	Leptospirosis	Required			
Birds	Avian flu	Recommended	○	○	
All species	Salmonella	Recommended	○	○	

(iii) Necessary medical supplies for the case of bites or other accidents shall be available.

(iv) The contact network for the case of bites, zoonosis, or any other accidents shall be organized and posted.

Article 4. The laboratories shall be identified by its signage, “Laboratory”.

Article 5. The laboratories shall satisfy the following requirements.

(1) Ventilation

Ventilation facilities shall be installed.

(2) Lighting

(i) There shall be sufficient inside lights.
(ii) It shall be maintained proper illumination.

(3) Experimental Facility
(i) There shall be equipped for experiments such as laboratory tables, etc.
(ii) There shall be the structure to be easily cleaned and disinfected.

(4) Prevention Measures for Escaped Animals
(i) The doorway has a structure that shall be able to lock the doors.
(ii) Drainage shall have drain covers so that animals cannot escape.
(iii) Prevention measures according to animal species shall be taken. (e.g., rat guard, anteroom, etc.)
(iv) There shall be capture tools for escaped animals. (e.g., capture net, etc.)

(5) Hygiene Control
(i) There shall be cleaning supplies.
(ii) There shall always have disinfectant-ready.
(iii) There shall be waste exclusive containers.
(iv) Odor control measures shall be taken by installing ventilation facilities or air cleaners, etc.
(v) Waste and animal carcass shall be incinerated or sent to a professional disposal company.
(vi) There shall be a refrigerator for a temporary storage of animal carcasses.

(6) Emergency Manual
A Plan for measures in case of emergency such as earthquake, fire etc., shall be established, posted and disseminated.

(7) Zoonosis Control Measures
(i) Necessary medical supplies for the case of bites or other accidents shall be available.
(iv) The contact network for the case of bites, zoonosis, or any other accidents shall be organized and posted.

National University Corporation Kagoshima University Animal Experiment Committee Regulation

April 1, 2004

Regulations No. 23

(Purpose)

Article 1. Based on "*National University Corporation Kagoshima University Organization Regulation"* Article 21 No.3 and "*Regulations Relating to Animal Experiments at Kagoshima University*" (hereafter referred as "the regulations") Article 5 No. 2, this regulation shall prescribe necessary matters on the National University Corporation Kagoshima University Animal Experiment Committee (hereafter referred as "the Committee").

(Organization)

Article 2. The Committee shall be comprised of the following committee members.

(1) A trustee appointed by the President.
(2) A person appointed by the director of Research and Education.
(3) A person elected from amongst professors, associate professors and instructors of each department, graduate school, and the University Hospital
(4) Two persons well versed in animal experiments
(5) Two persons well versed in laboratory animals.
(6) Several scholars and experts other than animal experimenters.
(7) People deemed to be necessary by the President.

2. The committee members stipulated in preceding items (3) through (7) shall be appointed by the President.
3. The committee members stipulated in items (3) through (7) of paragraph 1 shall have two years of term of office and the term may be renewable. However, in case where a vacancy occurs, the term of a substitute committee member shall be the remaining term of his/her predecessor.
4. The committee members of items 2 and 3 of paragraph 1 may concurrently serve as any of the committee members of items 4, 5, or 6 of the same paragraph.

(Discussing Matters)

Article 3. The Committee, as requested by the President, shall discuss and investigate the following matters, and report to, and advise the President.

(1) Ethical review of the conformity of the animal experiment protocol to the laws and regulations, the Care and Management Standards, basic guidelines, and the regulations herein regarding animal experiments
(2) Implementation status and results of the animal experiment protocol.
(3) Establishment and demolition of the facilities and laboratories.
(4) Care and management of laboratory animals.
(5) Proper conduct of animal experiments, proper treatment of laboratory animals, educational contents of related laws and regulations and its implementation.

(6) Self-assessment/evaluation, verification by external experts, and information disclosure
(7) Other necessary matters needed for proper conduct of animal experiments.

2. The Committee shall strive to mutually provide necessary information with other relevant committees related to animal experiments that require attention to safety management as necessary.

(Chairperson)

Article 4. The Committee shall have a chairperson, the committee member as specified in Article 2, item (1) of paragraph 1.

2. The chairperson shall call and preside over meetings.
3. If the chairperson is unable to perform his/her duties, the duties shall be performed by a member designated by the chairperson in advance.

(Parliamentary Proceedings)

Article 5. The Committee shall be formed with the attendance of a majority of the Committee members, and the decisions shall be effected by a majority of the members present. In the case of a tie, the chairperson shall be entitled to the casting vote.

2. The Committee members shall not be involved in the review of animal experiment plans in which they are responsible for the animal experiments, to avoid any conflict of interest.

3. The Committee members shall not disclose or divulge any information about the animal experiment plans to any third party.

(Substitute Attendance)

Article 6. Substitute attendance shall be allowed in case of an accident.

(Attendance Other Than the Committee Members)

Article 7. As deemed necessary by the Committee, attendance other than the committee members shall be allowed.

(Clerical Work)

Article 8. All the clerical work of the Committee shall be dealt by the Research Cooperation Division.

(Miscellaneous Provisions)

Article 9. In addition to the matters provided herein, any other necessary matters shall be prescribed separately.

Supplementary Provisions
The Regulations shall become effective from April 1, 2004.
Supplementary Provisions
The Regulations shall become effective from April 1, 2005.
Supplementary Provisions

The Regulations shall become effective from April 1, 2006.

Supplementary Provisions

1. The Regulations shall become effective from April 1, 2007.
2. Any Associate professor who became a member before this regulation became effective shall continue to be a member until his/her term ends.

Supplementary Provisions

The Regulations shall become effective from November 28, 2007 and be applied from April 1, 2007.

Supplementary Provisions

The Regulations shall become effective from April 1, 2008.

Supplementary Provisions

The Regulations shall become effective from April 1, 2012.

Supplementary Provisions

The Regulations shall become effective from October 1, 2016.

Supplementary Provisions

The Regulations shall become effective from April 1, 2017.

Supplementary Provisions

The Regulations shall become effective from September 28, 2017.

Supplementary Provisions

The Regulations shall become effective from November 29, 2018.

Supplementary Provisions

The Regulations shall become effective from November 1, 2021.

Arrangements Relating to Animal Experiments at Kagoshima University

August 10, 2018

Adopted by Animal Experiment Committee

"Other animals" defined in Article 3 item (5) of "*Regulations Relating to Animal Experiments at Kagoshima University"* (Reg. No. 23, 2008), are stipulated as follows.

1 the Animals subject to be controlled in accordance with *"Act on the Prevention of Adverse Ecological Impacts Caused by Designated Invasive Alien Species"* (Act No. 33 of 2005) and *"Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms"* (Act No. 97 of 2003)

e.g. bullfrog, cane toad, bluegill, largemouth bass, brown widow spider, signal crayfish

2 the Animals that animal experimenters wish to get screened by Animal Experiment Committee of Kagoshima University

i.e. animals which are required to be screened by Animal Experiment Committee of Kagoshima University for study report or publication

Guidelines for Animal Experiments with the Use of Hazardous and/or Carcinogenic Substances

January 1, 2018
Animal Experiment Committee
Kagoshima University

(Purpose)

1. The guidelines prescribe the necessary matters to protect experimenters, others, non-target animals and environment from the risk of exposure to hazards in the animal experiments utilizing hazardous substances as follows: carcinogen which may endanger the health and lives of students, staff, faculty, and non-target animals at National University Corporation Kagoshima University; toxic heavy metals which may cause environmental contamination such as arsenic, mercury, lead, cadmium and the like; endocrine disruptors, etc. (hereinafter collectively called "Hazardous and/or Carcinogenic Substances").

(Definition)

2. In these guidelines, the meanings of the terms listed below shall conform to the following definitions:
(1) Carcinogen: the agents in Group1 (Carcinogenic to humans), Group2A (Probably carcinogenic to humans), and Group2B (Possibly carcinogenic to humans) classified by the International Agency for Research on Cancer (IARC)
(2) Toxic heavy metals: the persistent, bioaccumulative and toxic elements which cause major health problems, such as mercury, lead, and cadmium, etc.
(3) Endocrine disruptors: the exogenous chemicals which interfere with the endocrine system, then result in harmful adverse effects on organisms, including humans, such as disorders of any system in the body.
(4) Other harmful materials: the harmful materials reported in the standard managed by The Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and categorized into Germ cell mutagenicity, Carcinogenicity, Reproductive toxicity, and Hazardous to the aquatic environment, etc.

(Application)

3. "Application Form for Animal Experiment with the Use of Hazardous and/or Carcinogenic Substances" (appended Form 2-1) in addition to the Animal Experiment Protocol stipulated by Animal Experiment Committee shall be submitted to Animal Experiment Committee, prior to conducting the animal experiment with the use of Hazardous and/or Carcinogenic Substances (hereinafter referred to as "Animal Experiment with administration of Hazardous and/or Carcinogenic Substances").

(Screening)

4. The screening of Animal Experiments with administration of Hazardous and/or Carcinogenic Substances at Animal Experiment Committee shall be conducted in conformity with "Standards and Operating Guidelines for Animal Care Facility, and Laboratory Equipment" (appendix in the following page). In any cases where it is deemed necessary at the aforementioned screening process, Animal Experiment Committee may hold the hearing of opinions from experts in the field of Hazardous and/or Carcinogenic Substances.

(Animal Experimental Laboratory involving the administration of Hazardous and/or Carcinogenic Substances)

5. For occupational safety and health management of users, including experimenters, students, staff, and faculty, and contamination and pollution control in the animal care facilities and laboratories, the necessary measures or actions in accordance with "Standards and Operating Guidelines for Animal Care Facility and Laboratory Equipment" (appendix in the following page) shall be taken whenever Hazardous and/or Carcinogenic Substances are utilized. The experimenters shall provide appropriate care to laboratory animals at animal care facilities and laboratories, and monitor them under the responsibility of the Person Responsible for Animal Experiment.

(Report)

6. The experimenters engaged in Animal Experiment with administration of Hazardous and/or Carcinogenic Substances shall promptly report to the managers of Care and Management Facilities or Laboratory and Animal Experiment Committee as soon as any extraordinariness or uncommonness in the laboratory management is identified.

(Discontinuation of Experiments)

7. Animal Experiment Committee may suspend the experiments and take other necessary measures in the event that inappropriate or irrelevant Animal Experiments with administration of Hazardous and/or Carcinogenic Substances are being conducted.

(Miscellaneous)

8. In addition to the matters provided for in these guidelines, Animal Committee may prescribe any necessary matters separately in relation to Animal Experiments with administration of Hazardous and/or Carcinogenic Substances.

(Appendix)

Standards and Operating Guidelines for Animal Care Facility and Laboratory Equipment

1. When utilizing Hazardous and/or Carcinogenic Substances and conducting the treatment of animals dosed with the aforementioned substances, the experiments shall be performed within negative pressure devices such as biosafety cabinets, etc. in principle for safely working.

2. The Laboratory Animals dosed with Hazardous and/or Carcinogenic Substances shall be kept and reared within the negative pressure rearing apparatus essentially utilizing disposable animal cages, etc. during the period in which there are risks that the Laboratory Animals may excrete or evacuate the aforementioned substances. Any apparatus, including disposable animal cages, etc. shall be handled as infectious waste mutatis mutandis immediately after the aforesaid period.

3. All the equipment and the materials, including floor mat and bedding, etc., contaminated with Hazardous and/or Carcinogenic Substances shall be retrieved and handled (e.g. incineration, etc.) with the same level of care as infectious waste disposal.

4. The criteria of the water pollution control regulated by Kagoshima City shall be met in order for the drainage and the waste fluids with the above-mentioned substances from the Laboratory to be safely poured down the drain below the effluent standard. In cases where such content of the discharge water potentially exceeds the maximum allowed contamination levels, that runoff shall be collected and dealt with properly.

5. During the period in which there are risks that the Laboratory Animals may excrete or evacuate the Hazardous and/or Carcinogenic Substances, it shall be banned in principle to take such Animals outside of the designated area.

6. The Animal Experimenters shall exercise good rearing management in the laboratory and the designated area.

7. All the laboratory workers, including students, staff, experimenters, etc. engaged in the experiments with the use of hazardous substances in the laboratory and the designated area shall be well-versed in rearing laboratory animals and in handing hazardous substances.

Ⅱ. QUARANTINE FOR ANIMAL EXPERIMENTS

1. Purpose of Quarantine

It is necessary for the newly arrived animals to be quarantined and inspected before staring an experiment. The most important purpose for quarantine is to prevent the introduction of zoonosis (see IV.in this chapter). Especially, wild non-human primates and rodents may be carrying dangerous pathogens for humans, so that quarantine must be conducted carefully for sufficient time (see IX.in this chapter). In addition, not only would pathogens introduced by infected animals possibly infect other healthy animals resulting in a confusion in the experiment results, but also the introducing infected animals could negatively affect other ongoing animal experiments in the same facility.

The purpose of quarantine is not only to identify infectious diseases but also to make those animals familiarize with the environment. If the adjustment is not done properly, animals might not react well and might negatively affect the results.

2. Quarantine Strategies

Strategies of quarantine differ depending on the animal species, microbiological quality grade, purpose of the experiment and its contents. It is desirable to conduct quarantine after deciding the quarantine measure, its duration, its items and procedures for abnormal cases. Recently, for small laboratory animals, SPF (specific pathogen free) animals can be purchased from trusted contractors, but for conventional animals, more attention is required, since they might carry subclinical infectious agents even if they look healthy.

Before the introduction of animals, it is necessary to confirm the report such as a microbe examination report that is provided by a contractor.

Ⅲ. SAFETY MANAGEMENT OF VARIOUS LABORATORY ANIMALS

1. Cattle

Though cattle are usually docile and easier to handle, it is important to pay extreme attentions not to cause any accidents. The common injuries (Table 1) while handling cattle are being kicked by the hind legs, stepped on, or knocked over with the horn or head. The general causes of these accidents came from forcing cows to move, or handlers' carelessness and fatigue. To avoid these accidents, it is recommended that students become familiar with cattle behavior not to frighten or threaten them. It is also important to know their characteristics and individual temperaments and always be aware of the surroundings when handling cattle.

Table 1. Examples of Accidents Occurred While Handling Cattle at Kagoshima University

Types of Accidents	Types of Works	Injured Area
1. Being Kicked	- hoof trimming	feet, arms, head
	- artificial insemination	feet
	- milking	feet, arms

	-	breast examination	feet, arms
	-	body measurement	feet, arms
	-	nose print	feet
	-	blood sampling	feet, arms
2. Being stepped on	-	milking	tips of toes
	-	body measurement	tips of toes
	-	putting on bridle	tips of toes
	-	capturing in a narrow area	stomach (stepped on when one fell)
3. Being pushed	-	calf measurement after birth	chest (by a mother cow)
	-	opening the headlocks	chest
4. Being bitten	-	oral examination	fingers

1) Rearing Management

During daily care of cattle, students should wear heavy-duty working clothes, boots, hat, and gloves for safety reasons. Students should gently talk to cattle. Cattle will become tame during the course of getting exercises or training outside and being groomed or trimmed.

When cattle are housed, the size of the facility should be as wide as possible for safety, and the bedding should be placed. Their hoof should be trimmed regularly (Figure 1), and extreme attentions should be paid to the hygiene control, such as pest control and disinfection, in order to avoid zoonosis and pest infestations.

Dehorning is recommended wherever possible to avoid any unnecessary fights among cattle and dangers to humans. Castration is recommended when bulls are young to tame their temper.

Newly arrived cattle and mother cows in the perinatal and weaning period are instinctively very sensitive and stressed, so students should treat them with more care and attention.

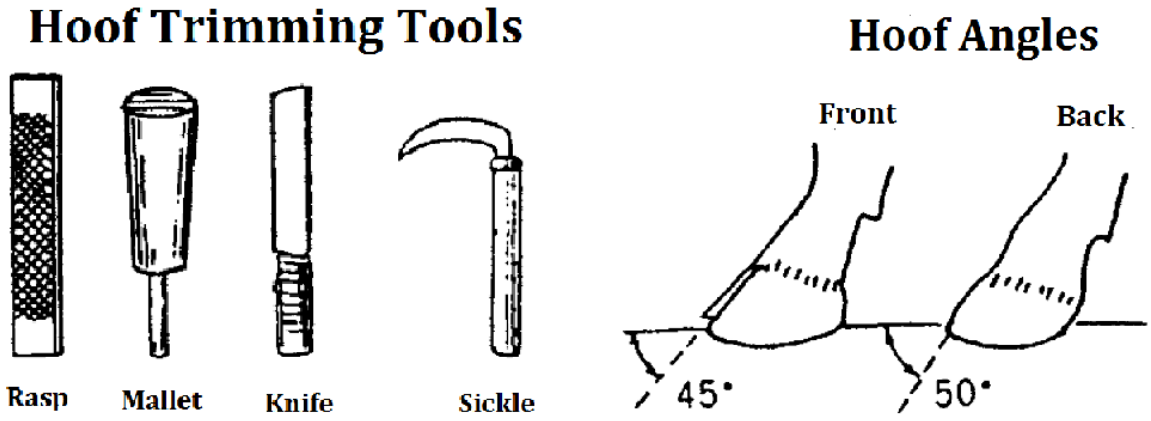


Figure 1. Hoof Trimming

2) Restraint

It is crucial to learn how to restrain the cattle's head. There should be several sizes of nose rings and bridles ready depending on the size of cattle and the purpose of use. When capturing cattle, gently approach by calming them to a wall or a safe place where they cannot back away, and restrain their heads first.

Grazing cattle should be also driven into a corner or small/narrow space, not being chased around for capturing. During the treatments such as insemination, vaccination, and blood sampling, the specialized restraining stall should be used. The rope from the nose ring should be tied tight to a pole to restrain cattle's head, and as needed, use the rope to restrain the tail and hind legs.(Figure 2)

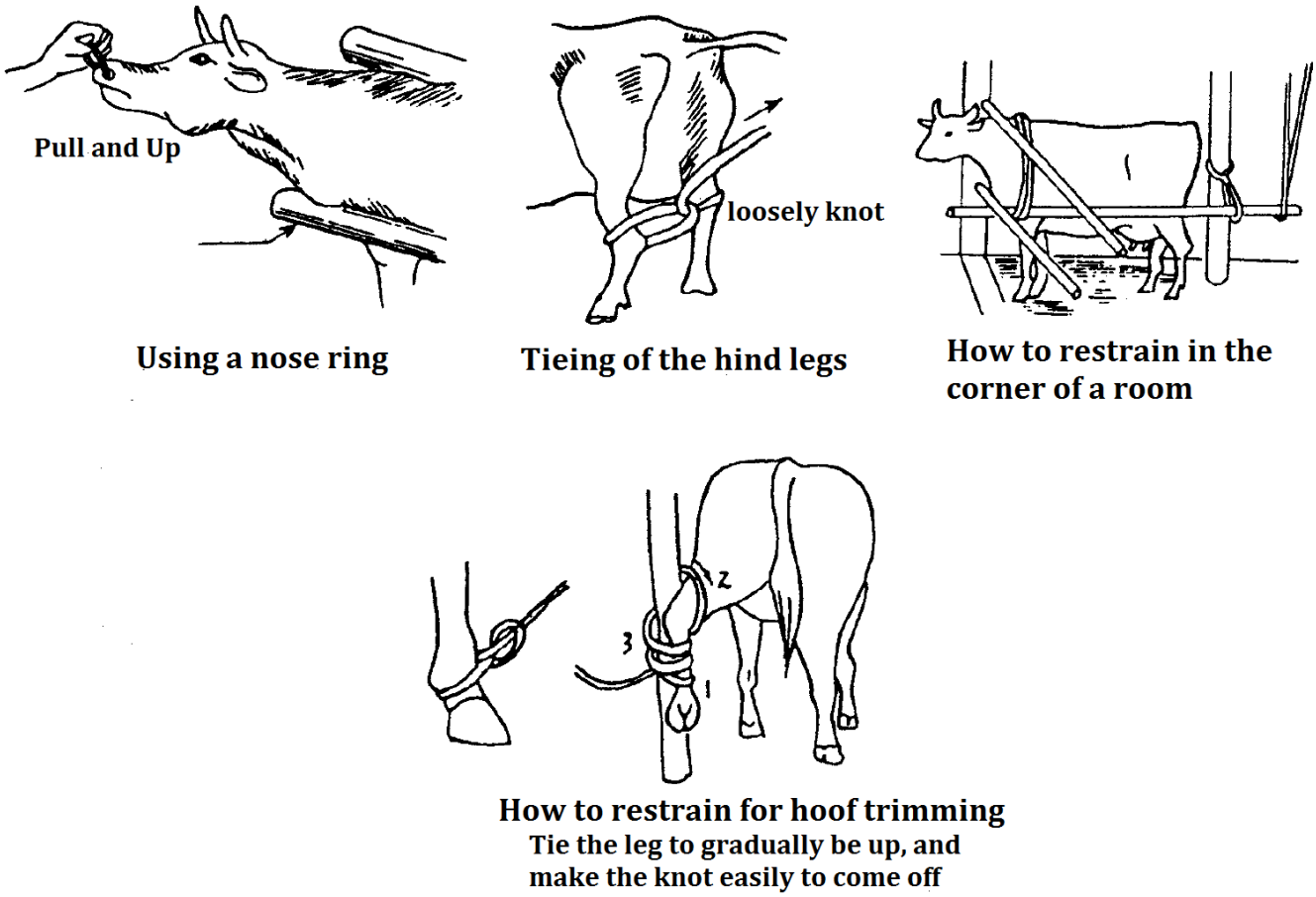


Figure 2. How to Restrain Cattle

3) Other Considerations

It is also important to learn how to tie ropes (cannot be untied by cattle themselves but handlers) and how to make bridles (Figure 3) in accordance to the purposes. Also, it is necessary for students to learn how to use agricultural tools used in rearing and managing cattle, such as sickles, shovels and lawn mower.

Figure 3. Bridles

[Notes]

1) New Chikusan Zusetsu, New Agricultural Education Research, Agricultural Library (1992)
2) Kotori Wagyu Jyouzuna Kaikata, Takamichi Ueda, Rural Culture Association (1993)

2. Horses

1. Rearing Management

(1) Approaching Horses (both Inside and Outside a stable)

1) Be aware that horses are very timid and cautious.

2) Look at the horse's face and talk to calm down. Once settled, approach the horse quietly from the left shoulder and lightly rub the neck. Place a rope around the neck (Figure 4) and put on harness (Figure 5).

3) Always pay attention to movements of the horses but do not timidly approach the horse nor make a big noise because horses get scared easily and become dangerous.

4) When horses pretend to bite or act in a malicious manner, they put their ears down and have a sharp look that it is usually easy to tell. When this happens, look at their eyes and scold the horse with a loud voice.

(2) Handling Horses

1) Rough handling will cause horses to be scared of humans and raise suspicions.

2) Act confidently, and at the same time, try to understand horses and show affectious attentions.

(3) Interacting with Horses

1) Horses will understand handler's intentions and feelings from facial expressions, voices, and behavior, if constantly being talked by humans, and those interactions develop interdependent relationship between humans and horses.

2) Read horses' emotions and behaviors.

(4) Care for Horses

1) Horses should be firmly tied in a way that the tie cannot come loose when pulled by horses, but easily comes loose by humans (Figure 6).

2) Brushing – Start from the left side to the rear side, then to the right side

3) Hoof Care – Wash with water and apply oil.

(5) Forage – Appropriately feed the mixture of concentrated and coarse feed (appropriate amount and ratio).
Water – Constantly feed clean and fresh water. When using a water bowl, make sure that the bowl is always washed and cleaned.

Figure 4. How to put and hold on to a lope

Figure 5. How to put on harness

Figure 6. How to tie a rope

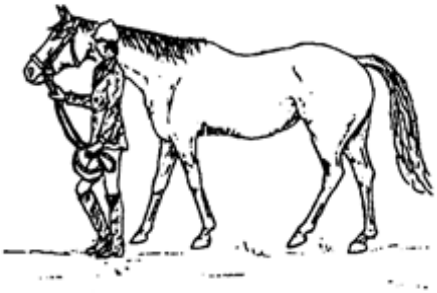


Figure 7. Pull a horse side by side

(6) Exercise

1) Take horses to a pasture by pulling (Figure 7).

2) Exercises are necessary for horses to stay healthy.

2. Restraint

(1) Restraint in the stable – How to put halter and lead rope.

(2) Restraint at a hitching post – Make sure how to tie appropriately to the post.

3. Horse handling can be managed safely by close observing their facial expressions and movements.

(1) Ears

Being aware – Both ears are up and are facing the same direction.

Being anxious – Each ear is moving separately.

Being cautious – Ears are put down backwards.

(2) Eyes

Sharpness of the eyes can somewhat tell their emotions and healthiness.

(3) Nose

Anxiety and Fear – Open the nostril and breathe shallowly.

Unpleasant – Waggle taut upper lip.

Pleasant – Curl back the upper lip and muffle, inhales with the nostrils and squint (Flehmen reaction).

(4) Tail

Pleasant/Excited – Raise the tail horizontally.

Fear – Curl the tail between legs.

Anxiety and Unpleasant – Swing the tail constantly.

Prepare to kick – Put the tail between legs and curl it.

(5) Feet

Scratching/Pawing the ground with forelegs – Desire, pain

Movement with hind legs – Preparing to kick, fear and anxiety

4. When horses bite or kick, they are expressing self-defense and distrust towards humans, so it is important to treat them gently and know when to pet and when to discipline.

3. Goats

(1) Behavioral Habits and Management

1) Goats are very active and move around quickly, and less gregarious than sheep. Goat herds have a clearer hierarchical structure compared to the other domesticated animals. The competition among individuals is intense, and this tendency especially appears when feeding. Therefore, it is important to ease the individual competitions when managing them in a large herd. For example, they could be tied to a peg when feeding, or could be dispersed with more fodder containers.

2) Goats tend to prefer dry environment and dislike swampy lowland. Moreover, they hate rainwater so much that they stop moving when there is a paddle in front of them. It is proverbial that rain would fall if a goat bleats. Therefore, goat farming requires some humidity countermeasures. For example, inside the shed should be always dry by having good swage system, and build a goat shelter to get out of the rain.

3) Goats tend to prefer browsing on tree leaves, especially hardwood. They tend to eat buds, shoots and barks. Feeding only high-moisture green grasses can cause softened stool and sometimes diarrhea. Therefore, feeding half-dried or dried food is recommended rather than fresh greens, and feeding tree leaves, from time to time, is even better. Goats are particular about cleanliness, so it is recommended that feeder racks should be designed in a way that forages do not get spilled and dirty. And it is also important to mix feeds so that they do not get tired of eating the same thing.
4) Goats tend to prefer high, dry places, such as rocky terrain, and prefer to rest outside rather than staying inside. Therefore, their housing should include a playing field and heaping up earth or rocks in the part of the field is even better.
5) Goats do not show signs of pain. Therefore, it is difficult to detect their illness and sometimes that can lead to death. Checking their behaviors regularly is recommended for early detection of their illness.

(2) Restraint

Goats are fast and hard to restrain. They jump high with front legs when captured. For restraining bucks, two handlers required: one handler puts their neck between the legs and the other holds the rear legs with both hands. In general, goats should be collared for care and management rather than bridles.

4. Sheep

(1) Behavioral Habits and Management

1) Sheep tend to stay in flocks compared to other domesticated animals, so it is easier to do herd management. There are some social pecking orders, but they compete less when comparing to goats and fit for herd management. In addition, sheep have a strong instinct to follow the leader sheep.
2) Sheep tend to prefer dry highland, high plateau and dislike low wetlands. Therefore, sheep farming requires some humidity countermeasures. For example, inside the shed should be always dry by having good swage system, and building a rain shelter is recommended. Sheep excel in selecting foods; they eat grass, leaves, grains, roots, and even seaweeds. Sheep have bigger stomach and longer intestine, so that they have a better digestive system compared to other animals. Sheep tend to prefer short grass to long grass, and in some cases they are used as clean-up clippers after cattle graze.
3) Sheep are highly adjustable animals that are very easy to tame.
4) As for diseases, they do not show signs of pains well, so it is difficult to detect illness, and in some cases that behavior can lead to death. Checking their behaviors regularly is recommended for early detection of their diseases.

(2) Restraint

Sheep are calmer compared to goats, so they are easier to restrain. So for restraint, press your elbow onto the flank closer to you, grasp the other side of flank with one hand, turn the sheep head to the side with the other hand. You can restrain the sheep if you twist and lay the sheep on your knee. For blood collection or vaccination, restrain in the same way as goats.

5. Swine

(1) Rearing Management

Pigs by nature are very curious and sensitive animals. Once they are bullied, they will never forget about it, so do not abuse or treat them harshly. Pigs grow fast and become matured in a short period of time. For example, in a week a piglet can weigh twice as much. Therefore, types and amounts of food and housing space require individual attention. Pigs have a habit of digging dirt with their nose and strong enough neck to lift fences or break food containers, so farming equipment and building structure have to be tough. Always clean the facility to prevent pest infestation, such as flies, mosquitos, cockroaches, or rats/mice. And also make sure there are no cobwebs. Be careful with hog tusk, especially when hogs walk by sows in heat.

(2) Restraint and Handling

1) Piglets

Hold or lift the piglet by a front or back leg. You could also use a restraining snare made of thin wire (placing snare loop over the top jaw and snout), or V-shaped restrainer (Figure 8).

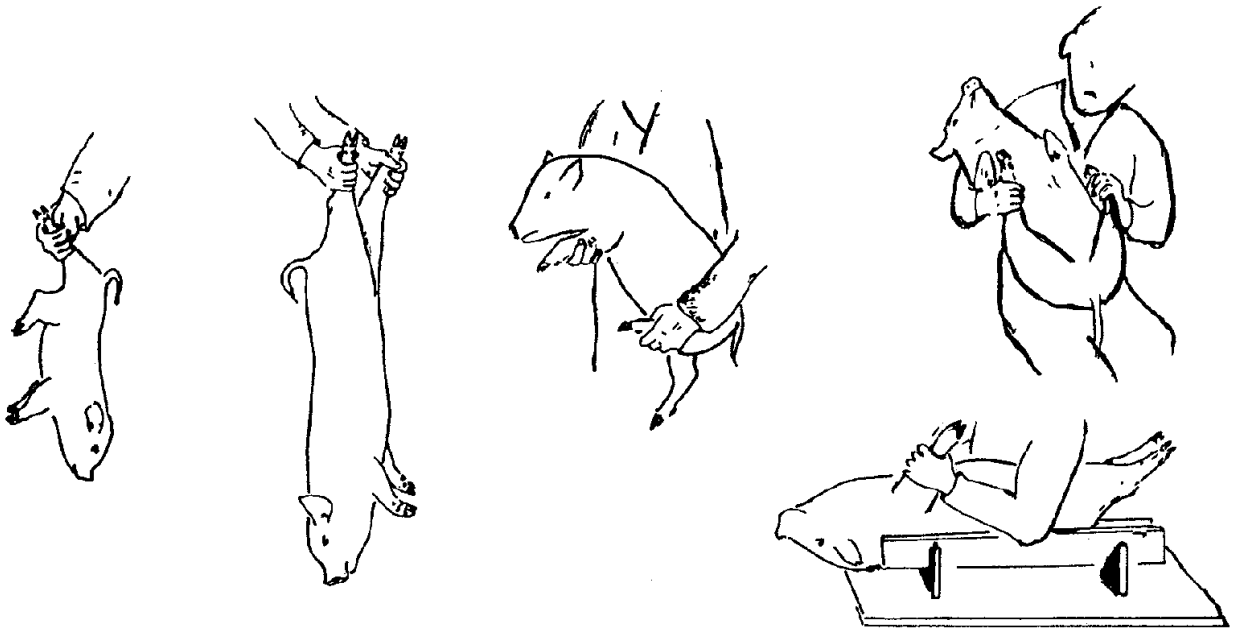


Figure 8. How to restrain piglets (less than 20kg: various ways to lift/V-shaped restrainer)

2) Medium sized to Adult Pigs

Use thick wired restrainer, nose restrainer using a rope, hammock style or box style restrainer. (Figure 9)

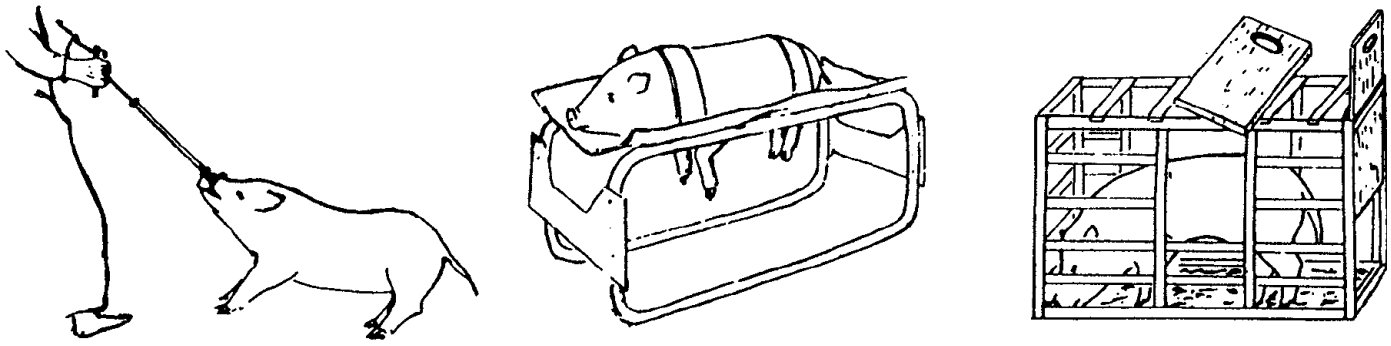


Figure 9. How to restrain bigger pigs (left: nose restrainer, middle: hammock, right: box)

3) How to Use a Nose Restrainer

① Approach quietly and stand near the right or left shoulder.

② Put loop of restrainer or the rope around the jaw and tie the loop.

③ Stand facing the pig and hold the restrainer, placing the hand, restrainer, and the pig's body in a straight line. The pig will try to step backward so you should pull towards yourself. In the law of action and reaction, the pig won't be able to move.

④ Manual restraint is difficult is for medium-sized to bigger pigs, so fix the rope tight to the frame of pigsty.

4) Transferring Pigs

Use a big board or stick to lead pigs (Figure 10).

[Notes]

1) HolzW., Pig and Minipigs, p501, The UFAW Hand Book on the Care and Management of Laboratory Animals (6ed), Longman Science and Technical (1987)

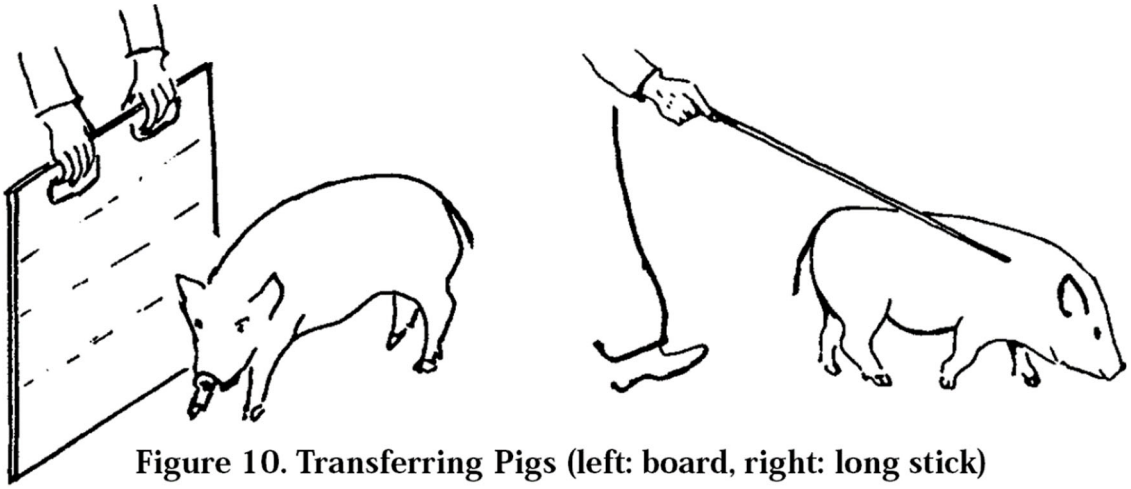


Figure 10. Transferring Pigs (left: board, right: long stick)

6. Dogs

(1) Rearing Management

The sizes of the cage for dogs are referred to the standards of NIH (Table 2). The floor material is generally metal mesh. However, in the case of dogs, they tend to develop abscess between fingers

(interdigital furuncle), so for the kennel floorings, the materials that touches whole bottom of their feet should be used. The cages need to have sufficient height where dogs can comfortably stand upright.

Table 2. Recommended Minimum Space for Dogs

Weight (kg)	**Height** (cm)	**Minimum Floor Area** (m^2)
＜15	＜15	0.74
15－30	15－30	1.2
＞30	＞30	2.4

ILAR (Institute for Laboratory Animal Research)

Adult dogs should be fed once a day in a nutritionally set amount. Change the water twice a day if there are no automatic water feeders. If dogs cannot exercise freely, they should be taken out regularly. Depending on the rearing place, the consideration of dogs barking is necessary.

(2) Restraint

Restraint without anesthesia heavily depends on the degree of how much the dog is tamed. The flexibility in handling different dogs personalities is required. The behavior of dogs on the run can be split into roughly two groups: those in one group show abjectly submissiveness, and those in the other group show evasiveness until cornered, but then show some aggressiveness when completely cornered. It is not uncommon to get bit when catching the dog running through you. Dogs bite so quickly that it might be impossible to avoid without any warnings. When being chased, they tend to run to escape from the catcher. Fierce dogs or very fearful dogs might still try desperately to bite even after getting held on the neck. Even in cases where the dogs seem to be obedient first when they are put in a new environment, they could be no longer obedient later.

Figure 11 shows the typical restraining ways for intravenous injection and blood sampling. In each way, restraining should be done by handlers knowledgeable about dog not to give any unnecessary fear. For dogs, fear and stress can easily cause red blood cell mobilization from spleen.

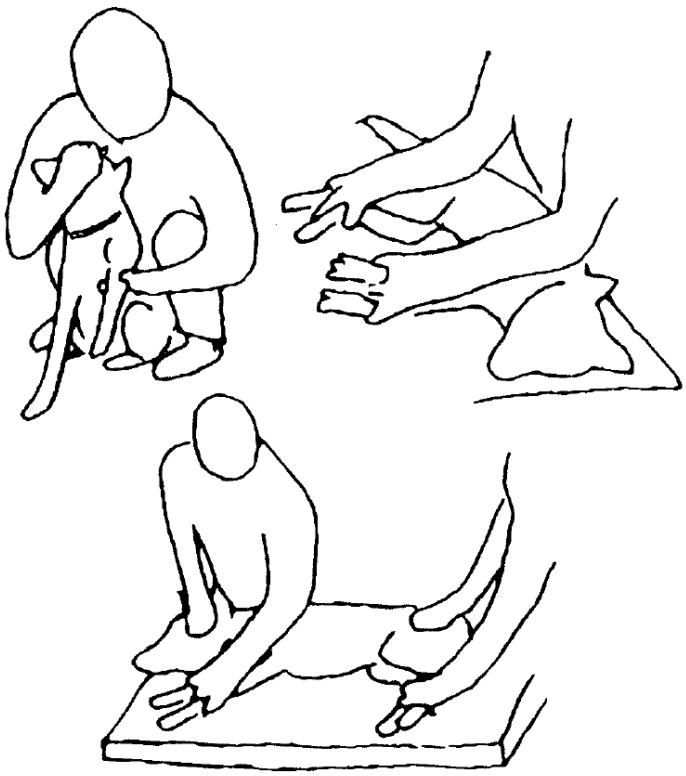


Figure 11. How to restrain dogs

(3) Other Considerations

Even dogs produced for animal experiments should be treated with a full understanding of animal welfare and animal rights. Any researchers who conduct animal experiments with dogs need to have knowledge on their ecology, physiology and ethology, and a thorough understanding and experiences on the daily work of dog care and management. Dogs are very sensitive to the daily breeding tasks, including caretakers and can be greatly influenced from those tasks. From the perspective of animal welfare, the researchers are responsible for all aspects, including not only the experimental procedures themselves but also care and management.

7. Cats

(1) Rearing Management

Rearing management of cats is the same as dogs. There are single cat housing and group cat housing, and the size of the cage should be based on the NIH standards (Table 3). In the corner of the room or a cage, there should be a litter tray. And ideally, cat housing should provide vertically open space, such as some shelves that cats can climb on. Unlike dogs, cats should be fed twice a day in a nutritionally fixed amount. Food consumption might vary even though they are in normal conditions, so daily observation is needed. Water should be changed twice a day if there is no automatic water feeder.

Table 3. Recommended Minimal Space for Cats

Weight (kg)	Minimal Floor Space (m^2)	Minimal Cage Height (m^2)
＜4	0.28	60.9
＞4	0.37	60.9

ILAR (Institute for Laboratory Animal Research)

(2) Restraint

The same restraining method as dogs is used for tamed cats. Tame but scared cats could cling with their claws. For furious cats, use squeeze cage or capture net. Laundry nets and bath towels would also work by

wrapping them (Figure 12). When capturing an escaped cat, wear leather gloves, hold onto the cat's neck, then immediately grab the back legs and hold down. Injuries by cats' teeth and claws could be deep, and even after disinfecting the area, it might be swollen.

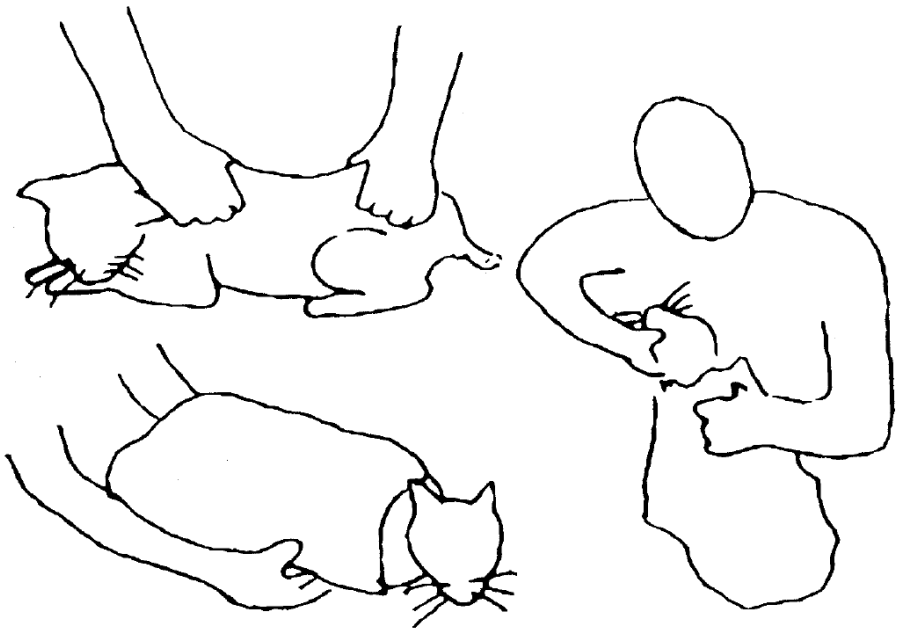


Figure 12. How to restrain Cats

(3) Other Considerations

Same as dogs, cats should be treated with a full understanding of animal welfare and animal rights. The researchers are obliged and responsible to properly conduct/manage daily rearing duties and experimental procedures with a full understanding of the ecology, physiology and ethology of cats.

8. Rabbits

(1) Rearing Management

Cages should be made of metals, and for flooring metal mesh, metal plate with small holes, or drain boards should be used so that urine won't accumulate. If the drain board has sharp corners or wire is too thin, it might hurt the rabbits' limbs and even humans (when washing cages), so meticulous care should be taken. There should be a resting board in the cage if the floor is made of metal mesh. There are several types of metal cages on the market, such as portable rabbit hutches for putting on the floor and the stackable ones which can be attached to rearing shelves.

Feed rabbit pellets on the market. As for waterers, use polyethylene bottles (500ml). Glass bottles could be dangerous since rabbits might bite/chew or bottles might fall and break. Recently, automatic water dispensers are becoming popular, but it is still important to make sure if it's properly working, and inspect regularly.

(2) Restraint

1) How to Grasp a Rabbit (with hands)

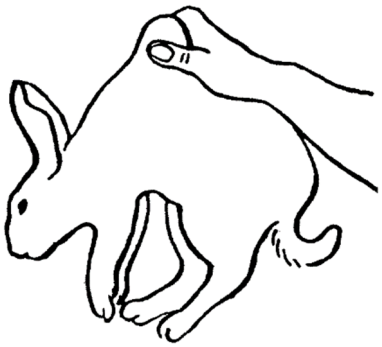


Figure 13 - a. How to lift a rabbit (1)

When taking rabbits in and out, lift the rabbit by grasping the skin of the shoulder with one hand, and place the other hand on the buttocks to support the weight (Figure 13 a, b). Rabbits would react wildly if grabbed by the ears or the skin around stomach, and might injure the experimenters with their claws. Such handlings accordingly should be avoided. And careful attention should be paid when taking the rabbit out of the mesh floored cage, not to cause the rabbit's back legs to be caught in the mesh. When carrying rabbits inside, carry them either in the arms as shown in (Figure 13 – c), or in a shopping bag or a bucket with a lid.

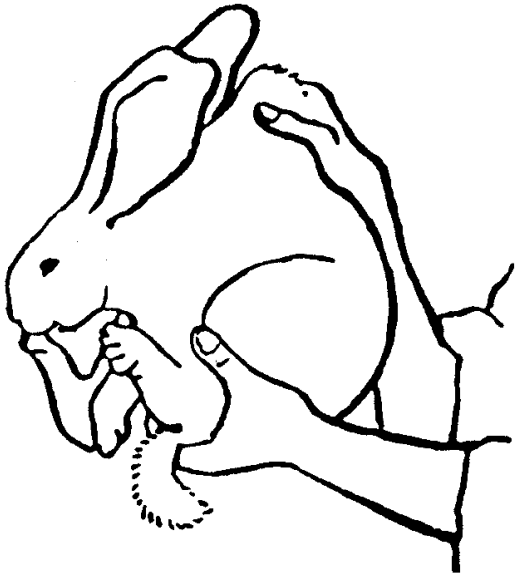


Figure 13 - b. How to lift a rabbit (2)

Figure 13 - c. How to carry a rabbit

2) Restraint Using Restrainers

There are four types of restrainers: round type (Figure 14-a), boxed type (Figure 14-b), collar type (Figure 14-c), and face-up type (Picture 14-d). Round and boxed types are made to expose the head to outside, so they are used for experiments using ear veins or ears. A collar type restrains a rabbit in a natural position, so that the rabbit can be strained in the same position for a long time. A face-up type can restrain a rabbit in a face-up position to tie the limbs firmly onto a table, and is used for a blood sampling from a carotid artery.

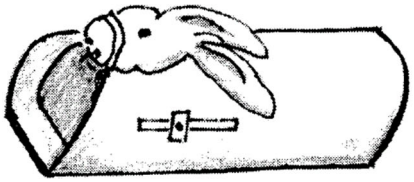


Figure 14-a

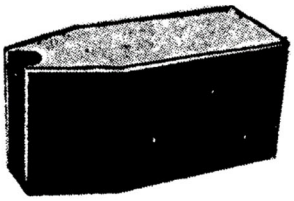


Figure 14-b

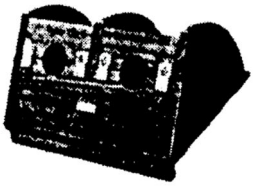


Figure 14-c

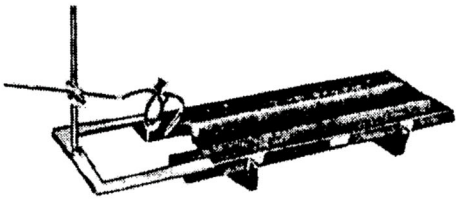


Figure 14-d

Figure 14. Restrainers for rabbits

3) Precaution to the Risks of Vertebral Dislocation and Fracture

Rabbits have thin bones and often suffer from vertebral dislocations and fractures (the seventh lumbar vertebrae) by powerful kicking due to inadequate restraint and improper handling. This is caused by strong muscular contraction when kicking with the hind legs. In order to prevent these injuries, proper restraining technique is necessary. Symptoms include paralysis of the hind limbs and loss of muscle control, such as anal sphincter and bladder.

9. Guinea Pigs

(1) Rearing Management

There are several types of guinea pig cages on the market; for example, a portable cage made of metal wired mesh with litter tray underneath for placing on the floor, and a stackable cage, which can be installed onto rearing shelves. Usually, five to six guinea pigs weighing around 300g are housed in one cage. Feed guinea pig pellets on the market. Polyethylene water feeders are recommended for use since glass made might cause injuries.

(2) Restraint

1) How to Grasp/hold a Guinea Pig (with hands)

When grasping a relatively bigger guinea pig with one hand, slip the hand under the belly, place the thumb onto one side, and the pinky and third fingers onto the other side, then grasp with the index and second fingers catching one of the hind legs in between (Figure 15-a). When grasping a smaller guinea pig, lift the body by grasping the back with one hand, catching one of the forelims with the index and second fingers (Figure 15-b).

2) Restraint Using Restrainers

Rat restrainers could be used for guinea pigs.

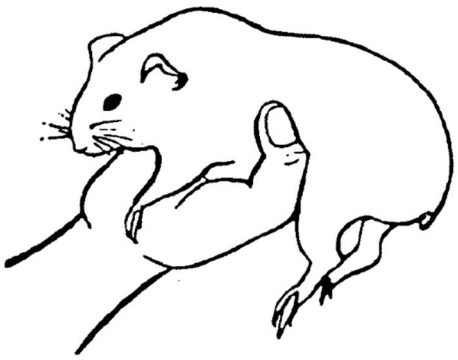


Figure 15-a. How to grab a guinea pig (1)

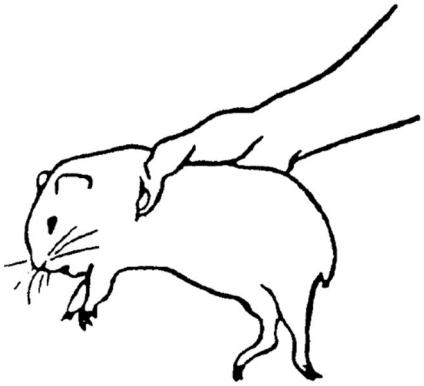


Figure 15-b. How to grab a guinea pig (2)

(3) Other Considerations

Guinea pigs are so sensitive to noises that the handlings have to be careful. Even slight noises can scare guinea pigs and they run around the cage kicking the floor mat, so it might be better not to use the floor mat during the experiments of infectious microorganism. Guinea pigs tend to defecate in the corner of the cage, so using drain boards with the four corners cut off is a good idea to minimize the work to change the drain boards.

[Precaution for cardiac puncture blood collection]

Because of the risks of serious side effects (such as cardiac tamponade) from cardiac puncture blood collection and extremely high stress on animals, it is currently limited to the final blood collection (for euthanasia in the last step of the experiment) and has to be done under anesthesia.

10. Mice

(1) Rearing and Management

Cages have to be habitable, able to prevent animals from escaping or invading, easy to clean, and resistant to high heat and disinfectants. The cages on the market are made of hard plastics (i.e. polypropylene, polycarbonate) and metal (i.e. aluminum, stainless steel). In recent years, there are automatic cage washers available, but attentions have to be paid since they could be dangerous during the work. The feeder should be filled up with solid feed at least once a week.

(2) Restraint

1) How to Grasp a Mouse

Mice tend to bite, so the experimenters should treat them carefully. First, stop the movement by picking up the middle of the tail with the thumb and index finger, and lift up (Figure 16). In another way, lift up by slightly pinching the skin from behind the ears to the back (Figure 17). Even in both ways, do not add any extra power, treat them with care, and handle them quickly while checking their movements.

Figure 16. A typical way to grab a mouse

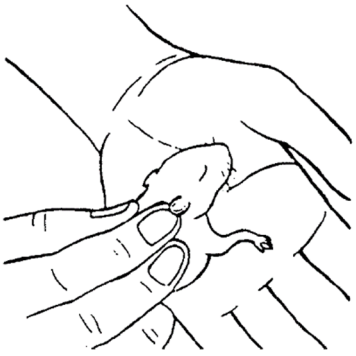


Figure 17. How to grab a baby mouse

2) Restraint (with hands)

Hold the tail with the right hand, place on the cage lid, and gently pull the tail back. Then, the mouse will reflexively attempt to pull away to stretch the body. Gently pinch and lift the scruff with the thumb and index finger of the left hand (Figure 18-a) while maintaining a grip on the buttocks and tail. Grasp the left limbs with the ring and pinky fingers, and push forward with the thumb and index finger (Figure 18-b).

Figure 18-a

Figure 18-b

Figure 18. Restraining a mouse

3) Restraint Using Restrainers

Restrain the mouse face up or down tying each limb with strings onto the wooden board. There are some types of restrainers made of plastic and metal available in stores.

11. Rats

(1) Rearing Management

There are cages with metal wired floor and flat floor. The former are made for the filth to drop, so litter trays should be attached underneath. In the metal wire floored cage, there should be resting boards. The flat floored cage should be made of aluminum or plastic. In order to prevent escape, there should be metal lid stopper attached such as clasp. Generally, rats are fed in ceaseless feeding style, which means animals are fed plenty of food and water at all times.

(2) Restraint

1) How to Grasp a Rat

When grasping a rat, gently grasp around the back to axillae with the thumb and middle finger, and lift the body up spreading both forelimbs. Then, place the other palm on the waist and hold the hind limbs with the thumb, index and middle fingers (Figure 19). When rats are calm, the handler can try grabbing from the stomach as shown in Figure 20.

2) Restrain Using Restrainers

Fix the rat tying four limbs with strings onto the board. There are restrainers with stand available in stores.

(3) Other Considerations

Rats' tails are delicate to be accidentally cut off if picked up by the tip of tail, unlike mice tails. If picked by the base of tail, it will not come off. Rats might be reservoirs carrying pathogen of zoonosis such as hemorrhagic fever with renal syndrome virus, so make sure to purchase SPF animals from trusted vendors.

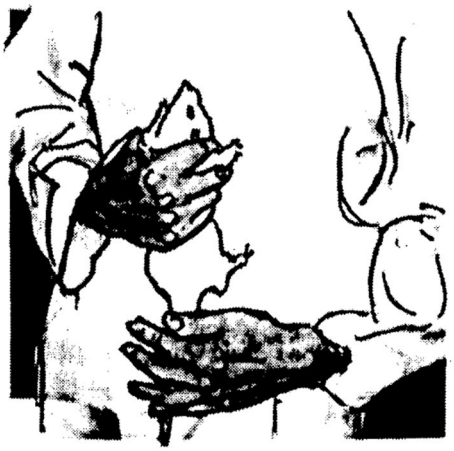


Figure 19. Common way of restraining a rat

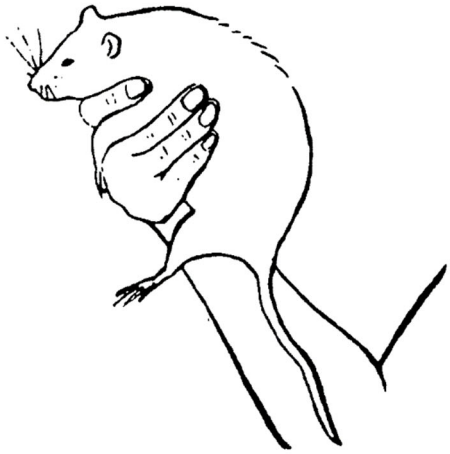


Figure 20. An example of handling a rat

12. Hamsters

Two strains of hamsters are known to be used in the laboratory: the Syrian or golden hamster and the Chinese hamster.

(1) Rearing Management

Hamsters have tough enough teeth to bite off the wire. Therefore, tough metal cages with a stopper are one of the best choices to prevent hamsters from escaping. Aluminum cages can be chewed for escape through the hole. Generally, hamsters are fed in ceaseless feeding style.

(2) Restraint with Hands

1) How to Grasp a Hamster

Quietly place a hand on the back and envelop the body with one hand (Figure 21).

2) Restraint with Hands

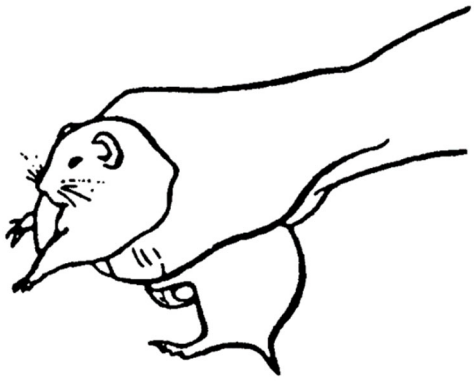


Figure 21. How to grab a hamster

Hold the back of head with the left hand, slide the thumb and index finger from back on the axillae and firmly grab the scruff. Use other three fingers to grip the back. Be sure to grasp firmly otherwise hamsters might bite if they can move the head with the loose skin.

3) Restraint Using Restrainers

Tie the four limbs with strings while restraining with hands, and place the body face-up onto the board. Restrain the head with strings if needed.

(3) Other Considerations

Hamsters have a strong ability to push the cage lid with their heads to escape. Therefore, it is necessary to secure the cage lid with a stopper. Hamsters are usually calm in nature and can be easily accustomed to humans, but at the same time very cautious. When hamsters are lying on their back while squealing, it means they are on alert. Be careful when they are excited and squealing, they might bite.

13. Non-Human Primates

There are about 200 species of non-human primates, ranging from chimpanzees to small tupaia, but here we talk about cynomolgus monkeys, in particular, which are mainly used for experiments.

(1) Rearing Management

It is recommended that cages should be approximately 45 – 60cm wide, 60cm deep, 60 – 70cm high, made of stainless steel, and include squeezing device. They should be fed solid feed and 300g of raw sweet potatoes or fruits for vitamin C supplement.

(2) Restraint

Monkeys possess sharp canines, strong grip, and are agile in movement, so close attention should be paid when handling. Capturing by two or more experimenters is strongly recommended.

1) How to Grasp a Monkey

Without an anesthesia, the handler should wear leather groves, hold down the monkey in a capture net (Figure 22), quickly grab the back and push it down on the floor (Figure 23-a). Then, as shown in Figure 23-b and 23-c, the handler should put the monkey's arms together behind the back, hold the upper arms tight and lift it up with both hands.

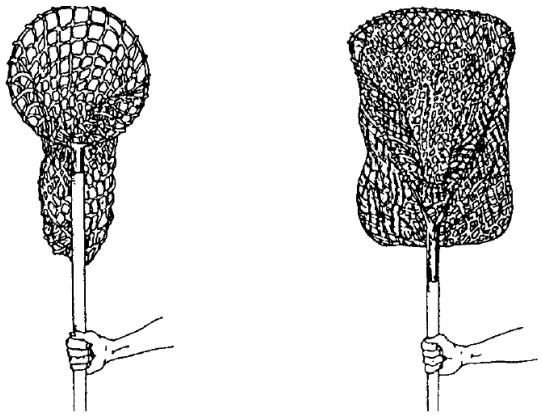


Figure 22. Capturing net for monkeys

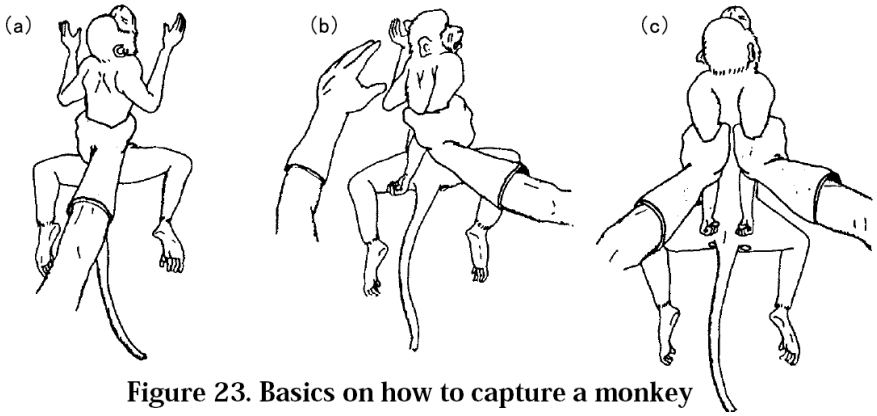


Figure 23. Basics on how to capture a monkey

2) Restraint Using Hands

After capturing the monkey according to the method described in 1), pinion the monkey's arms behind the back and restraint with one hand, and with other hand, grasp the scruff.

3) Restraint Using Restrainers

If the animal is housed in a cage equipped with a squeeze mechanism, use the device and inject an anesthetic (ketamine) into the muscles of the buttocks or the femur prior to its removal from the cage. Monkey chair could be used when the handler wants to restrain the animal for a long period of time without anesthesia, but it is not recommended unless it is absolutely necessary for the experiment. For surgery, restraint the animal on the surgery table under anesthesia.

(3) Other Considerations

Many of the non-human primates used for experiments are caught from the wild. Therefore, precautious handling should be performed as described below.

1) Non-human primates have a risk of infection with various zoonotic diseases, so strict quarantine is required.
2) Adequate time is necessary for the animals to get used to the artificial environment.
3) The numbers of wild non-human primates are gradually decreasing and they are even endangered. Careful experimental planning is essential to avoid wasting precious animal resources.
4) The Cynomolgus monkey is designated as invasive alien species (mammal). Therefore, there are many restrictions on its use.

14. Chickens and Quails

(1) Rearing Management
   1) It is desirable to have poultry house in a sunny and well ventilated spot. Depending on the breeds and purposes of farming, it is necessary to determine the housing, such as individual breeding cages or flat deck housing.
   2) Feed should vary depending on their growth stages: chick starter for baby chicks; starter-grower feed during the teenage stage; grower feed for grown chickens; layer feed for adult chickens (at full maturity). Feed likewise for quails. They should be fed plenty but not be overfed. Water should be kept always fresh and make sure not to run out of water especially during summer.
   3) Lighting is the important factor in egg laying. Lighting for 14 to 15 hours is generally appropriate when there are different ages and breeds of poultry in the same house.
   4) Fields have to regularly get soil-sterilized with calcined lime.

(2) Restraint
   1) When chickens are housed in individual breeding cages, they are easier to be captured than the ones housed in flat-deck cages. However, it is not preferable for poultry to be stressed by being chased when they are in flat-deck cages or free ranged, so herd them into an enclosure first, then capture.
   2) Firmly grasp the joints of both legs, gently envelop and hold the wings. Be careful with a spur when handling cocks.
   3) Quails and baby chicks should be restrained gently.

[Notes] Figure13 – 22: Adapted from “Laboratory Animals Textbook – Beginner –, Japanese Association for Laboratory Animal Science Board of Education Edition, Tokyo (1981)” and “Basic and Techniques of Laboratory Animals II, Specifics, Japanese Society for Laboratory Animal Resources, Maruzen, Tokyo (1991).”

Ⅳ. ZOONOSIS AS BIOHAZARD

Zoonoses are infectious diseases that can be transmitted from animals to humans or vice versa. During the veterinary education curriculum, students will have plenty of opportunities to handle various animals in practical trainings. Students should fully understand the characteristics of zoonoses when handling animals and take appropriate measures not only to protect oneself from zoonoses, but also to prevent the spread the pathogens in the general environment. In this chapter, animals are roughly categorized in laboratory animals (non-human primates, rodents), farm animals (cattle, swine, horses, poultry), and companion animals (dogs and cats). Noteworthy infectious diseases in each category will be discussed.

1. Zoonoses in Laboratory Animals

Figure 24 shows zoonoses that are important in laboratory animal care and management. Many of the pathogens that non-human primates carry could be very dangerous to humans, so the handlers have to be especially careful when handling wild-caught non-human primates. There are no guarantees that rodents

bred and raised in the university and private businesses are free of pathogens. Latent infections could happen and spread without any awareness, so attention needs to be paid well.

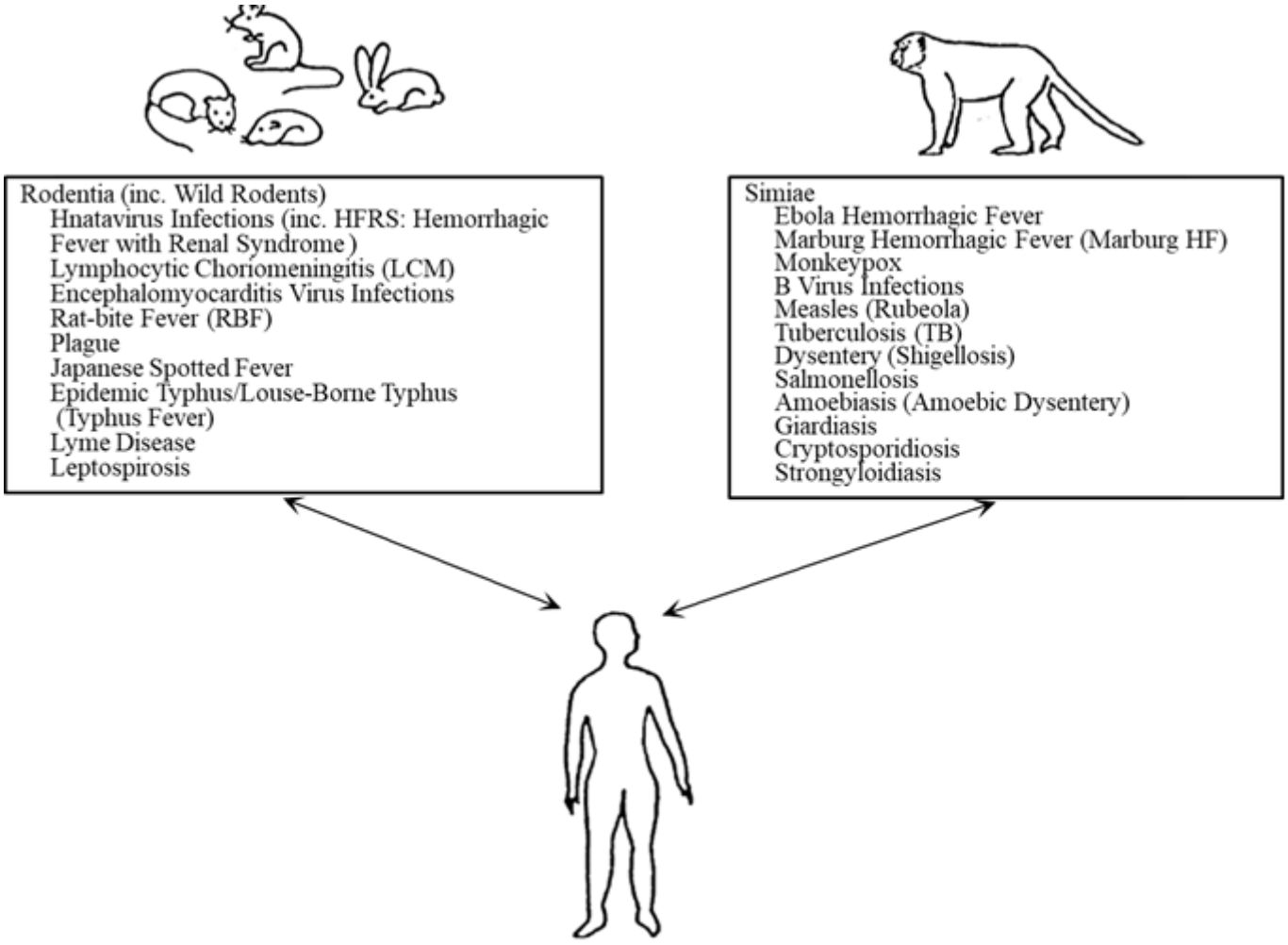


Figure 24. Zoonoses that Transmit to Humans from Laboratory Animals

(1) Salmonella

Rodents tend to carry salmonella typhimurium and cause food poisoning from oral infection. Prevention measures will be hygiene control and careful hand washing.

(2) Hantavirus Infections (inc. Hemorrhagic Fever with Renal Syndrome [HFRS])

Hantavirus Infections are caused by hantaviruses, which is transmitted to humans from laboratory rats. HFRS broke out in some animal experiment facilities in Japan in the past, and there were fatal cases reported. Rats show no obvious signs of infection. The infection is transmitted to humans by bites and via the respiratory route. Symptoms include high fever, diarrhea, stomachache, albuminuria, and subcutaneous hemorrhage.

(3) Lymphocytic Choriomeningitis (LCM)

LCM is a rodent-borne infectious disease caused by Lymphocytic Choriomeningitis Virus (LCMV). It is mainly transmitted by mice and hamsters, and usually latent. The virus is discharged from nasal secretions/mucus, saliva, urine, and feces. It is transmitted to humans through oral intake, inhalation, percutaneous exposure or conjunctiva, and symptoms are influenza-like fever, muscle ache, and nausea.

(4) Shigellosis

Originally, shigellosis is a human-to-human infection that causes severe diarrhea, but it can also infect monkeys. However, there are many latent cases among non-human primates, so close attention is required. The Infection route is oral. A stool examination is necessary when monkeys are used for experiments.

(5) B Virus Infections

B virus is found among macaque monkeys (rhesus macaques and cynomolgus monkeys), which are the natural host of this virus. Infected monkeys usually have no symptoms, but there are some cases of forming blisters and ulcers on the oral mucosa or the tongue. Transmission to humans is caused by bites. In humans, symptoms include swelling of the lymph nodes, fever, nausea, ataxia, and paralysis, and the case-fatality rate is high. Even after recovery, there remains some severe sequelae. If blisters or ulcers are identified on monkey's oral mucous membrane, those animals should be immediately euthanized before moving onto the experiments.

(6) Marburg Hemorrhagic Fever

There are no recorded outbreaks in Japan so far, but it is designated as a Class 1 disease under the Infectious Diseases Control Law. The transmission routes of Marburg virus from the animal hosts to humans are unknown, but it was first reported that the disease was contracted via direct contact with the blood and tissues of African green monkeys. The Symptom onset is sudden and marked by fever, chills, headache, and myalgia, followed by a maculopapular rash. Symptoms become increasingly severe and may include jaundice, general prostration, mental disorder, hemorrhaging, multi-organ dysfunction. This virus is considered extremely dangerous and causes fatal disease.

2. Zoonoses in Farm Animals

Figure 25 shows common zoonoses among farm animals and explains briefly on information that especially needs attention during practical trainings.

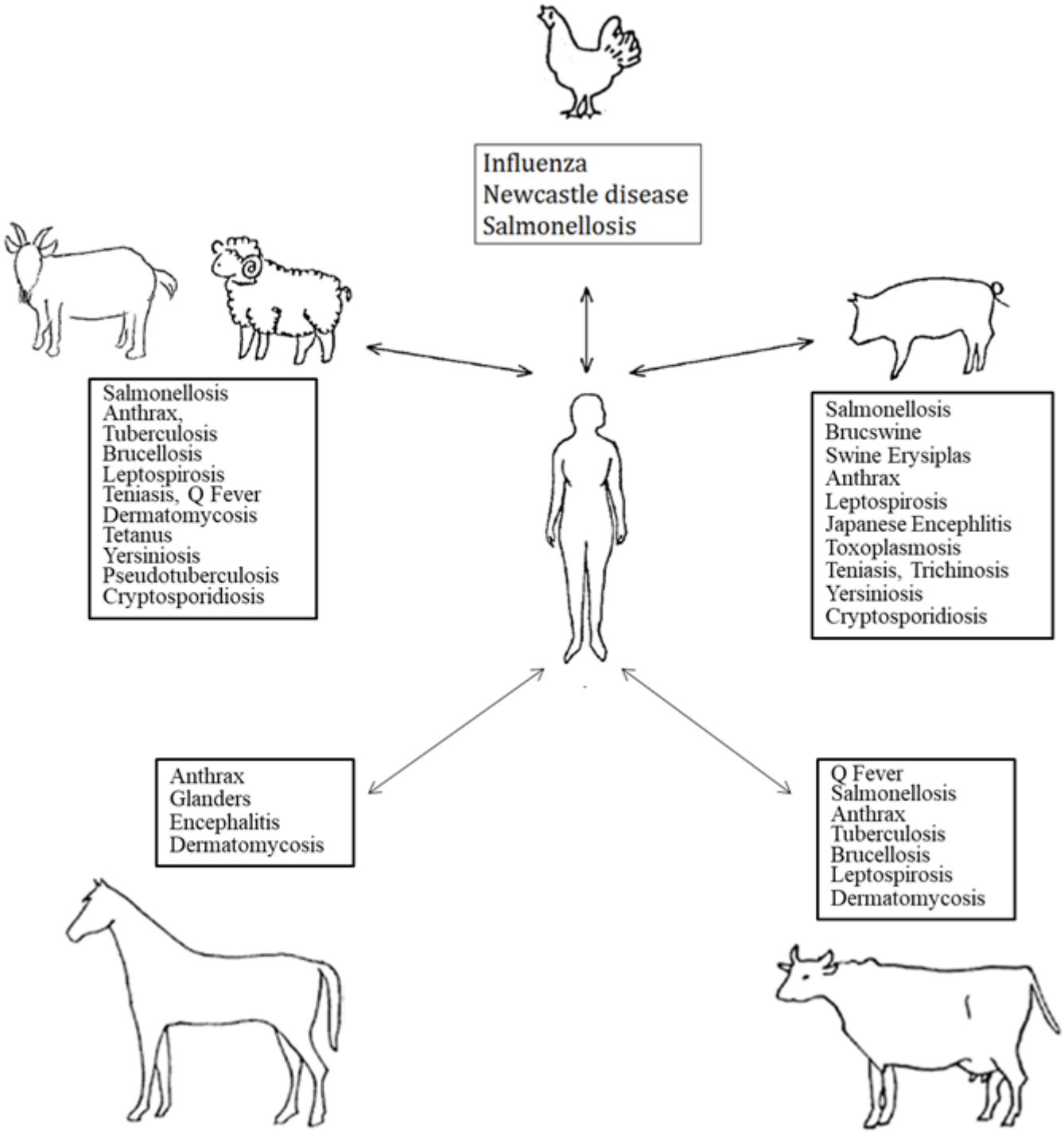


Figure 25. Zoonoses that Transmit to Humans through Farm Animals

(1) Japanese encephalitis

Japanese encephalitis is a viral disease. Horses and swine are very sensitive to this pathogen. Especially, swine is the cause for its widespread and poses public health problem. It causes an encephalitis in horses, stillbirths or miscarriages in pregnant pigs, and spermatogenesis failures in boars, but in fatted pigs, there are close to no symptoms. In humans, Japanese encephalitis is transmitted by mosquitos and can be divided into the following three characteristics.

① High fever, meningitis, headache, vomiting, and loss of consciousness.
② Result in death by respiratory paralysis within 4 – 5 days after the onset.
③ Fully recover with some after-effects. Vaccines are available for both humans and animals

(2) Q fever

Q fever is a disease caused by infection with *Coxiella burnetii*. Animals (cattle, sheep, goats, and companion animals) are reservoirs of the bacteria with silent infection and the source of infection for humans. Ruminants tend to get bacteremia, but the symptoms are latent and they excrete bacteria in milk, feces, and lochia at the time of birth. Humans get infected by breathing in bacteria scattered with contaminated milk and dust. Symptoms are headache, fever, cough, and chest pains. For the milk from dairy cattle raised in areas where Q fever is endemic, sterilizing at ultra-high-temperature is required for the infection prevention. Antibiotics such as tetracycline are effective on humans.

(3) Salmonellosis

Farm animals, especially pigs and cattle, pose public health problems as reservoirs of the bacteria. In pigs *S. typhimurium* and *S. derby* cause diarrhea and chronic enteritis, but symptoms are mild and many of them become healthy carriers of salmonella. In cattle, infections are caused mainly by *S. dublin, S. typhimurium* and *S. enteritidis,* and particularly developed among calves, resulting in fever, enteritis, septic deaths, pneumonia, encephalitis, arthritis, and mastitis. There are many cases of miscarriage due to *S. dublin*. In humans Salmonella is transmitted orally, and cause food poisoning, fever, diarrhea and stomachache. Salmonella is frequently used for experiments and trainings, so great care has to be taken to avoid infection in the laboratory.

(4) Anthrax

Anthrax is a disease caused by *Bacillus anthracis,* and infects cattle, horses, pigs, and sheep. In Japan, most cases are reported in cattle more and it causes sudden death with an acute septicemia. Pigs have more natural resistance, so they develop localized lesion (ulcers) in the intestines or other parts of the body, and asymptomatic (atypical anthrax infection). They are often found during meat hygiene inspection. Humans can get infected by contact with infected animals, inhalation of spores on animal skin, furs, hairs, and bone meal. Other cases are resulted from inadvertent dissection of and consuming meat of infected animals. Special care has to be taken when dissecting the suddenly dead animals that are bleeding from natural orifice. In humans, anthrax can be grouped as the following depending on the infection route: cutaneous anthrax (contact), pulmonary anthrax (inhalation), and gastrointestinal anthrax (oral). There are vaccines available for animals, and antibiotics such as penicillin and streptomycin are effective.

(5) Glanders

Glanders is an infectious disease in genus Equus, caused by *Burkholderia mallei* and primarily affects horses. It is a chronic infection and forms glanders node in nasal cavity, respiratory mucosa, internal organs, lymph node, and skins. *B mallei* is transmitted to humans by inhalation of infected aerosols or dust contaminated by infected animals, and through cuts or abrasions in the skin and mucosal surfaces. There have been reported cases of infection in laboratories during nasal infection experiments in mice, which were transmitted by the inhalation of affected animal's sneezing. It is rare to be infected by contact with infected animals. Symptoms in humans are fever, lymphadenopathy, pneumonia, and septic death from skins ulceration. There are no reported cases in Japan.

(6) Tuberculosis (TB)

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis, M. bovis,* and *M. avium-intracellulare,* and affects cattle, goats, and pigs. TB in cattle causes emaciation, decrease in milk production, coughing and lymphadenoma enlargement on the body surface, but in most cases, no clinical abnormalities are observed. There are pulmonary, intestinal, mastitis, and cutaneous tuberculosis as well as internal organs miliary tuberculosis. In pigs, the lesions are localized to mesenteric lymph nodes, and often asymptomatic. Pulmonary tuberculosis with *M. tuberculosis* infection is common among humans, but there are rare cases of tuberculous cervical lymphadenitis, bone and joint infections, meningitis and intestinal tuberculosis in children through raw milk from infected cows with *M. bovis*. There have been cases of infections in laboratories in the past, so that much attention needs to be paid during infection experiments.

(7) Brucellosis

Brucellosis is an infectious disease in farm animals, caused by *Brucella abortus* (cattle), *B. suis* (pigs), and *B. melitensis* (goats), resulting in miscarriages and infertility. Transmission to humans can occur most commonly through ingestion of raw milk from infected animals (gastrointestinal infection), but there are also cases of transmission from mucous membranes and skin. *B. suis* and *B. melitensis* infections in humans are mostly severe, lasting months to years. Infections with *B. abortus* are usually mild and symptoms are hardly apparent. Common symptoms are fever, headache, myalgia, chill, sweating, enlarged cervical lymphadenopathy, enlarged spleen, and hepatitis. There are some cases of infection in laboratories and among breeders. Oxytetracycline and streptomycin are effective.

(8) Swine erysipelas

Swine erysipelas is an infectious disease caused by *Erysipelothrix rhusiopathiae*, and transmitted through mouth, wounds and blood sucking insects. This bacteria can also spread to poultry, rodents and fish. In pigs, there are septicemia, hives, arthritis and endocarditis. Transmission to humans can occur through contacts with infected animals or wounds when handling fish (erysipeloid). This disease is known as occupational disease for workers at slaughterhouses and pig farms. There are also cases of infections in laboratories. Penicillin is effective.

(9) Leptospirosis

Leptospirosis is an infectious disease caused by animals such as wild mice and dogs that excrete *Leptospira interrogans* and infect cattle. It is transmitted from mucous membranes, skin and wounds and symptoms are hemoglobinuria, anemia, jaundice and miscarriage, but there are many cases of latent infections. In humans, a wide range of symptoms from mild form to severe form can be identified: flu-like symptoms, Jaundice, haemorrhagia, kidney failure.

(10) Dermatophytosis

Dermatophytosis is a fungal disease that occurs in cattle, horses, pigs, and laboratory animals by infections with dermatophyte. In farm animals, symptoms are mainly hair loss (not for pigs), desquamation, and scabbing. In humans, inflammation of the skin and irregular patches of hair loss can be shown.

(11) Toxoplasmosis

Toxoplasmosis is an infectious disease caused by *Toxoplasma gondii*. Most of the infections are latent, but some apparent infections have been observed in pigs and sheep, and regarded as important for public health. By orally ingesting oocysts of *Toxoplasma gondii* parasite, piglets can get fever, loss of appetite, nasal mucus discharge, congestion of retina, coughing, difficult breathing, diarrhea, or constipation. When symptoms worsen, there appears red and purple spots on the body surfaces resulting in paralysis and other neurological symptoms, and death. In humans, there are very few adult-onset cases, but there is a risk to infect the unborn baby by mother-to-child transmission during pregnancy, resulting in stillbirth or miscarriage at worst afterward.

(12) Influenza

The major natural hosts of Influenza A virus are wild aquatic birds such as ducks, but it can be transmitted to mammals such as pigs and horses, poultry such as chicken and ducks, and humans. Many cases are spread through droplet or airborne transmission.

3. Zoonoses in Companion Animals

Figure 26 shows common zoonoses in companion animals. Dogs and cats are usually treated in clinical training, but they may also be handled as laboratory animals. Especially noteworthy diseases are roughly explained here.

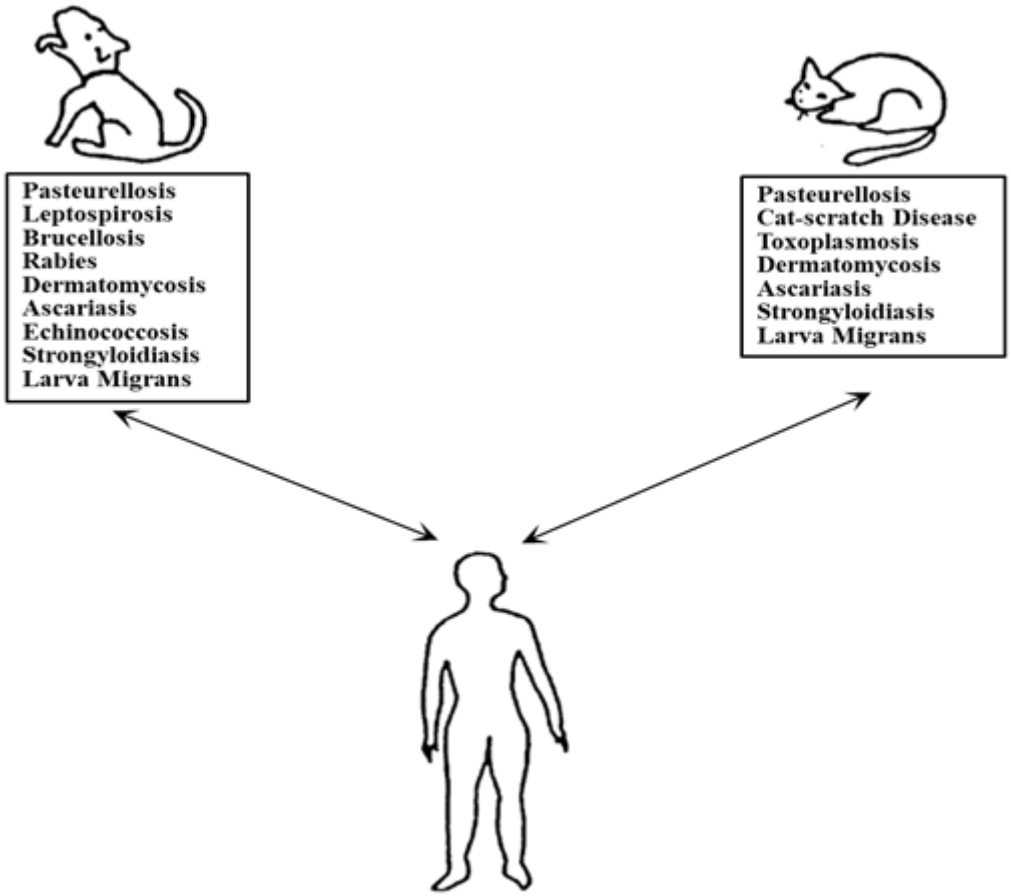


Figure 26. Zoonoses that Transmit to Humans from Companion Animals

(1) Pasteurellosis

Even healthy dogs and cats can carry the bacteria of the Pasteurella genus in their respiratory tracts. Transmission to humans can occur by bites and scratches or wounds. Symptoms are regional flare, ache, swelling, and sometimes swollen lymph nodes.

(2) Leptospirosis

Leptospirosis can be transmitted to dogs from most commonly wild rodents and contaminated environments (water, soil, etc). Infected dogs develop hemorrhagic jaundice, vomiting, diarrhea, and ulceration in the oral mucosa. Bacteria discharged from infected animals' urine contaminates the environment, and infect humans through skin and mucous membranes. Even in humans, fever, myalgia, vomiting, diarrhea, or jaundice are also observed.

(3) Canine Brucellosis

Canine Brucella is transmitted through mouth or copulation among dogs. It causes a long-term bacteremia, miscarriages, and epididymitis. The bacteria are excreted from miscarriage fluids and urine, and can be transmitted to humans orally and transdermally. In humans, fever and swollen lymph nodes are observed.

(4) Toxoplasmosis

Toxoplasmosis affects cats, dogs, pigs and many other animals. The Toxoplasma gondii parasite reproduces in the digestive tract of felines, which are the definitive hosts, and forms oocysts, which are shed in feces into the environment. Once animals and humans, the intermediate hosts, ingest sporulated oocysts orally, the zygote initiates its development within the oocyst. During zygote development, the oocyst becomes infective. It is rare for adults to show symptoms. However, if a pregnant woman gets infected, the child can get infected, resulting in miscarriage or death.

(5) Ascariasis

Ascaris lumbricoides parasitize in the small intestine of canids (Toxoplasma canis and Toxocara lionii) and felids (Toxocara cati and Toxascaris leonina). The source of human infection is the roundworm eggs excreted in the environment. Once infected, the parasites can migrate in various organs and tissues, and cause various symptoms. Thorough antiparasitic treatment and proper disposal of feces are important.

(6) Cat Scratch Disease

Bartonella henselae (bacteria bartonella) can be transmitted by cat bites and scratches. Symptoms include the formation of a small pustules and ulcers on the skin, causing local lymph node enlargement.

(7) Rabies

Almost all mammals can be infected with rabies. It is transmitted to humans through bites by rabid animals and show nervous symptoms afte a-month long incubation period. Once the clinical signs of rabies appear, it is 100% fatal. There have been no cases of rabies reported in Japan in recent years, but preventive vaccination is recommended for those who conduct experiments witht a high risk of rabies infection.

V . BIOLOGICAL HAZARDS PREVENTION

1. Biohazard Prevention Measures and Basic Concept

The term ‘biohazard’ has been traditionally used for human infections within laboratories by pathogenic microorganisms. However, biohazard includes not only the human infections during infectious experiments, but also zoonoses that are transmitted from animals to humans, and any infectious diseases that spread among animals. Therefore, during animal experiments, the handlers have to be aware of facilities and equipment as well as handling animals.

There are two fundamentally important concepts in biohazard prevention measures: 1) to be familiar with risks of handling microorganisms (to both humans and animals); and 2) to physically or biologically contain the microorganism. The following are just concepts and the details are explained later.

(1) Classification of Microbial Hazards

There are four levels of pathogen hazard classifiation established by WHO (the World Health Organization) and OIE (the World Organisation for Animal Health) guidelines and for each level, examples of various pathogens are determined. For the risk classification of infectious diseases of humans and farm animals, “National Institute of Infectious Diseases Safety Control of Pathogens” and “Rules for Handling Microorganisms at the National Institute of Animal Health” have been regulated However, these rules and

classifications are constituted to show the risks to humans and animals during in vitro experiments, not for animal experiments. More cautions have to be paid for animal experiments compared to in vitro experiments, because of the amplification of pathogenicity by animals and the risk of transmissions among animals.

(2) Containment of Microorganisms

In order to prevent the spread of dangerous microorganisms outside, they need to be contained. In this case, the level of containment has to be as strict as the degree of risk of the microorganism. There are two ways of containment.

1) Physical Containment

According to the risks of the experiment and microorganisms to be handled, there are four levels of physical containment: P1, P2, P3, and P4 (P stands for Physical) There should be appropriate facilities or equipment to control the experimental areas and the personnel entering the areas, isolate workers from the experimental space, and confine dangerous aerosols.

2) Biological Containment

The concept of biocontainment is used for recombinant DNA experiments, in which host-vector systems with a particularly low viability in the natural environment are used. This is to prevent the spread of recombinant DNA. Even if it leaks into the environment, the host containing the recombinant DNA is not likely to survive in the natural environment.

2. Risk Classification of Pathogens

(1) Classification Criteria for Biosafety Levels of Pathogens at National Institute of Infectious Diseases (NIID)

Biosafety levels (BSL) of pathogens are classified according to the following criteria based on the standards for humans, when dealing with normal amounts of pathogens in vitro.

1) Level – 1 (Low risk to personnel and the environment)
   Definitely no risk of causing a disease in healthy adults.
2) Level – 2 (Moderate risk to personnel and minor risk to the environment)
   Low risk of or likely causing a disease in humans.
3) Level – 3 (High risk to personnel and low risk to the environment)
   Endogenous and exogenous pathogens with potential for aerosol transmission: Cause serious symptoms or potentially fatal if it develops.
4) Level – 4 (High risk to personnel and the environment)
   Exotic pathogens that pose high risk of life-threatening, aerosol transmissible and related pathogens with uncertain risk of transmission within laboratories.

   Notes;

   ① Pathogens of foreign diseases are classified at the higher level.
   ② Pathogens that can cause nosocomial infection are classified at the higher level.
   ③ Other pathogens that are not included here are considered on a case-by-case basis.
   ④ Clinical specimens are dealt with at Level – 2. When a high-risk pathogen is suspected based on clinical diagnosis, it should be treated in accordance with the risk level.

(2) Criteria for Determining Biosafety Levels for Animals at National Agriculture and Food Research Organization (NARO)

1) Risk group 0 Pathogenicity untested strains (except for Risk 1 – 4)
2) Risk group 1 Nonpathogenic, live vaccine strains, and residential strains with little or no possibilities of infections or onset.
3) Risk group 2 Strains that meet any of the following conditions.
   ① Possibility of developing the disease is low even if infected, but can be serious if the disease develops.
   ② Possibility of infection and disease is high, but generally the symptoms are light.
4) Risk group 3 Pathogens that are prescribed in Article 2 of "*Act on Domestic Animal Infectious Diseases Control*" and the equivalents, but excludes pathogens that can be fully confined in the regular experiment operating steps.
5) Risk group 4 Pathogens of overseas virulent infectious diseases (1975/9/16 50LivestockA Article3483 and 1976/7/5 partial revision 51LivestockA Article2760) and the equivalents, but , in principle, excludes attenuated strains.

Reference) Simplified List of (2)

Risk Level	Pathogenicity	Infection	Transmission	Disease	Symptoms
0	Not tested	Not tested	Not tested	Not tested	Not tested
1	None	Hardly none	Hardly none	None	None
2	Yes	Low or high	Low	Low or high	Light or serious
3	Yes	High	High or low	High	Serious
4	Yes	High	High	High	serious

3. Facilities and Equipment

According to the risk level of the pathogen, animals should be confined in a certain area when conducting animal experiments with pathogens. The WHO guidelines specify animal biosafety levels (ABSL). The primary barriers include cages, metal lids, and filter caps to prevent animals from escaping, and confine any coarse dusts. The secondary barrier is containment of contaminated aerosols with isolators (Figure 27) and cabinets (Figure 28) for infected animals. The tertiary barriers should be the architectural and equipment isolations from the environment, which requires airtight structure, sterilizable walls and floors, airlocks, locker rooms, germicidal lights, differential pressure air conditioners, and sterilization of exhaust air and drainage. Here, the "Safety Facilities Equipment and Operation Standards for Laboratories Handling Pathogens" by National Institute of Infectious Diseases (NIAID) is shown. However, those

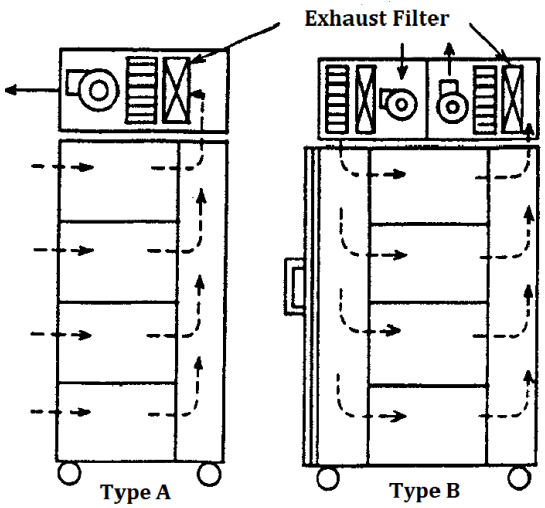


Figure 27. Isolators for Infected Animals

standards are constituted to show the risks to humans during in vitro experiments, so more understanding and precautions are needed for animal experiments.

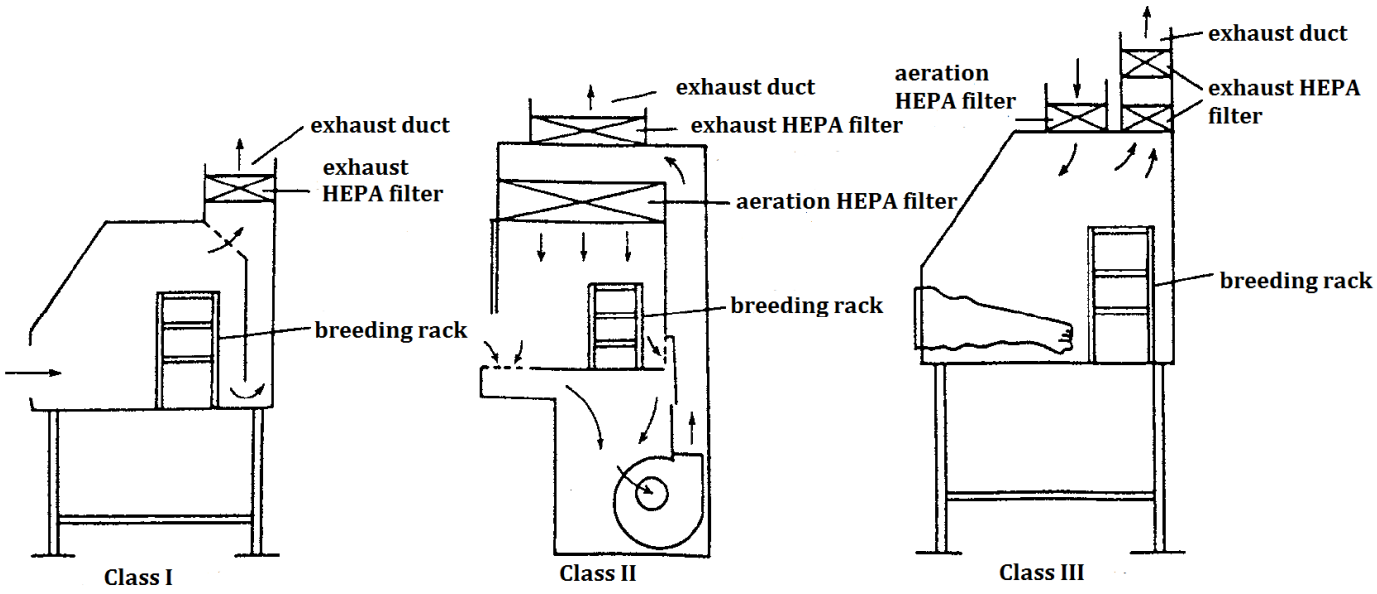


Figure 28. Safe Cabinet for Infected Animals

National Institute of Infectious Diseases

Safety Facilities , Equipment and Operation Standards for Laboratories Handling Pathogens (BSL)

Level 1 (1) Use an ordinary microbiological laboratory and no special isolation is required.

(2) General visitors can access with permission from the manager.Level 2 (1) Use an ordinary microbiological laboratory under limited conditions.

(2) Put on personal protective equipment (protect coat, mask, gloves, etc).

(3) Conduct inside the biological safety cabinet if the experiment might produce aerosols.

(4) Equip the lab with autoclave.

(5) Display the biohazard symbol.

(6) Lock the entrance to the laboratories.

(7) General visitors are not allowed to access the laboratory during an experiment.

Level 3 (1) Use the laboratory which is isolated from the outside by restricted access to hallways, double doors or airlocks.

(2) Put on specialized personal protective equipment.

(3) All the surfaces of walls, floors, ceilings and working tables have to be easily washable and sterilizable.

(4) Maintain enough airtightness to conduct gas sterilization.

(5) Control the exhaust system for constant inflow of the air to the laboratory from outside, and never circulate the exhaust.

(6) The exhaust from the laboratory should be filtered through HEPA (High Efficiency Particulate Air) filter before being released into the air.

(7) Laboratory wastewater should be sterilized or autoclaved, and processed in the wastewater treatment and sterilizing device, and released into the drainage.

(8) Experiments should be conducted inside the biological safety cabinet.

(9) Autoclave should be equipped inside the laboratory.

(10) Display the international biohazard symbol at the entry of BSL 3 room.

(11) BSL3 rooms should be lockable.

(12) Access by those not listed in the staff list are prohibited.

Level 4 (Omitted) The operation is not approved in Japan.

4. Protective Clothing and Equipment

Protective clothing and equipment prevent experimenters from contacting with pathogens and also protect the experiment itself from contamination. They are effective and essential for safety measurement to some extent if the pathogens and experimental methods are selected, but overreliance on them is dangerous. The U.S. NIH (National Institute of Health) guidelines for recombinant DNA experiments (1976) specify the wearing of protective clothing and equipment for each level of physical containment of pathogens, as shown in the Table 5.

Table 5. Wearing Protective Clothes and Equipment for Each Physical Containment Level

Physical containment Level	Wearing Protective Clothing and Equipment				
	Hand	Head	Body	Feet	Respiratory
P1			Free to wear a lab coat or not with any designs		
P2			Wear a lab coat but need to take it off when going anywhere outside of the laboratory.		
P3	Wear surgical gloves during the experiment and immediately take them off afterwards and decontaminate		Wear protective clothes that completely cover the street clothes (cover whole body with long sleeves); All-buttoned type is not appropriate. The protective clothes should be only for the laboratory-use and make sure to sterilize before taking it out outside.		Prepare gas masks for emergency.
P4	Wear surgical gloves during the experiment.	Wear a cap. Do not go outside with the cap on.	Change to the laboratory only-underwear, pants and shirts. Take everything off and leave the clothes inside and shower off.	Change to the laboratory use only footwear and do not take them outside	Prepare gas masks for emergency.
	In the case for P4 suit laboratory style, using the positive pressure suit and life support system (supply and exhaust system), sterilize the outside of the suit with chemical liquid shower.				

Adapted from “Biohazard Measurements Handbook, Akira Ootani, Kindai Shuppan, Tokyo (1981)”

The following are biohazard precautions for major protective clothes and equipment.

(1) Lab attire/Protective clothes: Overall-type, which can cover the whole body is desirable. Or, it should be buttoned at the back so that there is no front opening. The sleeves should be long, and the wrists and neck should be well-fitted and closed.
(2) Gloves and shoes: Gloves should be long enough to cover up the wrists and forearms as well as the fingers to prevent exposure, and easy to work with. For materials natural rubber (latex) or synthetic rubber (neoprene) should be used. Latex gloves are easier to work with for finger work, but neoprene is more durable. When working with animals, wear leather or canvas safety groves on the top of rubber gloves. Rubber boots are sufficient for footwear.
(3) Protective equipment for face and eyes: Aligning with mouth and respiratory system, eyes and face are important as entryways in laboratory infection. It happens when liquids, solids or fine dust contaminate the mucous membranes of the head, face or eyes. Many materials are used to develop facial screens, protective caps, goggles and eyeglasses, but none of them have the function of preventing infectious aerosols, so they should be understood as supplementary gears to avoid physical contact with infectious materials.
(4) Protective equipment for respiratory system: Cloth and non-woven fabric masks are commonly used in surgery and hospital rooms, but those masks only minimize the spread and contamination of exhaled air and prevent from directly getting chemicals or contaminated fluids into mouth and nose, so that they cannot be expected to be effective as a safety measure for experimenters. In order to totally eliminate aerosols and prevent respiratory infections, the handler has to use a HEPA filtered respirator or positive-pressure hoods with air supply.

5. Laboratory and Animal Room Cleaning

The laboratory should be organized in a consistent manner, and decontaminated and cleaned immediately after the experiment is completed so that it's ready for the next experiment.

Cleaning of laboratories is categorized into everyday cleaning and decontamination after the experiment. The former can be done by regular cleaning staff, who do not have expertise in pathogen hazards and safety measures, but the director of the room should be responsible to determine where to be cleaned by cleaning staff. The latter has to be done by the experimenters themselves. For safety measures, all the steps of cleaning and decontamination should never be skipped to prevent a major flaw. The handlers should remember that the most important thing for biohazard measurements is to faithfully adhere to the prescribed measures.

Organizing and cleaning of animal rooms is necessary more than in general laboratories and can be rather difficult in a technical aspect. Not only the animal rooms, but also the anteroom and hallways have to be cleaned, sterilized, and always maintained in a sanitary condition. The experimenters should always maintain the clean environment after any experiments. To be specific, carefully sweep the floor not to stir up dusts (there are electronic vacuum cleaners with high performance filters available). Mop the floor with disinfectant and wipe with tightly squeezed cloth. Wipe the walls, ceiling, and air supply and exhaust port of the room with the cloth perfused with disinfectant at least once a month to prevent the accumulation of dust. Do not spray the disinfectant in the room when animals are around, in order to prevent their being splashed.

The exhaust vent in rabbits rearing room can be easily clogged with hairs, so it is best to install a wire netting. Make sure to clean the wire netting at least once a week.

In case of group rearing of cats and dogs, clean the floor every day to remove any filth. Make sure to dry the floor well in order to avoid high humidity in the room. If the room is designed to have a groove, make sure to brush, wash, sterilize it, and spray disinfectant after the work is completed to prevent the accumulation of excrement.

6. Aseptic Technique

There are countless microorganisms living around us, and if any of them get mixed into experimental materials, the result could become confused, and the experiment itself could be meaningless. Also, when dealing with pathogenic microbes, humans and animals can get infected causing serious problems. Aseptic manipulation is the procedure to prevent those risks, which is the most basic experimental technique in microbial experiments. Aseptic manipulation is closely connected to sterilization, which is described in the next section, and is the most required technique for isolation and culture of bacteria and viruses, subculture, media preparation, dissection or surgery of animals, production and rearing of germ free animals. More specifically, aseptic handling of inoculation loops, test tubes, petri dishes, flasks, pipettes, scalpels, and scissors is the main aseptic manipulation technique.

The following matters shall be strictly followed in order to properly conduct the aseptic manipulation.

(1) Acquire the correct aseptic manipulation techniques. Adherence to an inexperienced own fashion can cause accidents or failures. The handlers have to learn the correct techniques to handle tools and equipment with accurate understandings of their functions.
(2) Clarify the procedures in the laboratory and make sure to follow them.
(3) Always wear the laboratory-specific clothes in the laboratory and rearing rooms. Do not go outside in the lab cloths. Eating, drinking, smoking, touching any mucous membrane including wearing contact lenses or make-up are not allowed in the laboratories or rearing rooms.
(4) Minimize conversations to avoid any scattering of mouth bacteria.
(5) Clean any cultural medium, tools, experiment tables, filth, and carcasses thoroughly after the experiment to prevent any contaminations.
(6) Always keep the laboratories and rearing rooms clean and organized. Any reagent bottles, test tubes, and other containers should be clearly labeled with the contents, name of the handler, and the date.
(7) Conscientiously disinfect hands, experiment tables and floors before and after the experiments.
(8) If the aseptic manipulation fails, humbly reflect on the situation, conduct the post-failure procedures calmly, and never take care of the situation in the own way.

7. Sterilization and Disinfection

Sterilization and disinfection are one of the major procedures of biohazard measurements. Sterilization is the destruction of all microorganisms regardless of pathogenic or nonpathogenic. Disinfection means to eliminate pathogenic microorganisms outside the body by physical and chemical ways. There are many ways to sterilize and disinfect as shown in Table 6. Depending on the species and strains of microorganisms,

the resistance might often differ. Therefore, their application should be selective based on the nature of the pathogenic microorganisms, especially the physical and chemical resistance.

(1) Sterilization Method

1) Flame sterilization: A method to destroy the microorganisms by heating with aflame. In the laboratory, gas burners and alcohol lamps are used to sterilize inoculation loops and needles. When heating an inoculation loop with bacteria on with a gas burner, first, heat it in a low temperature reducing flame (blue flame) and then with high temperature oxidizing flame (red flame) (Figure 29). The bacteria might burst out and contaminate the surroundings if heated in the reverse order. If the inoculation loop is dipped into liquid right after burning, aerosols might be generated, so that close attention is required.

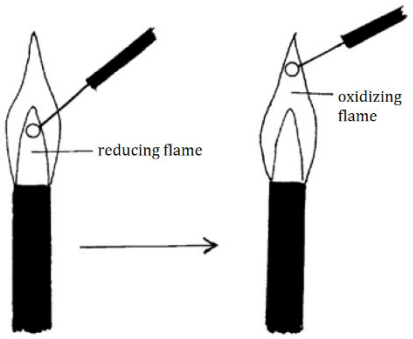


Figure 29. Flame Sterilization of an Inoculation Loop

2) Dry-heat sterilization: A method to destroy the microorganisms in dry, high temperature air using a dry heat sterilizer. It is like an oven that directly heat using city gas or electricity. Usually the temperature is raised to the specified level: at 160~170℃ for 2 – 4 hours, or 180~200℃ for 0.5 – 1 hours. After maintaining the temperature for a certain period of time, turn it off and take it out when the temperature drops to room temperature (See Dry Heat Sterilization section in XI.).

3) High pressure steam sterilization: The most complete sterilization method used for sterilization of heat resistant chemicals, cultural media, tools, contaminated materials, and waste. Autoclave is a heat-resistant machine equipped with a manometer, thermometer, vacuum pump, and other accessories. It is conditioned to sterilize for 30 minutes at 115℃ (1.55 kg/cm²) or for 20 minutes at 121℃ (2.1 kg/cm²). Be careful when handling because of high pressure (See Autoclave section in XI.).

4) Gas sterilization: A method to sterilize using sterilizable gases such as ethylene oxide and formaldehyde. Ethylene oxide is suitable for sterilizing plastic syringes and petri dishes made of heat-sensitive synthetic resins using a specialized sterilizer. Formaldehyde is suitable for sterilizing the entire laboratory, a storage and an animal rearing room. In all cases, those gases themselves are very toxic to living organisms, so that it should not conducted at individual discretion.

5) Filter sterilization: When sterilizing materials that cannot be heated such asserum, or culture media containing non-heatable materials that oxidize if heated, or when removing bacteria and other viruses from virus emulsions or toxin liquids, the materials have to be passed through a fine filter that does not allow bacteria to pass through. Membrane filters are easy and convenient to use, and there are several types of them. Set the filter carefully.

6) UV sterilization: A method to sterilize surfaces of tools that cannot be heat-sterilized. There are some specialized devices to prevent UV from reaching the outside, but simply place the object to irradiate under the sterilization light in a sterilized space. Sterilization effects of UV are the strongest at a short wavelength around 260mm. It is important to place the object to be sterilized as close as possible to the light so that there are no areas that are not irradiated. UV can be harmful to humans (cataract or dermatitis), so it is important to block out UV rays, wear protective glasses, and cover skins to avoid direct exposure to UV.

(2) Disinfecting Method

This section describes the major disinfectants used on a daily basis and their characteristics. Make sure to be careful with handling them not to get on fingers or clothes.

1) Alcohols

① Ethanol: It is most effective at concentrations between 60 – 90 %. Most bacteria get killed in 15 seconds, but it is ineffective against bacterial spores and filamentous fungi. Ethanol kills miecoorganisms by denaturing their proteins, inhabiting enzyme activity, and dissolving their liquids.

② Isopropanol: It is used at concentration between 30 – 70%. It is slightly stronger than ethanol. Sterilizing effects are the same as ethanol, but its toxicity and irritation is stronger than ethanol.

2) Iodine tablets

① Iodoform: It is bonded and compounded with a nonionic surfactant, which is effective against molds and viruses at high concentrations. e.g.) isodine, diazan, wescodyne at 75 – 150ppm

② Iodine tincture, lugol solution

3) Chlorine compounds:

① Sodium hypochlorite: It is very effective and bactericidal. It is effective against spore-forming bacteria at high concentrations, but ineffective against tubercle bacilli. The effect will be weaker in the presence of organic matter. It needs to be stored in the shade at low temperature. Use at 500 – 1000ppm for disinfecting contaminated tools.

② Trichlorohydroxydiphenyl ether (Irgasan): It is an organochloride and effective against nutritional types of bacteria and the hepatitis B virus.

4) Metals and Metal Salts

① Mercury compounds: It is extremely strong disinfectant, but highly toxic. Because of the pollution problem by heavy meal disposal, it is hardly used nowadays. e.g.) mercurochrome

② Silver compounds: It is used to disinfect mucous membranes. e.g.) silver nitrate, silver protein

Table 6. Summary of Disinfecting and Sterilization Methods

Classification		
Physical Methods	1) Mechanical factors: sound wave, high pressure, surface tension, filtration 2) Ionizing radiation: X-ray, α-ray, y-ray 3) Nonionizing radiation: visible rays, UV	
Physical Chemical Methods	1) Heat Treatment	Dry Heat: Flame (burning, incineration); High Heat Air
		Wet Heat: Boiling; Steam: Circulating Steam, High Pressure Steam
	2) Freeze-Thawing 3) PH 4) Salinity 5) Filtration	
Chemical Methods	1) Denatured Protein: formalin, urea, phenol 2) Oxidizing Agent: hydrogen peroxide, halogen 3) Alkylating Agent: nitrogen mustard, ethylenediamine, ethylene oxide, beta propiolactone 4) Organic Solvent: ethylene alcohol, methyl alcohol, acetone 5) Surfactant: deoxycholic acid, NP40, laurylsulfate, Tween-80 6) Enzyme: trypsin, papain, nagarse, pronase	

[Principle and Reality of Biosafety, National Institute of Infectious Diseases]

5) Boiling sterilization (disinfection)

It is a method to destroy microorganisms by heating the tools in boiling water. Boil for at least 15 minutes at 100℃. However, only nutritional types of bacteria, some viruses and parasites in trophozoites are destroyed at this temperature and time, and the spores or viruses are usually not destroyed under this condition, and some of them can survive in boiling water for even a few hour, so careful handling is needed.

8. Pipetting Techniques

When liquid samples include any pathogens, improper pipetting techniques can be a cause of biohazard. In order to minimize any errors during the experiment, always prepare the same volume of standardized products in the sterilizing box. Also, plug up the pipette's mouth with a cotton plug to avoid any contaminations. For any experiments that deal with pathogens that are classified as BSL2 or higher, or recombinant DNA, conduct the experiment with safety pipetter in the safe cabinet. It is desirable to use a specialized, easy to use small pipette in the cabinet. It is recommended to use a disposable micro tip when using small amounts of sample.

Make sure not to shoot the liquid sample out into the air from the tip of the pipette. Also, make sure not to foam the sample or spill bubbles. Stop the experiment when contamination is suspected, and immediately disinfect the pipette by soaking into disinfectant. Be careful not to ignite the disinfectant, and disinfect the contaminated area.

After using a pipette, gently put it into the sterilizer not to spill or splash any liquids. Sterilize disposable pipettes and microtps under high pressure.

9. Syringes and Needles

Use disposable, plastic syringes and needles. Carefully handle the syringe in inhalation and exhalation of samples to avoid splashing or foaming the sample. Conduct the experiment inside the safety cabinet when the sample is pathogenic. When removing air bubbles, wrap the tip of the needle with an alcohol impregnated cotton.

When giving an injection to an animal, it is important to restrain the animal properly beforehand not to accidentally stick the handlers themselves by unexpected movements of the animal. First, disinfect the area to be injected with an alcohol swab, and after injection, disinfect the area well not to leave any pathogens .

To avoid needlestick injuries, do not replace a protective cap, and sterilize the syringes with needles on under high pressure after every usage. Separate the syringe and needle from other test tubes and pipettes for proper disposal.

10. Centrifugal Sedimentation

Regularly inspect and maintain the centrifuge and follow the directions in order to prevent any contaminations. Be careful with properly balancing, the position of the bucket, and the permissible numbers of rotation. When the sample is pathogenic, conduct the experiment in the safety cabinet. Be careful not to spill when putting in and out from the centrifuge tubes.

There are possibilities of infection or contamination by aerosols, even during proper machine operation. Always put the cap to seal the centrifuge tubes to prevent leakage of the liquid inside. Consider the angle of the tubes and pour the sample liquid less to avoid any spillage. Glass and plastic centrifuge tubes have different strengths against gravity, so check the durability of each tube under centrifugal force of each tube. Before and after of experiments, disinfect the inside of the centrifuge and its rotor.

11. Mortar and Homogenizer

In the process of homogenizing, a large amount of infectious aerosols is generated in the air. Therefore, use a sealable metal blender cup. Yet the aerosols can still leak out of the cup, so make sure to conduct the homogenizer in the safety cabinet. Wait for more than 10 minutes for the aerosols to settle before opening the cup. After the process, immediately sterilize all used tools and disinfect the areas around the homogenizer to get rid of any contaminations.

12. Ultrasonic Treatment

Ultrasonic treatment can be the biggest source of aerosols. Therefore, conduct the safety measures that are the same as the homogenizer, and conduct the experiment inside the safety cabinet when the sample is pathogenic. Do not use an ultrasound generator with an ultrasound drill in an open container. Use the device that put the ultrasound on the bottom of the screwed lid cup.

13. Preservation of Microorganisms

The most important factor for preservation of pathogenic microorganisms is to maintain their biological, immunological, and genetic characteristics. Common methods are subculture, cryopreservation, and drying.

(1) Subculture Method

Subculture is mainly used for preservation of bacteria. It is convenient in that a new cell or new culture can be made by regularly transferring some or all of the cells from a previous culture to fresh growth medium, but there are following disadvantages.

1) Due to constant cell transplantation, the risk of laboratory infection and the opportunity for various bacterial contaminations increase.
2) It is likely to decrease pathogenicity, productivity of bioactive substance, and produce genetic variation.
3) It is likely to make errors in subculture, such as taking and passing on the wrong bacterium by mistake, and incorrectly writing down the names of bacteria and the strain number.

(2) Cryopreservation Method

1) Refrigeration (-20 ~ -85℃)

In the case of pathogenic bacteria viruses, and some parasites, the test tube culture itself is often frozen. However, it is desirable to prepare the liquid which has more pathogen contents adding protective agents (dispersion medium) such as sterilized glycerin, saccharides solution, skim milk, serum, or dimethyl sulfoxide (DMSO) for cryopreservation.

Use the good quality of ampules for freezing, such as cryopreservation vials or ampoules that will not break during the freezing process or storage period. It is safe to keep the right amount of liquid in each ampule. As other precautions, it is better not to add any electrolytes (e.g. NaCl) unless needed to avoid denaturation (salt damage) during the process of freezing.

2) Preservation Using Liquid Nitrogen

It can be widely applied to various bacteria, viruses, protozoans, and animal cells. Here are precautions for using liquid nitrogen:

① Use specialized plastic vials as storage containers. Plastic straws are used to preserve livestock semen.
② The vials with outer caps can be storage in the gas phase and the vials with inner caps can be storage in the gas/liquid phase.
③ Do not use glass beakers when thawing the frozen sample in warm water. It is dangerous if the ampule breaks.

3) Preservation by Drying

There are freeze-drying method, liquid-drying (L-Drying), gelatin disk drying, and paper disk method (filter paper preservation method). Freeze-drying method reduces mutations of microorganisms during preservation and enable a long-term storage, so that it is used for various bacteria and viruses. There are multi-tube type dryer and chamber type dryer. Multi-tube-type is convenient for drying using ampules. Here are common precautions for using the multi-tube type dryer;

① When dispensing the microorganism suspension into an ampule, be careful not to touch the tube wall.
② Use a small and simple dryer for easy sterilization. It is safer if the device can be installed in the germ-free hood.
③ Loosely plug the mouth of ampule with cotton and dry as shown in Figure 30 for highly poisonous strains, especially spore-forming bacteria.

④ Wear rubber gloves to avoid any contaminations or injuries from glass fragments when taking off the sealed ampule from the multi-tube type dyer after drying.

⑤ The area between the ampule inlet and the cold trap is easily contaminated by dried bacteria. After drying, sterilize the melted drain in the cold trap by pouring into a container with disinfectant.

⑥ When opening the ampule, be cautious not to allow airborne bacteria to enter the ampoule, and not to allow dried bacteria to become airborne (aerosols). If possible, open the ampule inside the clean bench. First, wipe the neck of the ampule with an alcohol swab, file it, wrap the neck with sterilized gauze or a plastic sheet and break it. Or file it and place the incandescent glass stick onto filed area for easy crack.

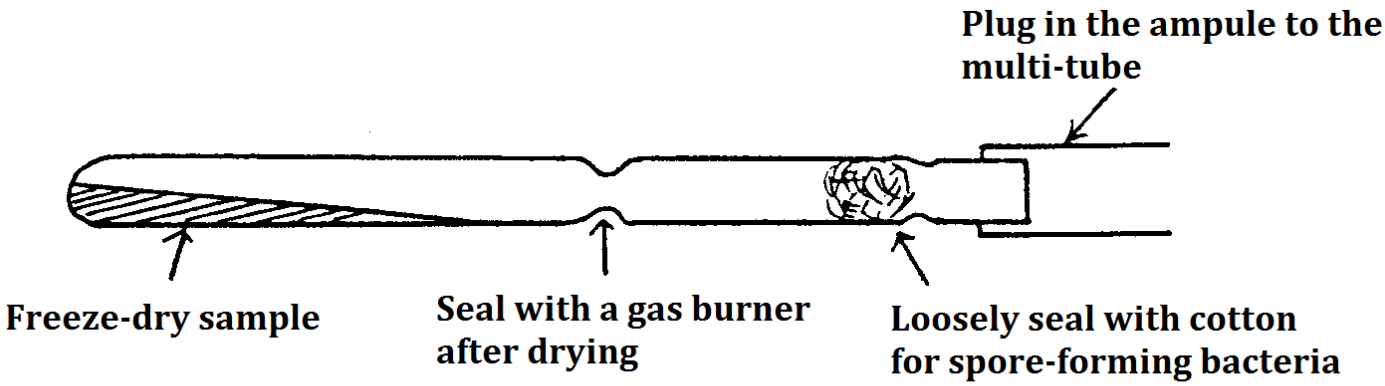


Figure 30. Ampule for Freeze-Drying

14. Preservation, Management and Transportation of Pathogenic Microorganisms

(1) Preservation and Management of Pathogenic Microorganisms

From the perspective of biohazards, the preservation and management method pathogenic microorganisms should require all the possible measures to ensure safety of humans and animals. Therefore, preserved strains and microorganism strains during and after the experiment should be stored and treated under the appropriate management system.

1) The person in charge of storing the pathogenic microorganism strains shall preapre a logbook and regulate the management rules, and conduct experiments according to these rules.
2) Store the pathogenic microorganisms in labeled containers not to mix with others.
3) Pathogens after use in experiments should be treated in accordance with the most effective disinfecting method for each pathogen.

15. Microorganism Resistance

Resistivity of microorganisms against physical and chemical effects depends on the type of microorganism, strains, and the existing conditions. For example, nutrient bacteria are destroyed in 10~30 minutes at 100℃, but bacillus and clostridium spores require 15~20 minutes at 121℃. Similarly, spores have the strongest resistance against medicine, so effective medicines are limited. Viruses without envelopes have higher resistance compared with viruses with envelopes. Table 7 shows the resistance of various microorganisms against disinfectants. Effectiveness of disinfectants is affected by concentration, temperature, duration, pH, presence of organic matter and saline.

Table 7. The Antiseptic Effect of Each Disinfectant

Types of Disinfectants	gram-positive bacteria	grave-negative bacteria	tubercle bacillus	Spore	Fungus	Viral Envelope	No Envelope
Phenol	+	+	+	−	+	+	−
Cresol soap	+	+	+	−	+	+	−
Phenol derivative	+	+	+	−	+	+	−
Chlorinated pesticide	+	+	+	+	+	+	+
Iodophor	+	+	+	+	+	+	+
Inversed soap	+	±	−	−	−	+	−
Amphoteric soap	+	+	−	−	−	+	−
Chlorhexidine	+	+	−	−	−	+	−
Alexidine	+	+	±	+	+	+	−
Quicklime	+	+	+	+	+	+	+
Caustic soda	+	+	−	+	+	+	+
Aldehyde	+	+	+	+	+	+	+

(Notice) + ; Effective - ; Ineffective from [Dairy Farm Matters, Iizuka (1993)]

16. Human Health Management

There are various potential risks that can affect human health in animal experiments and rearing management of animals. Especially, there is a great riskof getting infected while working with animals injected with pathogenetic microorganisms or animals naturally infected with zoonosis. Table 8 shows the number of accidents caused by various microorganisms by cause. We should pay attention to the majority of accidents by animals and external parasites, not to mention the overwhelming number of accidents that occurred during the handling of microorganisms or due to unforeseen circumstances. Pathogens can stick to the animal hairs and can remain suspended in the air as aerosols, or might come out in infected animals' saliva or urine.

In addition to damage caused by animals come from these pathogens, it includes allergies to animal hairs and feces, respiratory disease caused by inhalation of dust and ammonia gas when changing floor covering, and bites by animals. To protect from these hazards, it is crucial to learn how to handle animals: always stay cautious when in contact with animals; do not touch the animals unless needed; wear specialized clothes, hat, mask, boots, groves, and other protective tools as needed; work with as little dust as possible; and always clean and sterilize hands after work. Needless to say, eating, drinking, and smoking in the animal rooms are prohibited. Get periodical medical exams and regularly manage your health. Depending on the animals and the types of pathogen to be used in the experiment, consult with the professor in charge and ask for doctor's advice as needed. Any experimenters that might be in danger of exposing to radiation should get a special periodical medical exam at the University Health Center.

Table 8. Infectious Factors That are Confirmed or Estimated as Human Cases of Laboratory Infection

Causes	Types of Microorganisms							
	Bacteria	Virus	Rickettsia	Fungus	Chlamydia	Parasite	Others	TOTAL
Accident	378	174	45	33	14	38	21	703
Animals or External Parasite	149	249	66	15	32	11	1	523
Clinical Material	90	175	2	1	0	19	0	287
Used Glass Tools	34	10	2	0	0	0	0	46
Human Anatomy	56	9	4	0	0	1	5	75
Deliberate Infection	14	1	0	0	0	4	0	19
Aerosol	101	92	217	88	22	2	0	522
Working with Microorganisms	381	213	100	62	43	28	0	827
Other	7	1	7	0	1	0	0	16
Unknown or No Record	459	125	130	18	16	12	7	767
	1,669	1,049	573	217	128	115	34	3,785

Pike, R.M. (1976): Biohazard Measures Handbook, Edited OOtani, etc. (1981)

Ⅵ. OTHER ANIMAL EXPERIMENTS THAT REQUIRE SPECIAL ATTENTION

1. Experiments That Use Toxic Chemical Substances

Kagoshima University has established new guidelines “Guidelines for Animal Experiments with the Use of Hazardous and/or Carcinogenic Substances” (promulgated on January 1, 2018), in order to prevent hazards to experimenters/others, non-use purposed animals as well as environmental pollution in animal experiments using carcinogenic substances which may have a risk to humans and other animals, harmful heavy metals such as arsenic, mercury, lead, cadmium which may have a risk of causing environmental pollution, and hazardous materials such as endocrine disruptors (hereafter called ‘hazardous and/or carcinogenic substance’).

Make sure to mention the classification of the substances to be tested in the animal experiment plans and to apply with an application form for “Animal Experiment with the Use of Hazardous and/or Carcinogenic Substance”, when carrying out animal experiments using hazardous and/or carcinogenic substances.

(1) Handling of Toxic Chemical Substances

It is important to pay close attentions when handling any toxic chemical substances that include carcinogen and mutagenicity (genotoxicity). These substances should be handled in the draft chamber in the laboratory. Place a polyethylene paper filter on the experiment table to avoid any contaminations by toxic chemical substances, and make sure to wear protective groves and an apron. Furthermore, depending on the characteristics of the substances, wear a protective mask (dust-proof or gas-proof). When using as a solution, use a pipette and do not suck in with your mouth. Prepare a decomposition resolution beforehand, put the used tools in the solution, and wash after the substance is completely decomposed. After handling any toxic chemical substances, wash your hands thoroughly with a soap and change the lab-wear.

(2) Toxic Chemical Substances Injection Experiments

Toxic chemical substances that are injected into laboratory animals are metabolized and accumulated in the body, but some are excreted into feces and milk. The handler accordingly should keep in mind that hairs of laboratory animals are also contaminated, so always wear protective gloves when handling experimental animals. Floor mats, any rearing tools, and devices that have come into contact with toxic chemical substances and feces should be fully discomposed, and disposed or cleaned. When toxic chemical substances are mixed into their power feed, make sure not to spill or splash. Carefully clean the feces not to raise dusts and make sure to wear a dust protective mask.

During the toxic chemical substance injection experiments, display the signs on the room, frames, and cages, which indicate that toxic chemicals are being used, in order to inform caretakers and other relevant personnel of the danger. Also, the person in charge should explain the danger of each toxic chemical substance to all relevant personnel. Access to these rooms should be restricted to relevant personnel and staff only. Eating and smoking in these rooms are strictly prohibited.

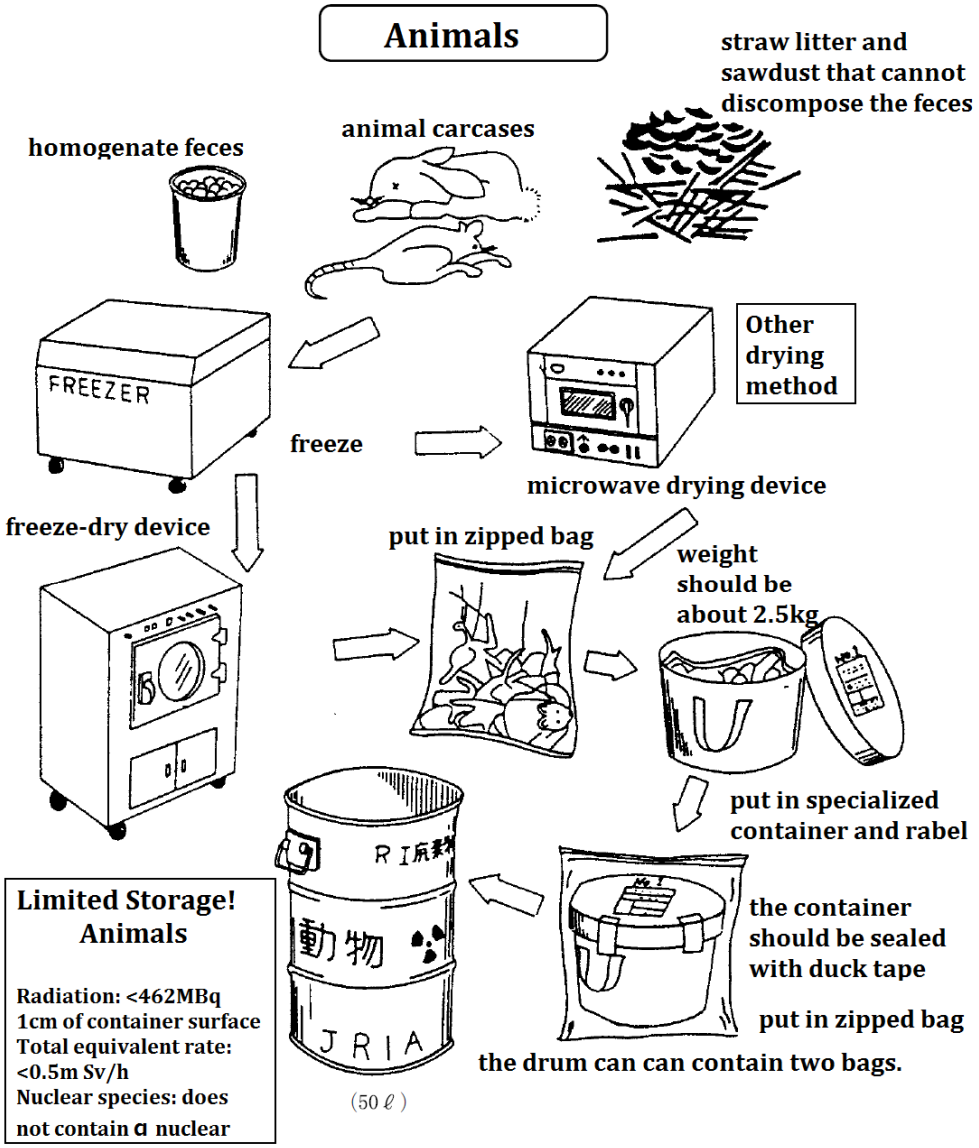


Figure 32. How to Process Feces and Carcasses of Laboratory Animals at RI Animal Experiments

2. Experiments with Radioisotope

Animal experiments using radioisotope (RI) are effective for research to observe the absorption, utilization, and metabolism of substances in the body by directly injecting the RI-signed chemical compounds (medicine, chemical substances) into laboratory animals. However, when starting an animal experiment using RIs, it is important to note that not only the utilized RI but also the RI-injected laboratory animals themselves are radiation sources, and that RI substances might be excreted in the exhaled air and feces of the animals. Therefore, animal experiments with RI should be conducted in the special controlled area, and the handling, rearing, and disposal of RIs and the injected animals are strictly regulated by the national regulations, "*Act on Prevention of Radiation Hazards due to Radioisotopes, etc.*", just as in general RI laboratories. Accordingly, animal experiments with RI should be conducted in the special facility (RI Experiment Facility).

Refer to the *Radioisotopes Handbook* by the Japan Radioisotope Association for RI handling, laws, facility and equipment of RI laboratory rooms. This section explains the matters directly related to animal experiments using RI.

When conducting an animal experiment using RI and RI compounds, the most important points in the terms of radiation hazard prevention for the handlers are to assure the safety of the handler and others around him/her, and to prevent any contaminations of laboratory animals and the environment by the radiation hazard accidents in the rearing environment.

(1) Precautionary Points in Terms of Radiation Hazard Prevention

1) Fully understand the characteristics and features of the nuclides to be used in the laboratory animals.
2) RI dosage should be well calculated beforehand to minimize to be applied and fully maximize the efficiency of the radioactivity measurement.
3) Get used to handling animals to prevent any radiation accidents when injecting RI.
4) Since a part of the injected RI substances is excreted through the exhaled air, feces, and urine, all rearing tools and devices that are used for the experiment should be treated with utmost care.
5) When dissecting an animal, it can easily evoke radioactive contamination of surrounding environment, so extreme caution needs to be exercised.
6) Other precautions are the same as the regular RI experiments.

(2) Processing Method of RI Waste

RI wastes are categorized in general waste, animal feces, and carcasses. These wastes have to be processed in drying, liquefaction, or mummification before disposal. Figure 32 shows the specific procedures to process feces and carcasses of RI laboratory animals.

(3) Importance of Pilot Study

It is more likely to cause radioactive contamination accidents if the RI experiment is not well-operated. Prior to the start of an experiment, it is important to practice and become proficient in rearing management, restraint, administration method and dissection of the laboratory animals without using RI, and to move onto the actual experiment only after becoming sufficiently proficient in the whole procedures. Whenever possible, conduct the experiments with in vitro or small animals (such as mice).

3. Experiment Using Recombinant DNAs and Transgenic Animals

(1) Registration and Approval of Experimental Plans

Recombinant DNA experiment should be conducted in accordance with "*Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms (2007/4/20, Ministry of Finance, Ministry of Education, Culture, Sports, Science and Technology, Ministry of Health, Labor and Welfare, Ministry of Agriculture, Forestry and Fisheries, Ministry of Economy, Trade and Industry, Ministry of the Environment Ordinance No.1*" *(2003/6/18 Act No.97)*. Based on the Ministerial Ordinance "*Act on the Contamination Measures to Be Taken in Type 2 Use of Living Modified Organisms for Research and Development, etc*" *(2004/1/29 Ministry of Education, Culture, Sports, Science and Technology(MEXT), Ministry of the Environment Ordinance No.1)*, it is mandatory to take prevention measures of proliferation in the experiments. Be precautious for penalties and fines. It has to be approved by

the minister of MEXT and the school president. In Kagoshima University, it is also required to be reviewed by the GM Experiment Safety and Management Committee. Transgenic animal experiments are recombination experiments in animal genomes.

On campus gene-modified experiments webpage
http://hp.kagoshima-u.ac.jp/jimu/bu03/ka01/post-2.html

Ⅶ. SAFETY MEASURES IN PATHOLOGICAL ANATOMY

In animal experiments, there are autopsies (post mortem examinations) and dissections after euthanizing the ante mortem cases. When dissecting the ante mortem cases, select the appropriate euthanizing method that fits the experimental purpose. It is indispensable to have staff with knowledge of zoonosis during autopsy. Especially, during the autopsy of laboratory infection or toxic experiment of hazardous materials, it is essential to conduct countermeasures against biohazard and chemohazard (refer to V., VI.).

1. Precautions Before Autopsy

(1) Clinical History of the Animal to be Dissected

Obtain information on the dates and time of the onset, symptoms, intake, descriptions of feces, and changes in weight.

(2) Treatment

Obtain information on the medication, types of medication, its amount and duration, and other treatments.

(3) Test Results

Examine test results of urinalysis, homological, serum-biochemical, microbiological, and physical exams. When suspecting any infectious diseases, prepare to collect samples for microbiological or serological exams.

(4) Necessity of Infectious Prevention Measures

When dissecting an animal suspected for any infectious diseases, it is important not to spread the infectious sources by dissecting. In particular, when dealing with an animal infected with highly infectious pathogenic microorganism, prepare the preventive measures not only for the other animals but also for humans.

2. Preparations

(1) Anatomy Room and Equipment

The anatomy room should be large enough, equipped with lights, ventilations, water supply and sewages, and air conditioning. The dissection table varies depending on the animal size, but there should be enough space for carcasses, dissecting tools, and any removed organs.

(2) Clothes and Mental Readiness

In general, when dissecting an animal, including experiments fraught with hazards, the handler should assume that there is a risk of human infection, so protective measures against infection should be taken, such

as wearing a waterproof or plastic apron on the top of the lab coat, boots, rubber gloves, a mask, a hat and goggles to protect the eyes.

It is important to conduct an autopsy in an appropriate order and avoid any contaminations from feces and blood. Conversation during the autopsy should be limited to the findings about the autopsy, striving not to miss any important lesions as well as paying close attention not to spread any contaminations from the carcass. Cautiously use the surgical knife and scissors, and immediately sterilize wounds when accidentally get injured.

If a specific livestock animal infectious disease (official disease) is diagnosed during an autopsy, or if there is a suspicion of such a disease, it is necessary to contact the related agency (Prefectural Agricultural Administration or Livestock Hygiene Service Center) and conduct appropriate treatments.

3. Processing After Dissection

(1) Processing of Carcasses and Wastes

Processing of carcasses and wastes is necessary to prevent any infections from bacteria, viruses, external parasites such as flies, cockroaches and mites, offensive odor, and ammonia gas generation. They should be put in plastic bags or containers and incinerated in an incinerator. When incinerating, be careful not to contaminate the surroundings with carcasses and wastes while transferring them to the incineration site.

(2) Sterilizing the Anatomy Rooms and Tools

Sterilization of the anatomy room and tools must be conducted after the experimental infections, especially after working with dangerous bacteria and viruses. Table 9 shows disinfectants by application. Boiling and high-pressure steam methods (high pressure sterilization) are also used. When conducting sterilization, follow the directions of each medicine and device, and pay close attention for hazard prevention.

Table 9. Disinfectant by Application

Indoor	**inversed soap, ampholytic surface active agent, phenol, chlorhexidine sodium hypochlorite**
Fingers and Skins	**ethanol, inverted soap, ampholytic surface active agent, iodine tablet phenol, hydrogen peroxide**
Metal Tools	**ethanol, inverted soap, ampholytic surface agent, phenol**
Non-Metal Tools	**ethanol, inverted soap, ampholytic surface agent, phenol sodium hypochlorite**
Surface of Cloths	**ethanol, inverted soap, ampholytic surface agent, iodine tablet, phenol**

Ⅷ. SAFETY MEASURES FOR CLINICAL TRAININGS ON ANIMALS

1. General Precautions for Clinical Training on Animals

(1) Preparation for Clinical Trainings

- It is important to treat animals with care and love.
- Interact with healthy animals regularly to become familiar with their handling and behavior.
- Always keep in mind that animals can behave unexpectedly, even when they seem familiar, so keep an eye on them. (Companion animals can bite and scratch. Farm animals can kick, step on, or push you off to the wall).
- Keep quiet not to overexcite animals.
- Be sure to touch or interact with the animals while talking to them. Do not suddenly touch them from behind.
- Behaviors to stimulate animals are shouting loud, laughing, approach running, waving hands, blowing a towel on the neck in the wind, rubbing sound of plastic bags, reflecting a raincoat/umbrella. Give full consideration to the fact that animals are constantly cautious of humans.
- Assume that farm animals might suddenly kick when standing near them. Be especially careful when walking behind the animals, they might suddenly kick. The same is true for cows as well as horses.
- Do not sit near the animals during a training. Always be in a half-standing position or higher to be ready for running when needed.
- When handling farm animals, it is likely to work as a group, so cooperate and communicate well with the members.
- Always follow the instructor's directions while performing the practical training. Observe and refer to their techniques and skilled personnel.
- Wash and disinfect the wounded area immediately with running water if bitten, scratched, stepped on, or injured. Report to the instructor and follow the instructions to see a doctor as needed. Make sure to keep in mind that even if the wound seem fine immediately after the injury, there is a possibility to get infected.

(2) Dress Code for Trainings

- It is desirable to always wear clean clothes that can be easily washable if soiled.
- Avoid wearing flashy, fancy, or shiny clothes made of reflective materials.
- Make sure to wear comfortable clothes for easy and agile movement. It is dangerous to wear a long lab coat since it might be blown in the wind and can give an anxiety to animals. It might also get caught in surroundings when running away from animals behaving violently.
- Wear a hat when going outside for the farm animal trainings.
- Wear appropriate footwear for trainings. Boots and safety shoes are usually worn for the farm animal trainings, so make sure to always wash and disinfect them.
- Avoid wearing an excessive perfume, long nails, and jewelries (small earrings, necklaces, piercings are fine, but big ones should be avoided).

(3) Precautions for Tools, Devices and Chemicals/Medicines Used in Clinical Practices

- Handle the tools and the equipment with care, sterilize them after every use to remove contamination, And put them back where they belong.
- Store all the tools properly to be able to use them right away in the next training.
- Report to an instructor immediately when you notice any malfunctions, failures or misconducts of the tools and devices.

- Dispose of any used syringes and needles into the designated containers after use. Medical wastes with blood on must be disposed of in the separated container for medical waste.
- Follow an instructor's directions when handling any chemicals or medications. Do not take them out of the training areas.
- Pay close attention to contamination of chemicals and medicines that are repeatedly used. Report to the instructor when you notice something wrong or unusual.

2. Farm Animal Practical Trainings

- It is important to understand the characteristics of each animal and acquire techniques for safe handling and restraint in the farm animal (cattle, horses, pigs, goats, etc.) trainings.
- Rubber boots (or safety shoes) are mandatory for the practical trainings. Use the tools and devices that are dried and sterilized, and be familiar with how to operate them.

Cattle Clinic

(1) Management

- Calculate nutrients according to the Japanese Feeding Standards for Cattle. Dietary restrictions and management might be necessary as a part of the treatments, so follow the instructions of the instructor.
- Place the rubber mats on the floor, be conscious for ventilation, and trim hooves as needed.

(2) Restraint

- Spend the time with the cattle on a daily basis to give them a sense of ease. Do not scream at or intimidate them with a stick.
- Strain with a bridle and a nose ring. Be careful not to be stepped on, butted, kicked or mounted.
- When tying to a station, make sure not to dangle a rope too long and to tie in a knot that can come undone easily by humans.

Equine Clinic

(1) Management

- Horses are very habitual animals so try to repeat the same behavior at the same time daily. (Horses can get used to the rhythm and will become easier to work with.)
- Follow the guidance on feeding since dietary restriction or special management may be necessary due to treatment, in some cases.
- Sudden movements around the horse is very dangerous (might bite or stand up). Especially, if approached from directly behind, horses may get scared and kick, so avoid approaching them from directly behind at all costs.
- When the horse's ears are facing to handlers, it means the horse is paying attention to them. Talk to the horses gently and check their ears are facing to handlers when approaching them.
- Do not approach horses when they pin their ears back, which means they are irritated or angry. If a handler notices this action while touching a horse, stay away from a horse slowly.

(2) Restraint

- When tying a horse to a post, make sure a rope is not too long. Tie it in a way that will not come loose when the horse pull it, but can be quickly released by humnas. When a horse is about to get entangled in the rope, because of being out of control, quickly release it from the human side.
- Use a Japanese halter on the stick called "*hananeji*" or a tranquilizer as needed. People around a handler should cooperate to make a horse calm.
- Restrain a horse with a lead rope or a halter when in the stall or outside. When a horse still moves, another person should grab the shoulder or raise the opposite limb to be photographed to make a horse still.
- When restraining at a post, a horse should be tied with a rope that holds the both sides of a halter. Take off one side of a rope from a patient as needed.
- When restraining to a stall, restrain a horse with the braid straps. If a patient falls in the stall or get tangled in a rope, cut the straps off quickly.

Swine Clinic

(1) Management

- Pigs are highly agile and may come towards humans in a state of extreme inflammation, so use care with them to avoid to get attacked.
- Pigs are usually calm, not aggressive, unless inadvertently standing in front them, or chasing them around to make them excited. Those actions can provoke them to an attack.
- Pigs use their head area (especially a nose/teeth) when they attack humans. The head may be violently thrust upward from below, or they may bite. Make sure to be careful, especially with hogs with untreated canine teeth. Also be aware that male pigs at mating time and sows with children can be rough.
- Pigs are very sensitive to sound, so always make your presence known, and do not scare them by quick movements or loud noises.
- In general, pigs will stand up when approached by humans, but when getting a sleeping pig to stand up, tap a back of pigs or raise its tail. Do not kick the buttocks or hind legs unnecessarily.

(2) Restraint

- The portable piglets should be strained on the knees or on a stable table.
- Grown pigs, which are not portable, should be restrained with a restrainer or a rope around the snout.
- If the pigs are kept in a large space, use a board (e.g. a thin wooden board) to herd them to a narrow space to be able to restrain them easily.
- Pigs can be violent right after released from restraint. When releasing the pigs, check your surroundings and be cautious not to injure anybody.

3. Companion Animal Practical Training

- Understanding the characteristics of each animal and acquiring the skills of safe restraining/handling are the first priority in the practical training of the companion animals.
- Wear clean clothes and avoid wearing a perfume or jewelries.

- Dogs are naturally very obedient towards humans, but depending on the types of dogs, the environment where dogs were raised, and their condition at the time they can become aggressive. Do not treat the dogs used for the experiments and practical trainings as regular companion animals we usually interact with, because of the risks of unexpected serious accidents. Observe the dogs carefully beforehand and always be cautious when handling them.
- Cats are very quick in action and can get into into narrow spaces, so keep an eye on them not to escape during practical training.

Canine Clinic

(1) Management

- When introducing canines, examine each dog clinically, and isolate the ones that is suspected with parasites or infections for a fixed period of time to give a treatment before being housed. Give an appropriate vaccination for prevention as needed.
- Feed according to their weight, age, and conditions.
- The patients should always have an access to fresh water. It is dangerous to touch the dogs while eating or suddenly take away their food dish. There may be a risk of an attack.
- Clean kennels/dog rooms twice a day to keep them clean.
- Observe the dogs' general condition and their bowel movements during feeding, cleaning, and excising. Communicate with dogs as much as possible to know their mental and physical conditions.
- Report to an instructor as soon as a handler notices something strange or abnormal.

(2) Restrain

- Restrain not to give any pains to animals, but in a way that a handler can accomplish all the tasks safely and efficiently.
- Check the condition of collars and leashes, and make sure no doors or windows are open so that the patients cannot escape.
- Make sure to relax dogs. Do not excite or fear them (Aggressive or fearful dogs are usually restless, bristle or bare the teeth).
- When restraining, quietly approach the dog, keep talking to relax the dog's wariness, and gently pet the whole body. Once the dog's wariness is decreased, gently hold the body in the arms and lightly restrain the head.
- Use a muzzle or an Elizabethan collar for any activities that accompany pains.
- Follow the directions of the instructor when restraining an aggressive dog, and use a tranquilizer or an anesthetic.

Feline Clinic

(1) Management

- When introducing cats, examine each cat clinically, and isolate the ones that are suspected with parasites or infections for a fixed period of time to give treatment before being housed. Give an appropriate vaccination for prevention as needed.
- Feed properly considering their weight, age, and condition.

- The patients should always have an access to fresh water.
- Clean the cat room twice a day to keep it clean.
- Observe the cats' general condition and their bowel movements while feeding, cleaning, and excising. Communicate with cats as much as possible to know their mental and physical conditions.
- Report to the instructor as soon as a handler notices something strange or abnormal.

(2) Restraint

- When handling cats, make sure to conduct in a small room with all doors and windows closed to prevent them from escaping.
- When transferring a cat, put it in a cage or pet carrier.
- When taking a cat out of its cage, open the top door and pick a cat up from above, since it is relatively easy way to take it out of the cage.
- Make sure to be careful not to be scratched or bitten when trying to pull a cat out of a cage from a side. Wear sturdy leather gloves as needed.
- When restraining an aggressive cat, wrap the body in a bath towel or put it in a mesh laundry bag. Conduct a treatment taking out one forelimb or hind limb each under this position.
- When restraining the head, hold the zygomatic arches on both sides with your palms over the head.
- Follow the directions of an instructor when restraining the aggressive cat which may get stressed by restraint, and use a tranquilizer or an anesthetic.

Additional Notes about Equine

- ✓ When approaching a horse:
  ① Call a patient's name to get her/his attention to a handler; ② Once a horse looks at a handler, his/her ears are faced to the handler and calm, slowly approach from the front left side of the horse. Talk to them with a low voice as approaches; ③ Once a handler is close enough, check the horse again, and if a patient is calm, pet or pat the neck. If possible, feed grass or a carrot before touching a horse to reassure him/her.
- ✓ If a handler is afraid of a horse, the patient can feel that fear that can make a patient nervous. Approach or touch a patient with confidence.
- ✓ When a handler walks a horse with a lead rope, put one rope under the jaw, or put two ropes on the both side of the face. Stand on the left side and lead a horse. (stand ahead of the shoulder). If a handler walks behind the horse shoulders, the horse may think that he/she is the leader pulling the others. When pulling a horse, walk straight ahead without looking at the horse. Slightly slacken the rope and control it with the right hand. Fold the rest of the lead rope several times in the left hand, but not wrap it around the hand. Horse might suddenly start running and drag you.
- ✓ Horses need moderate exercise. If possible, range the horses for few hours a day. Make sure there are enough water and grass on the rangeland.
- ✓ Daily management of horses are ①hoof picking, ②brushing, ③shoeing and hoof trimming.

① Hoof picking is the most important care that needs to be done every day. The back of the hooves easily get pebbles/feces which might lead to injuries or infections without daily cares. When picking out a hoof, do it with one limb at a time, but do not stand in front or behind the legs to avoid being kicked. Approach and touch the horse gently from the shoulder or lumbar area to the legs for hoof picking. Grab the fetlock or ball joint and lift the leg up. When the back of the hoof is visible, remove dirt and pebbles with a hoof pick/iron nail.

② Brushing has an effect on removing fallen hairs/dandruff and improving a blood circulation. First, touch the body with hands, then brush if the horse does not refuse. (Use several types of brushes, such as made of plastic or animal hair, depending on its use.) Start with the neck and back. Many horses do not like to be brushed on the stomach area (they will bend their necks to bite a handler), so pay special attention to this area.

③ Shoeing and hoof trimming are usually done by a specialized farrier or the equine management staff. The hooves grow about 1cm per month, so trim them to a proper length and shape and shoe them.

Ⅸ. SAFETY MEASURES FOR WILD AND IMPORTED ANIMALS

1. Oversea Malignant Infectious Diseases

This term refers to major livestock infectious diseases that pose an important threat to livestock, but do not endemic to Japan. These infectious diseases are watched over by international organizations such as OIE, WHO and FAO to promote international quarantine of those diseases. In recent years, importing of livestock and animal products has been growing in number and speed. That means the risks of unknown diseases coming into our country is getting higher. Rabies and glanders are two of the important zoonoses in terms of public health.

[Notes] Notification System for the Importation of Animals

(1) Laws and Regulations

Act on Prevention of Infectious Diseases and Medical Care for Patients with Infectious Diseases (Article 56. 2)

Ordinance for Enforcement of the Act on Prevention of Infection Diseases and Medical Care for Patients with Infectious Diseases (Article 28~31)

(2) Abstract

Since September 1, 2005, when importing “live rodents, rabbits and other terrestrial mammals,” “live birds” or “carcasses of rodents and rabbits” to Japan (see notes below), it is required to take following procedures (Applicable not only to sale and display, but also to all personal pets).

1) Submit a request that records the types and the numbers of the animals to the Quarantine Station of Ministry of Health, Labor and Welfare. Attach the health certificate issued by the government of the exporting country attesting to the safety of those animals with respect to infectious diseases.
2) The Quarantine Station shall inspect and confirm the submitted request form, the health certificate, and other identification certificates of the importer. If the submitted applications have no deficiencies, the Quarantine Station shall issue the certificate of acceptance to the importer.

3) The animals will be checked for compliance with other laws and regulations. Only after inspected by the Custom, the animals will be allowed to bring in to Japan.

Notes: If the animals are already quarantined, or prohibited from importation, those are not subject to this system.

(3) Animals Already Subject to Quarantine (Quarantine at the Quarantine Station of The Ministry of Agriculture, Forestry and Fisheries)

1) Animals subject to quarantine under *the Act on Domestic Animal Infectious Diseases Control*: Artiodactyl (cow, sheep, goat, giraffe, etc.), Perissodactyl equid (horse, zebra, etc.), rabbits, poultries (chicken, ostrich, duck, etc.).
2) Animals subject to quarantine under *the Rabies Prevention Law*: dogs, cats, raccoons, foxes, skunks
3) Animals subject to quarantine under *the Infectious Disease Control Law*: some non-human primates

(4) Animals that are prohibited from Importing (Article 54 of *Infectious Disease Control Law* No.54): ferret badgers, bats, raccoon dogs, masked palm civets, prairie dogs, mastomys, monkeys

(5) Other related laws and regulations

1) *Act on the Prevention of Adverse Ecological Impacts Caused by Designated Invasive Alien Species (Invasive Alien Species Act)* (Last revised: 2005/4/27 Act No. 33)
2) Cabinet Order for Partial Revision of the Enforcement Order of *the Invasive Alien Species Act* (2005/4/27 Cabinet Order No.169).
3) Cabinet Order for Partial Revision of the Enforcement Order of *the Invasive Alien Species Act* (Ministry of Agriculture、Forestry and Fisheries and Ministry of the Environment 2010/2/1)

Reference:
Ministry of Health, Labor and Welfare: Notification System for the Importation of Animals
http://www.mhlw.go.jp/bunya/kenkou/kekkaku-kansenshou12/index.html

2. Wild Rodents

(1) Main Hosts, Zoonotic Pathogens, Symptoms in Animals and Humans

Animals/Carriers	Zoonotic Pathogens	Symptoms
Mastomys (*Praomys natalensis*) It is a genus of rodent that habitats in the African continent. It is used for researches of protozoa diseases and tumors.	Lassa fever virus Classifications: Arenaviridae, Lassa virus (RNA)	This disease first infected humans in Lassa, Nigeria in 1969. Transmission to humans are through food consumption that contaminated by mastomys' urines. After 3~26 days of incubation period, the fever exceeds to 40℃ and lead to a hemorrhagic fever. The mortality rate is 40~70%. Lassa fever virus was carried to US though the exported patients and thought to be dangerous virus.
Striped field mouse (*Apodemus agratius coreae*) Wild striped field mouse that habitats in the Korean Peninsula.	HFRS viruses Classification: Bunyaviridae, Hantavirus (RNA)	It is called hemorrhagic fever with renal syndrome (Korean hemorrhagic fever) among humans. It spread after the Korean War in 1951 in former Manchuria to Korea and China. It transmits from exhaling of aerosols at grassland and fields that are contaminated by striped fields mouse's feces. After 2~4 weeks of incubation period,

		the infected individual would have shivering, high fever, sudden hemorrhagic diathesis and albuminuria.
Wild mouse (*Mus musculus*) Other wild rodents	lymphocytic choriomeningitis virus (LCMV) Classification: Arenaviridae, LCM virus (RNA)	It spreads among wild rodents. For infections to animals, it usually shows no symptoms and the viruses come out with feces. For humans, there are some cases of no symptoms, but usually cause fever, no appetite, fatigue, headache, muscle-ache, and chest pain. There are cases for swelling of the cervical node, vomiting, neck stiffness, Decrease in muscle reflection, and these can lead to meningitis.
Brown rats (*Rattus norvegicus*) Ancestors of laboratory rats	HFRS virus and rickettsia typhi	Brown rats have a high adaptability, habits in sewage and can swim, but they are not good at vertical movements and crossing electronic wires. They come through drain holes, gaps between buildings, and can dig holes.
Black rats (*Rattus rattus*) The body is smaller than brown rats, but ears are bigger.	plague bacillus, salmonella, leptospira, rickettsia, Angiostrongylus cantonensis, Capillaria hepatica	Black rats usually nest in higher places such as attic. They are good at climbing and crossing electronic wires. They enter from gaps and holes, and especially near roofs.
Voles (*Microtus montebelli*)	rickettsia typhi, leptospira	Voles live in fields and forests, and enter from gaps to buildings.

(2) Safety Measures Against Wild Rodents

Wild rodents might carry various pathogens that are not listed in the preceding table. Therefore, careful handling is required when using wild rodents as laboratory animals. Any gaps and holes in the facility should be filled or rodent repellers should be installed at entrances/exits in order to avoid any wild mice from entering the animal facility. Inside the facility should be always organized, cleaned, and dried without food items for mice so that they cannot live there.

3. Wild Non-Human Primates

(1) Main Hosts, Zoonotic Pathogens Symptoms in Animals and Humans

Animals/ Carriers	Zoonotic Pathogens	Symptoms
Green monkey (*Cercopithecus aethiops*)	Marburg virus Classification: Filoviridae filovirus (RNA) This virus carries one of the strongest pathogens. Natural host is thought to be wild rodents in Africa, but not apparent.	In 1967, green monkeys were imported from Uganda to produce vaccines for polio. There was an outbreak of hemorrhagic disease in Marburg and Frankfurt, Germany and Belgrade, Serbia at the same time, and led some patients to death. This disease is called Marburg virus. There are no specific symptoms among non-primate humans, but deadly. For humans, symptoms are high fever, headache, vomiting, diarrhea, and lead to death from subcutaneous and gastrointestinal blooding.
Chimpanzee (*Pan troglodytes*)	Hepatitis B virus	There was an outbreak of hepatitis that thought to be infected from chimpanzees in US air force base. After that, the outbreak spread to

	Classification: Hepadnaviridae orthohepadnavirus (DNA) Hepatitis A virus Classfication: Picornaviridae hepatovirus (RNA)	zoos, livestock clinics, primates'C research centers, and thought to be infected through chimpanzees and other non-human primates.
Crab eating monkey (*Macaca fascicularis*) Rhesus monkey (*Macaca mulatta*)	B virus Classification: Herpesviridae Varicellovirus (DNA), shigella (*shigella spp*.)	For non-human primates, there are blisters on lips, tongue and the oral mucous membrane, and forms ulcers and scabs, but there are no general symptoms. When humans are infected, it causes encephalomyelitis and have a high mortality rate. When suspected, it is better to euthanize the animal even before the inspection. Macaca monkeys tend to carry shigella. However, the case of infection at totally wild condition is rare. Generally the animal got infected after capture and transmitted from humans.

(2) Safety Measures Against Wild Non-Human Primates

Wild non-human primates might carry various pathogens that are not listed in the preceding table. Therefore, careful handling is required when using wild non-human primates as laboratory animals. The WHO suggests to have 9 weeks of quarantine period. Therefore, conduct various pathogens and antibodies detection during the quarantine and make sure of the safety before starting experiments.

4. Imported Companion Animals

Imported companion animals might carry zoonotic pathogens and become an infectious source for humans. It is important to pay close attention in clinic and autopsy. The following needs extra attention.

(1) Psittacosis; Avian chlamydiosis

This infectious disease is caused by *Chlamydia psittaci*. Hosts extends to a wide range such as: birds like parrots, parakeets, fowls and wild birds; mammals like human, caws, cats, sheep; and even amphibians like frogs. They are mostly latent infection, but when stress is added, the disease might develop. Especially, parrots and parakeets can be the infectious source of psittacosis. Humans can get infected by inhalation or orally from carrier birds, causing pneumonia and bronchitis, which can be fatal. There were cases of infection among veterinary students in the United States and Europe. Tetracycline, macrolides, new quinolone are effective.

(2) Tuberculosis

This infectious disease is caused by *Mycobacterium tuberculosis* and *M. bovis.* There are cases of infection in pet monkeys and deer at zoos. The disease is very severe, lesions can spread to other organs other than lungs, and become fatal. Tropical fish infected with *M marinum* can infect humans causing ulcers on the skin.

(3) Bacillary dysentery

There was a case in the 1970s in which imported pet monkeys from Southeast Asia got infected, transmitted to the family, and caused death.

(4) Salmonellosis

Reptiles such as lizards, snakes, and turtles are often carry salmonella, and become the infectious source to humans.

X. SAFETY MEASURES FOR MEDICINAL AND POISONOUS SUBSTANCES

1. Anesthetics

(1) Inhalational Anesthetics

Inhalation anesthetics are broadly categorized into gas anesthetics and volatile anesthetics. The former have lower critical temperature and boiling point, so they are gaseous at room temperature. The latter have higher critical temperature and their boiling point is closer to room temperature, so they are in liquid form at room temperature. Currently, the main gas anesthetic used in veterinary medicine is a nitrous oxide. For volatile anesthetics, halothane, isoflurane and sevoflurane are used. When using volatile anesthetics, an anesthetic is discharged with the animal's breath into the room. That means all the people in the room inhale it indirectly, whether anesthesia equipment has a semi-open anesthesia circuit or a semi-closed one. The concentration in the room air is extremely low, so it does not affect the human health, but it is desirable not to inhale as much as possible. Caution has to be taken since there are cases of liver injuries and miscarriages among workers in surgeries who are exposed to halothane, in particular, repeatedly. Therefore, the device that releases the exhaled air and extra gas to outside via a pipe needs to be installed to prevent from inhaling exhaust gas.

Ether is volatile, clear, irritating, and strong liquid. Its boiling point is 35℃ and it is 2.5 times heavier than air. It is highly flammable, and mixing with air or oxygen is dangerous and can cause an explosion. Therefore, do not use it as an anesthetic.

Volatile anesthetics have to be stored in a cool and dark place under the strict management.

(2) Intravenous Anesthetics

Following anesthetics are the types of intravenous anesthetics that are used in veterinary clinical practice, and physiological and pharmacological experiments.

1) Pentobarbital

It was used for dogs, cats, and small laboratoey animals as a short term injectable anesthetic. Although there was Somnopentyl available by Kyoritsu Seiyaku before, it currently suspended to sell as a medication. It should be stored at room temperature. Respiratory depression and hypotension are likely to occur when the rate of administration is too rapid or deep anesthesia is attempted. So never use pentobarbital on its own.

2) Thiopental

It is used for dogs and cats as a very short term injectable anesthetic. There is Ravonal available by Tanabe Seiyaku. It should be stored at room temperature, but since it cannot be dissolved to store, the powder and solution liquids should be sealed and stored in separate ampules. Precautions should be in accordance with the section on pentobarbital above).

3) Ketamine

It is used for dogs, monkeys, and cats as a short term injectable anesthetic. There are Ketamine infusions available by Fujita Seiyaku. It should be stored at room temperature. It is to be noted that it

produces little muscle relaxation, so that it is not suitable for laparotomy. Respiratory and circulation depressions are hardly observed, and the safety margin is wide.

Ministry of Health, Labor and Welfare has designated Ketamine to be the subject substance under the "*Narcotics and Psychotropics Control Law* (2006/6/14 Law No.69)" since January, 2007. Therefore, the handler has to be certified in the narcotics practitioner license, narcotic administrator license, and narcotic researcher license by the local authorities.

4) Urethane

It is used for laboratory animals, such as rats, as a long-term anesthetic, but due to its carcinogenicity, other options should be considered. A single dose can produce deep anesthesia state with excellentt muscle relaxation and little bronchial secretion. It should be stored at room temperature.

5) Others

Muscle relaxants and sedatives are used as adjunctive to the anesthetics. The common muscle relaxants are tubocurarine, pancuronium, suxamethonium, and decamethonium. It is necessary to wear a respirator when using the muscle relaxants. The most common sedatives are medetomidines. These drugs are extremely dangerous if misused or abused, so they must be stored separately from other drugs under adequate control to prevent thefts and loss. Specially, drugs designated as psychotropics by the above laws (pentobarbital, midazolam, etc.), along with drugs designated as narcotics, should be managed and stored in compliance with "the Regulations Relating to Handling of Narcotic and Psychotropic for Academic Research at Kagoshima University".

2. Other Poisonous and Deleterious Substances

According to the "*Poisonous and Deleterious Substances Control Law*," the distribution of these substances is controlled: substances with particularly high toxicity are designated as 'poisonous substances'; substances with toxicity equivalent to that of poisonous substances are designated as 'deleterious substances'. In medical drugs, the designation of poisonous and deleterious substances is made by the "*Pharmaceutical Affairs Law*". Some drugs used in the treatment, training, and experimentation of animals are designated as poisonous or deleterious drugs. These general criteria are as follows. The Lethal Dose, 50% (LD_{50}) of poisonous and deleterious substances: 30mg/kg or less and 300mg/kg or less respectively for oral administrations; 20mg/kg or less and 200mg/kg or less respectively for subcutaneous administrations; 10mg/kg and 100mg/kg or less respectively for intravenous administrations. Poisonous substances are labeled "毒 (Poisonous)" in white ink on a black background with a white frame, and deleterious substances are labeled "劇 (Deleterious)" in red ink on a white background with a red frame. These drugs are extremely dangerous if misused or abused, so they must be stored separately from other drugs under adequate control to prevent thefts and loss.

XI. DEVICE, EQUIPMENT AND SAFETY MEASURES

1. Microtome

A microtome is a device to cut paraffin-embedded tissue into extremely thin slices accurately for a microscopic observation. There are various types of microtomes and they are all precision machines. For

precision slices and safety, it is important to maintain the devices properly and operate them correctly. The beginners should always operate under the guidance of experienced people.

Most of accidents with microtome for optical microscopy happen when mounting/dismounting the microtome blade and blocks. The microtome blade is heavier for its size and sharp enough to cause severe injuries even with slight force. For a sliding microtome, place the knife holder on the corner of sliding base. For a rotary microtome, lock the flywheel before mounting/dismounting the knife and blocks.

Inside the cryostat used for making a frozen section is usually kept at low temperature (around -30℃), so it is likely to get frosted. The frost must be removed periodically so that the microtome can rotate smoothly. Do not touch the device (especially the metal parts) with wet hands, as it might stick and get frostbite.

The most important thing to pay attention when preparing sections for electron microscopy is the handling of glasses for making knives. The handler can cut his/her hand when moving a sheet glass, or the glass can break in an unexpected direction resulting in injuries with the sharp glass edge, so the handler should acquire the correct way to break glasses. Moreover, after making knives, there are small pieces of glass on the tables, chairs, and floors scattered, so that the cleaning has to be done thoroughly.

2. Dissecting Tools

Careful attention should be paid for handling dissecting tools since the most of tools are sharp knives. Tools should be sufficiently sharpened so that the extra force is not required during use. While pulling the blade, do promptly and do not point the sharp edge to the others. If injured, wash the cut with the running water and quickly disinfect to prevent any bacterial infections. See a doctor as needed.

(1) Scalpel

Medium-sized straight blades. It is used for dissecting muscles, tendons, internal organs, and evisceration and dismemberment.

(2) Skinning Knives

Medium-sized flange steel blades. It is mainly used for skinning, dissecting muscles, tendons, and the limbs.

(3) Surgical Knives/Scalpel

It is used for removing and incision of tissues. There is a rare case of using end of handle for peeling the connective tissues. Be careful to attach and detach the blade.

(4) Scissors

These are used for dissection of intestinal tract, blood vessels, nerves, muscles and tendons. There are dull and sharp-pointed edge ones, so use properly according to the purpose.

(5) Saw

It is used for dissection of bone tissues such as calvarium, pelvis, and long bones.

(6) Bone Scissors

It is used for isolation of the skull and vertebrae, and dissection of bone tissues and cartilage tissues for removal of brain or spinal cord.

(7) Tweezers

There are various types of tweezers, so use properly according to the task. It is used for detailed work such as removal of unnecessary connective tissues, blood vessels, and nerves.

(8) Forceps

It is used to pinch blood vessels and digestive track, and separation of connective tissues.

(9) Round Bar Files

A bar of files. It is used for polishing the surgical and skinning knives.

3. Gas Cylinder

(1) Precaution for High-Pressure Gas Cylinders

When using a high pressure gas cylinder as a source of gas supply, follow the directions from the suppliers of the gas cylinders and make sure not to cause any accidents. In order to safely handle high pressure gas, the handler has to follow the relevant laws and regulations for its use and storage of cylinders. When taking out the gas, the pressure has to be lowered closer to the atmospheric pressure, so it is important to carefully handle the valves attached to the cylinders.

The predicted accidents that could happen in a laboratory are: ① leakage, blowout, rupture due to high pressure; ② gas explosion or fire due to flammability; ③ suffocation from gas-poisoning and oxygen deficiency; ④ injuries due to the weight of cylinder.

Gases used in a laboratory can be categorize into compressed gases, liquefied gases, and dissolved gases according to their physical sate.

1) Compressed Gases (with a pressure of 10kg/㎠ or more at 35℃): oxygen, hydrogen, nitrogen, argon, methane, helium, carbon monoxide
2) Liquefied Gases (with a pressure of 2kg/㎠ or more at 35℃): carbon dioxide, propane, methyl chloride, methyl bromide, ammonia, chlorine, 1－pten, 1, 3－butadiene, dimethyl ether, ethylene oxide, hydrogen sulfide
3) Dissolved Gases: acetylene

(2) Classification of High-Pressure Gas by Risks

1) Flammable Gases (with a lower explosive limit of 10% or less when mixed with air, and with the difference of 20% or more between maximum and minimum limits): hydrogen, acetylene, ammonia, ethylene oxide, hydrogen sulfide, amines
2) Poisonous Gases (with an acceptable concentration of 200ppm or less): carbon monoxide, chlorine, hydrogen chloride, hydrogen sulfide, phosgene, ammonia, ethylene oxide, methyl amines, hydrogen cyanide
3) Combustion Supporting Gases: oxygen, air, chloride, nitrogen oxide (NO, N_2O_3、N_2O_5、N_2O_6) etc.
4) Corrosive Gases: chloride, hydrogen fluoride, hydrogen chloride, hydrogen bromide, hydrogen sulfide, phosgene, ammonia, ethylamine, methylamine
5) Inert Gases: nitrogen, carbon dioxide, argon, helium

(3) Precaution for Preventing Hazards

1) When placing the cylinder vertically, fix the cylinder to a wall or other suitable object with a chain or a rope not to fall.
2) Do not use and store the cylinder in the direct sunlight or at high temperature room. Keep the temperature of cylinder below 40℃, but not too low.
3) Do not place combustible gases such as oxygen and chlorine with flammable gases.
4) When moving the cylinder by hand, clean the area, and make sure that the cylinder body does not touch the ground. Whenever possible, use the cylinder carrier, but when it is not available, roll with the edge of the bottom and do not carry on your shoulder or kick. Do not drag it with the high pressure valve (Figure 33).

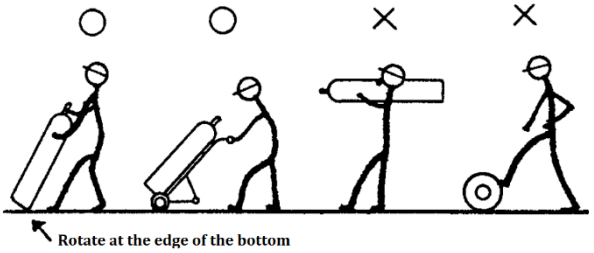


Figure 33. Good and Bad Examples of Carrying the Container

5) There is a high pressure valve on the head of the cylinder with a left-hand thread for all flammable gases and helium, and a right-hand thread for all regular high pressure gases and poisonous gases. All of them have screw threads on the outside of the filling port (gas outlet or intake port). On the other hand, liquefied petroleum gas cylinders have screwed left-hand threads, which is cut on the inside of the filling port, so-called "female thread."
6) Attach a regulating valve or a depressurizing valve to the gas outlet of the high-pressure valve when taking out gas. Each valve should be opened and closed gently.
7) Be cautious for ventilation and leakage to avoid oxygen deprivation. Especially, be careful when using non-irritating, colorless or smell-less substances (such as nitrogen, argon, methane).
8) Leakage testing should be done with soap water. Put an ammonia water close to hydrogen chloride and sulfurous acid, and concentrated hydrochloric acid to ammonia and amine gases. Check for the presence or absence of white smoke.
9) When handling poisonous gases, be sure to use the ventilation device such as draft and prepare a gas mask that are appropriate for the type.
10) Always insert the backflow preventer between a reactor vessel and a pressor regulator.
11) Leave a residual pressure of at least 1kg/㎠ for compressed gas and about 0.5kg for liquefied gas in the used cylinder.

(4) Cylinder Labeling

The color of the cylinder and the labeling of the filling gas are as follows.

Oxygen gas…… Black
Hydrogen gas…… Red
Liquefied carbon dioxide…… Green

Liquefied ammonia…… White
Liquefied chloride…… Yellow
Acetylene gas…… Brown
Other gas…… Gray

For fillings, flammable gases are labeled in red letters (hydrogen gas and acetylene gas are in white), other gases are labeled in white. Depending on the types of gas, poisonous gases are labeled as "毒 (Poisonous)" in black, flammable gases are labeled as "燃 (Burn)" in red (white letters for hydrogen and acetylene gas) (Figure 34, 35).

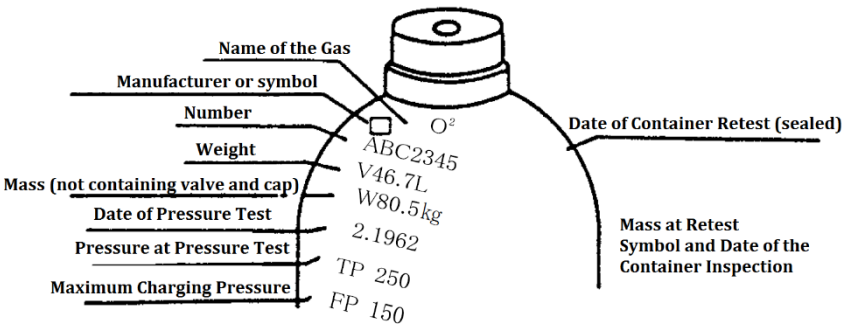


Figure 34. Cylinder Display

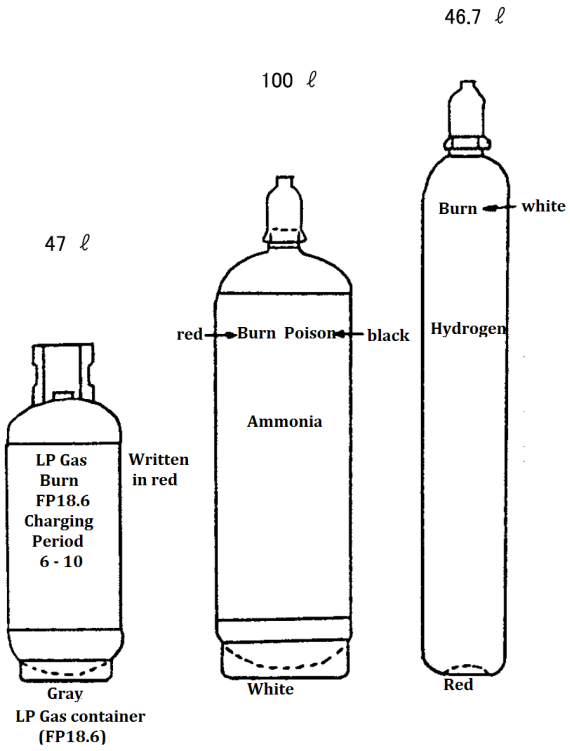


Figure 35. Explanations of Label

4. Autoclave

The autoclave, here, refer to a moist heat sterilizer (high-pressure steam sterilizer), in which the pressure is increased by about 1 atmospheric pressure, the high temperature of 115~121°C can achieved to sterilize the subject in about 15 minutes, except the special pathogens (such as prions of scrapie pathogens). Unlike the dry-heat sterilizer, anything that can fit into the device other than explosives can be sterilized. Generally, a small, vertical type is used for laboratories. This type sterilizer is filled with water, heated up by gas or electric heat source, and saturated with generated steam to sterilize an object with the steam. Large, horizontal autoclaves are used in facilities such as hospitals, laboratory animal facilities, and biohazard facilities that deal with dangerous pathogens, or biologic manufacturers that sterilize a big amount at once or sterilize big devices (such as beddings and cages), research devices such as a centrifugal rotor, and carcasses and organs of microorganism infected small animals. The steam is generated by a boiler and fills the chamber of sterilizer. These devices have been improved so that sterilization can be done safely automatically with some precautions. There are devices equipped with a dryer system, since the material is wet after sterilization.

However, since the equipment uses high pressure steam, there is a great risk of burns due to careless or inexperienced operation. So the following rules must be strictly adhered.

(1) Be sure to earth the electric autoclaves.
(2) Pour water (if possible purified water) to the specified level.
(3) Check if the exhaust valve and drain valve are closed. Even if the device is automatic, make sure to follow (2) and (3) to avoid accidental heating without water.

(4) Airtight containers involve a risk of rupture, so loosen the lid before sterilization, and then tighten it after sterilization.
(5) After sterilization, do not depressurize using the exhaust valve unless needed.
(6) Make sure that the lid is no longer high pressured before opening it. Always adjust the pressure meter and the thermometer to be accurate. Common accidents tend to happen at this moment. Hurrying to open the lid might burn arms or face (Autoclave is not designed to be opened under high pressure, but it can be opened under some high pressure).
(7) Drain after use and clean the inside. Rusting and corrosion of the lid hardly happen except in special cases, but it is definitely dangerous without meticulous check.
(8) It is desirable to monitor regularly if the sterilization is conducted sufficiently.

5. Dry Heat Sterilizer

A dry heat sterilizer has an electric or gas heating element at the bottom of the box, and the heat from the heating element is trapped inside the insulated box to sterilize the object placed inside. Generally, sterilization will be completed by maintaining the temperature at 180℃ for about one hour. This device is not dangerous when used correctly, but if misconducted, the sterilization could be incomplete and cause burns and fires.

(1) Do not pack a lot of items in the sterilizer. Adequate space for hot air circulation is needed. On the other hand, if the amount of items is too few, the surrounding temperature rises up too quickly For the temperature of the object inside to keep up, so avoid rapid heating.
(2) Once the heating starts, remove the inside cool air through the top air hole. Once the desired temperature is reached, close the hole and maintain the temperature.
(3) Do not put in anything burnable like paper. Use aluminum foil for alternatives.
(4) After sterilization, when the temperature is high (more than 100℃), opening the door to force cooling, or forcibly taking the inside object might break thick glass products. It is best to wait till the temperature naturally drops to room temperature before removing items. Accidents likely to happen when in hurry.
(5) Rapid rise and fall of temperature and over-packing of items can cause incomplete sterilization and accidents. Use a temperature monitor tape to check if the desired temperature has been reached. Placing tapes in multiple places in the chamber is recommended.
(6) Since the electric type uses a lot of electricity, the electric capacity of the laboratory should be enough for some extra use.
(7) Do not put anything burnable near the device. Communicate with each other to avoid any unnecessary long heating and vice versa. It is important that the person who started the device has to stop it.

6. Deep Freezer

There are various temperature setting freezers. Generally, freezer with temperature range of -20℃ ~ -30℃ are used to store serum. Freezers with temperature range of around -40℃ are economically suitable for storing frozen foods such as fish and meat. On the other hand, to safely store the infectious microorganisms for a few years, freezers with even lower temperature, so-called deep freezers (ultra-low temperature chambers), are essential, usually required at -70℃ ~ -90℃. Deep freezers are able to store

living cells for short period of time. There are upright types and “coffin” types. The former have the disadvantage of rising temperature when opening and closing the door, while the latter have a hard time finding the reserve.

It is ok to put serums in the deep freezer, but since the price of the device is high for its space, it is not desirable to put anything in the deep freezer. It is necessary to use properly for its purpose and value. Wearing thick gloves are essential to avoid frost bites. Here are some precautions:

(1) Do not turn on the power right after moving the freezer. Wait for 30 minutes to turn it on. Turning on and off frequently might cause a failure of the machine.
(2) Deep freezers release a lot of heat outside, so it is necessary to control the temperature in the room. It is not desirable to put in a small room, nor in direct sunlight.
(3) When putting a thing in and out, do it quickly. It is better to divide the inside of the freezer and make a “storage map.”
(4) It is better to divide the samples in smaller amounts to store. Glass containers might easily break, so it is desirable to use plastic containers. Close/seal the container tightly and use a tape to close firmly for rubber stoppers because they are easy to come off. Metal containers are not recommended.
(5) Even if the electricity is cut out for a few hours, the temperature of the inside will not rise much, so leave the door closed. If the power outrage continues, connect it to an emergency generator or put some dry ice inside upper area of the chamber to prevent the temperature from rising. If the sample is not well airtight, it might become acidic due to dry ice.
(6) Due to (2), some deep freezers might have placed in a hallway, it is important to secure an evacuation route for safety measures. It is also desirable to lock the deep freezer.
(7) It is easy to get frosted during a high temperature season, so regularly remove the frost. Otherwise, the door will not close.

7. Electron Microscope

The electron microscope is a device to observe ultrastructures of tissues and cells. There are two types of electron microscopes: the scanning electron microscope (SEM) for observing surface structures and cross-sectional surfaces; and the transmission electron microscope (TEM) for observing internal structures. They are both precision devices and have the parts that generate high voltage and high heat, so the handler needs to properly operate the device at all times to avoid any accidents. Especially, beginners have to always operate them under the guidance of experienced personnel or supervisor.

When replacing a filament, make sure to cool it down before replacing it, since the electron gun might be very hot right after using it for a long time. The vacuum oil for a pump needs to be changed to avoid a deterioration of vacuum level. Its waste oil has to be processed according to the regulations. Furthermore, cooling water is circulating in the device, so precautions should be paid for an electric leakage or corrosion of the parts due to water leakage.

8. X-Ray Generator

(1) Health Management and Operation Control

Radiations have brought various benefits to the mankind, but at the same time, they gave physical disabilities to handlers, reaffirming the risks of radiations. Since people cannot directly feel the radiation with the five senses, if the handlers are not sufficiently aware of and do not take measures to prevent the risks, it will result in endangering not only the handlers themselves but also other workers. Proper prevention, diagnosis/consultant, and treatment of radiation damages are not well established in the present state, so health management is crucial for those who have the opportunity to be exposed to radiations, such as health checkups.

When dealing with X-rays, it is very rare for the handler to be completely isolated from the radiation and to have zero exposed dose. Therefore, it is necessary to minimize the expose dose as much as possible.

Principles of operation control are as follows:

1) Keep a safe distance from the radiation source as much as possible (X-ray dosage will attenuate in inverse proportion to the square of the distance).
2) Shorten the time of exposure as much as possible.
3) Place an appropriate shield between the radiation source and the handler.

Combine these three ways to reduce the exposed dose close to zero.

(2) Damages to Human Bodies Due to Mishandlings of X-Rays

Acute Effects (When exposed to a large amount of radiation at once)

Loss of hair, erythema, inflammation, ulceration, decrease in white blood cells, temporary or permanent decline of fertility.

Tardive Effects (effects appear after exposure to a small amount of radiation at once for a long period of time and after the acute effects have disappeared or after a considerable period of time has passed even if no acute effects have appeared, with a latency period ranging from several months to several decades)

Cancer, shortening of life span, cataract, leukemia

Effects of uterine embryo or fetus exposures – malformations after birth, weak constitution, and carcinogenesis in newborns

Therefore, when engaging in the X-rays work, it is very important to follow the regulations and do not enter the radiation management area unless needed. When it is necessary to enter the management area, wear protective clothes that cover the whole body as much as possible.

9. Incinerator

Incinerator for animals is installed at the Joint Faculty of Veterinary Medicine. Here are some precautions.

1) Any objects that are stored in the refrigerator or freezer before incineration should be put in a designated plastic bag, cardboard box, or plastic container to prevent contamination of the storage area.
2) Make sure that there are no injection needles, metals or testing tubes to be carelessly mixed in.

XII. EUTHANASIA OF ANIMALS AND DISPOSAL OF CARCASSES

"Euthanasia" is a humane action to kill animals using the method that induce quickly unconsciousness to death without pain or agony. Euthanasia is planned and implemented from the perspective of animal welfare as a necessary measure at the end of the experimental progress, or the way to relieve animals from their suffering that cannot alleviate by administration of analgesics or sedatives, or therapy.

1. Criteria for Euthanasia

Animals will be euthanized only in the following cases.

(1) When the experiment has been completed or the experimental goal or the unique end point in an experiment plan have been achieved. The end point should be set in advance based on the degree of physical and behavioral obstacles (weight loss, symptoms, tumor size, etc.) from a humane perspective as a part of experiment plan.

(2) When animals are deemed unsuitable for experimentation because they are past breeding longevity, or too old.

(3) When determined too sick or no way for treatment or recovery.

(4) When the colony has been contaminated with pathogens and the animals need to be culled.

2. Precautions for Carrying Out Euthanasia

(1) With proper restrain and handling, minimize the distress such as nervousness and fear.

(2) Put the animals to complete loss of awareness quickly, easily, and certainly.

(3) Do not contaminate the environment or harm the surroundings.

(4) Secure the safety of the handler. Ethel is easily flammable, and chloroform is poisonous to liver, kidney and heart, so it should not be used.

(5) After euthanasia, confirm that the animals are completely deceased due to cardiac arrest or rigor mortis.

(6) Carry out euthanasia when other animals are not around, and where other people other than staffs cannot see.

(7) In cases of repeated euthanasia or emotional attachment to the animals, euthanasia can be a mentally challenging task for the person involved as an executor or a breeding staff. The responsible person should take it seriously and take action.

3. Methods

Specially, without justifiable grounds scientifically or medically, the method of euthanasia is aligned with the method mentioned in the "AVMA Guidelines for the Euthanasia of Animals" (2020 or the latest edition). And also, the best method should be chosen depending on the situation. Euthanasia has to be carried out in the way that does not cause pains and lose consciousness. The methods of euthanasia can be roughly divided into:

(1) Overdose of anesthetics

(2) Usage of other chemical substances

(3) Physical methods

Table 10 shows detailed methods with example of small rodents.

4. Disposal of Animal Carcasses

(1) It is desirable to incinerate the animal carcasses in the end, but it has to be careful not to produce offensive odors or create a living condition that is not favorable for humans.

(2) After the experiment is completed, the animals may contain pathogenic microorganisms or harmful substances, so it is necessary to make sure to prevent them from being dispersed. When it is known in advance that harmful pathogenic microorganisms are contained in animals, sterilize first before storing and disposing.

(3) Animal carcasses should be sealed in a plastic bag, water proof paper, or a container to avoid any leakages such as blood, feces, urines, and other contaminants. During the storage and transportation of the carcasses, make sure that the contents (carcasses) cannot be seen from outside (Refer to XI).

Table 10. The Example of Methods of Euthanasia: Small Rodents

	Method	Notes
1. Acceptable method of euthanasia	A. Excess administration of barbiturate (pentobarbital)	More than 3 times administration of amount of the anesthesia is necessary. Pain may be caused by the administration of it.
	B. Excess administration of dissociable drug	Administration of a mixture of α 2 agonist such as Ketamine, etc. is necessary.
2. Acceptable method of euthanasia with condition	A. Excess administration of inhalational anesthetic	Be sure to maintain the concentration in the anesthesia chamber.
	B. CO2 inhalation	Use the co2 cylinder (Don't use dry ice). The substitution rate should be 30-70% per minute relative to the chamber or cage volume. Neonatal is not suitable due to the resistance to the hypoxic condition.
	C. Cervical spine dislocation	Only veteran can perform for mouse and rats (weight: 200g or less)
3. Unacceptable method of euthanasia	A. Nitrogen and argon inhalation alone	
	B. Direct injection to the heart by KCI alone	
	C. Neuromuscular blocking agents injection alone or combining injection barbiturates and neuromuscular blocking agents	Despite the combining injection, it causes fatality before anesthesia, so be sure to apply it after being under general anesthesia surely.
	D. Urethane injection alone	Urethane is a carcinogen.
	E. α Chloralose injection alone	
	F. Opioids injection alone	

Reference Books

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CHEMICALS

I . HANDLING CHEMICALS

Chemical experiments involve the use of dangerous drugs, tools, and devices that carelessness can cause an accident. To minimize the risk of causing accidents, it is important to prepare with correct knowledge to deal with disasters in a calm manner. This chapter describes chemical agents and their characteristics.

Chemicals could be explosive, flammable, ignitable, poisonous, and corrosive. These dangerous chemicals are regulated by laws and regulations such as *the Fire Services Act*, *the Explosives Control Act*, *the High Pressure Gas Safety Act*, and *the Poisonous and Deleterious Substances Control Law*. These laws and regulations mainly regulate the production, sale, storage, usage, and disposal of substances in industrial activities, and do not cover the chemical usage in education and research institutes. However, the storage and handling of chemicals are regulated by the related laws and regulations, so it is important to be familiar

with them. Table 11 shows the relationship between the main laws and regulations and dangerous agents (drugs).

Table 11. Classification and Related Laws and Regulations on Hazardrous and Harmful Substances

Classification		Characteristics	Related Laws and Regulations
DANGEROUS	Dangerous Substances	Any liquids or solids that might be inflammable, triger a ignition, cause a fire or explosion.	Fire Services Act, the Explosives Control Law, Industrial Safety and Health Law Enforcement Ordinance
	High Pressure Gas	Any gases that can be pressurized or liquefied and might cause a sudden volume expansion. Any gases that might cause, fire, explosion, intoxication or hypoxia.	High Pressure Gas Safety Law, Industrial Safety and Health Law Enforcement Ordinance
HARMFUL	Harmful Substances	Any substances that have a strong toxicity and cause an acute poisoning. Any substances that have a weak toxicity, but if taken for a long period of time, cause health problems. Any substances that are harmful to human health.	Poisonous and Deleterious Substances Control Law, Industrial Safety and Health Law Enforcement Ordinance
	Environmental Pollutant	Any substances that affect human health and the ecosystem if exhausted to the environment.	Pollution and Environemental Related Laws

1. General Precautions When Using Dangerous Chemicals

(1) Check the characteristics of the chemicals to be used, especially their ignitability, flammability, explosibility, and toxicity. Check the "Material Safety Data Sheet, MSDS."

(2) Store in a cool place, away from any direct sunlight and fire resources, avoid mixing with other chemicals.

(3) When there are many types and large quantities of dangerous chemicals stored, they have to be categorized and stored in the predetermined storage according to the laws and regulations. Any poisonous and deleterious substances should be stored in locked medicine cabinets.

(4) When using any dangerous chemicals, use as small amount as possible. Conduct a preliminary exam when using any unknown chemicals.

(5) When using any dangerous chemicals, think any safety measures ahead and prepare all possible prevention measures for the worst, including protective masks, heat resistant clothing, and fire extinguisher for fires and explosions. Have rubber gloves, gas masks, and protective clothes for intoxication or injuries.

(6) Consider not to cause water and air pollutions when disposing wastes from any toxic chemicals.

(7) Report to the person in charge immediately if any of the dangerous chemicals are lost or stolen.

Ⅱ. HAZARDOUS MATERIALS

Hazardous materials regulated by *the Fire Services Act* are any chemicals (drugs) that have risks of causing a fire, and are categorized by their characteristics as category 1 through category 6 in Table 12. Handling of these chemicals listed in Table 12 is only allowed to the licensed hazardous materials handlers. When unlicensed handlers handle the materials, the licensed handler has to be present.

This section describes precautions for handling of hazardous materials by categorizing in ignitability, flammability, explosibility, and toxicity.

Table 12. Names of Products and Designated Amount of Hazadorous Materials

Class		Names of Products	Characteristic	Designated Amount
CATEGORY 1	1	Chlorates	Class 1 oxidizing solids	50kg
	2	Perchlorates		
	3	Inorganic peroxides		
	4	Chlorites	Class 2 oxidizing solids	300kg
	5	Bromates		
	6	Nitrates		
	7	Iodates		
	8	Permanganates	Class 3 oxidizing solids	1,000kg
	9	Dichromates		
	10	Other rights specified by a Cabinet Order		
	11	Substances containing any of those listed in the preceding items		
CATEGORY 2	1	Phosphorus sulfide		100kg
	2	Red phosphorus		100kg
	3	Sulfur		100kg
	4	Iron powder		500kg
	5	Metal powder	Class 1 combustible solids	100kg
	6	Magnesium		
	7	Other rights specified by a Cabinet Order	Class 2 combustible solids	500kg
	8	Substances containing any of those listed in the preceding items		
	9	Inflammable solids		1,000kg
CATEGORY 3	1	Potassium		10kg
	2	Sodium		10kg
	3	Alkyl aluminiums		10kg
	4	Alkyl lithiums		10kg
	5	Yellow phosphorus		20kg
	6	Alkali metals (excluding potassium and sodium) and alkali earth metal	Class 1 spontaneous-ignitable substance and water prohibitive substance	10kg
	7	Organometallic compound (excluding alkyl aluminiums and alkyl lithiums)		
	8	Metal hydrides	Class 2 spontaneous-ignitable substance and water prohibitive substance	50kg
	9	Metal phosphides		
	10	Carbide of calcium or carbide of aluminum		
	11	Other rights specified by a Cabinet Order	Class 3 spontaneous-ignitable substance and water prohibitive substance	300kg
	12	Substances containing any of those listed in the preceding items		
CATEGORY 4	1	Special inflammable materials		50 ℓ
	2	Class I petroleums	Water-insoluble liquid fuel	200 ℓ
			Water soluble liquid	400 ℓ
	3	Alcohols		400 ℓ
	4	Class 2 petroleums	Water-insoluble liquid fuel	1,000 ℓ
			Water soluble liquid	2,000 ℓ
	5	Class 3 petroleums	Water-insoluble liquid fuel	2,000 ℓ
			Water soluble liquid	4,000 ℓ
	6	Class 4 petroleums		6,000 ℓ
	7	Oil extracted from animals and plants		10,000 ℓ
CATEGORY 5	1	Organic peroxides	Class 1 self-reactive substance	10kg
	2	Nitric esters		
	3	Nitro compounds		
	4	Nitroso compounds		
	5	Azo compounds	Class 2 self-reactive substance	100kg
	6	Diazo compounds		
	7	Hydrazine derivatives		
	8	Hydroxylamine		
	9	Hydroxylamine salts		
	10	Other rights specified by a Cabinet Order		
	11	Substances containing any of those listed in the preceding items		
CAT. 6	1	Perchloric acid		300kg
	2	Hydrogen peroxide		
	3	Nitric acid		
	4	Other rights specified by a Cabinet Order		
	5	Substances containing any of those listed in the preceding items		

Ⅲ. COMBUSTIBLE SUBSTANCES

There are two types of combustible substances: the substances that ignite upon heating or shock; and the substances that ignite upon contact or mixture. Here are classifications, characteristics, and their specific examples of combustible substances shown below.

1. Strong Oxidant

Perchlorate [$MClO_4$] (M=Na, K, NH_4)

Chlorate [$MClO_3$] (M=Na, K, NH_4, Ag)

Inorganic peroxide [Na_2O_2, K_2O_2, H_2O_2]

Organic peroxide [alkyl hydro peroxide, R=O=O=H (t - butyl-, cumyl-)]
[dialkyl peroxide, R-O-O-R' (di – t – butyl -, dicumyl-)]
[diacyl peroxide, R-CO-O-O-CO-R']

Nitrate [MNO_3] (M=Na, K, NH_4)

Permanganate [$MMnO_4$] (M=K, NH_4)

[Precautions]

(1) Store them in cool and dark place away from any fire and heat sources since there are risks of explosions by heat or shock.
(2) It will catch fire if mixed with reducing agents (organic matters).
(3) Perchlorate produces carbon dioxide (CIO_2) and might explode. Permanganate produces ozone (O_3) and might explode.
(4) Peroxide produces oxygen (O_2) with water, and hydrogen peroxide (H_2O_2) with dilute acid, which generate heat and might catch fire. Make sure to maintain the storage moisture proof.
(5) Be careful with an organic peroxide generating as a side reactant while storing

2. Strong Acid

Fuming nitric acid, concentrated nitric acid (HNO_3)

Anhydrous sulfuric acid, fuming sulfuric acid, concentrated sulfuric acid (H_2SO_4)

Chromic anhydride (CrO_3)

Chlorosulphonic acid (HSO_3CI)

[Precautions]

(1) It might catch fire if mixed with reducing agents (organic matters). Store the container (glass) in a cool, dark place to prevent damage.
(2) Chromic anhydride will resolve and produce oxygen (O_2) if heated above the melting point, and might catch fire if there are organic matters around.
(3) If spilled, cover with sodium bicarbonate and dissolve with a lot of water before neutralizing.
(4) Wear rubber gloves when handling.
(5) When on fire because of these substances, pour large amounts of water.

3. Low Temperature Ignitable Substances

Yellow phosphorus, red phosphorus (P)

phosphorus sulphide (P_4S_3, P_2S_5, P_4S_7)

Sulfur (S)

Metal powder (Mg, Al, Fe, Zn)

Metal ribbon (Mg)

[Precautions]

(1) Store them in cool and dark place away from fire since it might catch fire if any heat sources are around.
(2) It will catch fire if mixed with oxidizer.
(3) Phosphorus sulphide and metal powder should avoid any contacts with moisture.
(4) Yellow phosphorus might catch fire in the air, so store it in water.

(5) Sulfur powder may absorb moisture and catch fire.
(6) Metal powder burns if heated in the air. If in contact with acids or alkalis, it produces hydrogen (H_2), so be careful with fire.
(7) Use sand or dry chemical fire extinguishers for metal powder.

4. Prophoric Substance

Organometallic compound RnM (R=alkyl group or allyl group, M=Li, Na, K, Rb, Al, P, Zn)
Reducing metal catalyst (Pt, Pd, Ni, Cu – Cr)

[Precautions]

(1) It will catch fire on contact with air, so follow the directions of experts when using it for the first time.
(2) Diluted organometallic compounds might catch fire if thesolvents volatilize, so it should be sealed to store. Do not put any combustibles nearby.
(3) When it is mixed with oxidizer, it might explode.
(4) Use dry sand or dry chemical fire extinguishers for seize a fire. Use a lot of water when the fire is small.

5. Water Prohibitive Substance

Metallic sodium (Na), metallic potassium (K)
Calcium carbide (CaC_2)
Quicklime (CaO)
Lime phosphide (Ca_3P_2)
Lithium aluminium hydride ($LiAlH_4$)
Sodium amide ($NaNH_2$)

[Precautions]

(1) Metallic sodium and metallic potassium react with water and burn violently, producing hydrogen (H_2). Metallic sodium should be sealed in petroleum and stored in cool, dark, and dry place above floor level.
(2) Metallic sodium and metallic potassium react with halogenide and might explode.
(3) Carbide may react with water, produce acetylene, and might explode.
(4) Calcium phosphide reacts with water, produce phosphine (PH_3) and might explode.
(5) Metal hydride reacts with water and might catch fire. When disposing of it, pour the small amount in ethyl acetate slowly, but not vice versa.
(6) Quicklime react with water but will not catch fire. However, if there are combustibles nearby, it might catch fire.
(7) Use rubber gloves and tweezers and do not touch with bare hands.
(8) Cover with dry sand when extinguishing fire. It is ok to use dry chemical fire extinguishers such as hydrogen carbonate but do not use water injection or water-based extinguisher.

Ⅳ. INFLAMMABLE SUBSTANCES

Risks of combustible materials are generally determined by their flash point. The lower the flash point is, the greater risks are. However, it should keep in mind that when the substance with high flash point is heated above its flash point, it is as dangerous as the substance with low flash point.

[Note]

Flash Point: The flash point is the lowest temperature of the liquid in a container at which a concentrated vapor that can ignite when mixed with air is formed at the top of liquid.

Kindling Point: The kindling point is the lowest temperature at which combustible materials get heated in the air and spontaneously ignites. It is also called the autoignition temperature.

1. Special Inflammable Material

It is a material witha kindling point of 100℃ or less, or a flash point of -20℃ or less, and a boiling point of 40℃ or less at one atmosphere pressure. Specific examples are:

diethyl ether, carbon bisulfide, acetaldehyde, pentane, isopentane, nickel carbonyl, alkylaluminium

[Precautions]

(1) The flash point and kindling point are very low with a high risk of ignition, so make sure to put out a naked flames/light when using.
(2) Since the boiling point is low and the explosive limit is wide, it is important to well ventilate so that vapors do not stay in the air.
(3) If it contacts with a light and air for a long period of time, peroxide will form and explode.
(4) If it is soaked in cloth, it might autoignite.
(5) Prepare a carbon dioxide or powder fire extinguisher to put out the fire.
(6) Once ignited, it is hard to extinguish the fire, so try to prevent the secondary disaster.

2. General Inflammable Substances

(1) High Inflammable Substances (with the flash point of less than 21℃ at one atomsphere)

[Class 1 Petroleum] petroleum ether, petroleum benzene, hexane, heptane, gasoline, benzene, toluene, alcohol (methyl ~ penthyl), dimethylether, acetone, methyl ethyl ketone, formate, acetate, pyridine, chlorobenzene

(2) Moderate Inflammable Substances (1 air pressure – the flash point is less than 21~70℃)

[Class 2 Petroleum] kerosene, diesel, turpentine oil, benzaldehyde, formic acid, acetic acid

(3) Low Inflammable Substances (1 air pressure – the flash point is more than 70℃)

[Class 3 Petroleum] heavy oil, spindle oil, ethylene glycol, nitrobenzene

[Class 4 Petroleum] gear oil, dibutyl phthalate

[Plant Oils] flaxseed oil, soybean oil, coconut oil

[Precautions]

(1) The inflammability is not as high as that of the special inflammable substances, but sparks of switch and static electricity and a cigar light can be ignition sources. Do not heat it directly.
(2) Moderate inflammable substances are likely to ignite when heating in an open vessel. Be careful not to retain any vapors.
(3) Low inflammable substances are likely to ignite by cracked gas at high temperature heating.
(4) When the gravity of vapors is high, it is easily retainable, so make sure to ventilate well.

(5) When the fire is small, use the carbon dioxide fire extinguishers, and when it spreads, pour a lot of water.

V . EXPLOSIVE SUBSTANCES

Peroxide, ozonide, chloric acid, perchloric acid, perchlorate, their esters, nitric ester, nitrite ester, nitrosamine, amine oxide, nitro compound, amine nitrate, sub-amine nitrate, hydrazine, diazo compound, azide, fulminate, and acetylide are unstable and explodes with heat and shock.

Ethers like diethyl ether and tetrahydrofuran are likely to generate peroxides when contacting with air. It should be distilled after decomposing the peroxide with a reducing agent.

Here are some examples of substances that are stable on their own, but explosive when mixed.

(1) Oxide and combustibles (perchloric acid and dimethyl sulfoxide)
(2) Ammonia and silver nitrate solution
(3) Alkali metal and carbon tetrachloride or chloroform

There are two types of explosions: combustive explosions that occur when combustible gases are mixed with air and reached the concentration within explosive limit; and decomposition explosions that occur when substances that are easily decomposed are instantly vaporized by heat or shock.

1. Combustible Gas

The High Pressure Gas Safety Act covers the gases with the lower explosive limit concentration of 10% or less, or the difference between the upper and lower limits of 20% or more.

[C, H as individual or Compounds]

Hydrogen, methane, ethane, propane, butane, ethylene, propylene, acetylene, butadiene

[Compounds of C, H, O]

Carbon monoxide, dimethyl ether, acetaldehyde, acrolein, ethylene oxide

[Compounds of C, H, N]

Ammonia, methyl amine, trimethylamine, hydrogen cyanide, acrylonitrile

[Compounds of C, H, X]

Methyl chloride, ethyl chloride, vinyl chloride, methyl bromide

[Compounds of C, H, S]

Hydrogen sulfide, carbon bisulfide

[Precautions]

(1) It there are any ignition sources, it leaks, remain and explode. Use or store with attention to ventilation of the room where the cylinders are stored.
(2) Do not heat or shock acetylene and ethylene oxide since they decompose and explode.
(3) When a large amount of gas leaks, stop the gas source and fire if possible, open the windows and wait, but evacuate immediately when the situation is worse or pressed for time.

2. Explosive Decomposition Substances

They are combustibles that are categorized as Class 5 by *the Fire Services Act*, and cause self-reaction by heat, shock, friction and light, resulting in heat generation and exploion. Nitric ester, nitro compound, azo compound and organic peroxide are the typical examples.

[Precautions]

(1) Explode by heat, shock, friction and light.
(2) Burn and explode by contact with strong acid.
(3) Byproducts may be produced during the various reaction process, resulting in unexpected exploion.
(4) Do not mix carelessly with acid, alkali, metal and reducing substances since they might explode when they come into contact with each other.
(5) Prepare and wear protective masks, heat-resistant protective clothes and gas masks as needed.

3. Explosives

Explosives are processed products made with explosive decomposition substances that are intended to explode, and explosives (gunpowder, explosive powder, chemical industrial products) are regulated by *the Explosives Control Law*.

Ⅵ. POISONOUS SUBSTANCES

It is reasonable that most of the chemical products used in laboratory can be considered poisonous substances. Usually, the amount of chemicals used in general experiments and researches is small, and there is little risk of poisoning by ordinary chemicals unless they are handled thoughtlessly. However, if a highly toxic chemical is used incorrectly, it can cause a fatal damage. Therefore, the handler has to be always aware of the toxicity of handling substances in advance.

1. Poisonous Gas

According to the High Pressure Gas Safety Law, the poisonous gas is the allowable concentration of 200ppm or less.

[Allowable concentration of0.1ppm or less] fluorine, phosgene, ozone, arsine, phosphine
[Allowable concentration of1.0 ppm or less] chlorine, bromine, hydrazine, acrolein
[Allowable concentration of5.0 ppm or less] sulfur dioxide, hydrogen fluorine, hydrogen chloride, formaldehyde
[Allowable concentration of10 ppm or less] hydrogen cyanide, hydrogen sulfide, carbon disulfide
[Allowable concentration of50 ppm or less] carbon monoxide, ammonia, ethylene oxide, nitrogen oxide
[Allowable concentration of200 ppm or less] methyl chloride

Here are poisonous gases that are relatively used often and could generate by careless mistake during experiments.

(1) Hydrogen sulfide and chlorine gas
(2) Cyanide compounds such as sodium cyanide and calcium cyanide generates hydrogen cyanide (hydrocyanic acid gas) if accidently thrown into the acid waste liquid container.

[Precautions]

(1) It generally causes asphyxiation, and highly toxic substance corrode skins and mucous membranes.
(2) If concentrated gas is inhaled, faint may occur instantly and it is unable to escape.
(3) Handle it in the ventilated device when using.
(4) Wear a gas mask when handling.

2. Nerve Gas and Regulations

Toxic chemicals such as sarin, soman, tabun, VX and precursors such as chlorosarin and chlorosoman fall under the specified substances. These are not allowed to manufacture, possess nor use without permission from the Minister of Economy, Trade and Industry even for a small amount (It's not feasible to be possessed).

3. Poisonous, Deleterious Substances, and Others

Poisonous substances are absorbed through the respiratory tract in the form of vapors and fine particles, through the digestive system in the form of aqueous solutions, and through skins by contacts, so that their handlings require extremely careful attention. The handler has to know which substances are designated as poisonous substances, and should be careful with similar substances. The table 13 shows the criteria for poisonous substances by t*he Poisonous and Deleterious Substances Control Act*. The table 14 shows the symbols and indications of reagent risks and their toxicities.

Table 13. Hazardous Materials by the Poisonous and Deleterious Substance Control

Name	Toxicity	Note
Specified poisonous substance	The following poisonous substances that have especially high toxicity and are at high risk of being hazardous.	tetraalkyl lead, pesticide
Poisonous substance	Oral: LD_{50} - less than 50mg/kg Percutaneous: - LD_{50} less than 200mg/kg	The toxicity condition is not rigorous. They are the substances that are designated by the law.
Deleterious substance	Oral: LD_{50} - 50 ~ 300mg/kg Percutaneous: LD_{50} - 200~1000mg/kg	

Poisonous and deleterious substances are to be managed according to the "*National University Corporation Kagoshima University Poisonous and Deleterious Management Regulations*." Table 15 shows the general chemical substances among the hazardous materials regulated by *the Poisonous and Deleterious Substances Control Law* and *the Industrial Safety and Health Law*, and categorizes into poisonous (●), deleterious (○), prohibited from manufacturing (×), permitted for manufacturing (■). It also shows the specific examples of inorganic compounds and organic compounds.

[Precautions]

(1) Poisonous (●) and deleterious (○) should be put in a sealed container, clearly labeled with the contents on it and stored in a locked chemical cabinet.
(2) Make sure to use any poisonous and deleterious substances under the supervision of the instructor. If the quantity of poisonous or toxic materials changes due to purchases, use, or disposal, it has to be recorded every time. If any of them got stolen, report to the instructor immediately.
(3) After using any corrosive substances, make sure to wash hands and face and gargle.
(4) Prepare or wear rubber gloves and gas masks.

Ⅶ. DISPOSAL OF CHEMICALS

Wastes (including solids) produced from education and research at the university include various hazardous wastes. The university has to try not to damage human health and the environment by these wastes. *The Basic Environmental Law* indicates the environmental standards that should be maintained. According to that, there are *the Water Pollution Control Law*, *the Sewerage Law*, t*he Waste Disposal and Public Cleansing Law*, *the Air Pollution Control Law, the Offensive Odor Control Law*, and the regulations for specified substances.

Do not mix the poisonous chemicals in the regular trash, nor release them to the sink nor volatilize in the air.

Experiment liquid wastes from education and research should be classified, stored, intoxicated and disposed of according to the "*National University Corporation Kagoshima University Liquid Waste Processing Regulations*." Table 16 shows the storing classification of the inorganic experiment liquid wastes, and the Table 17 shows the organic experiment liquid wastes. Store them until notified of inorganic and organic liquid wastes processing. Furthermore, when disposing of the empty drug bottles, wash them, take off the lids, separate them by materials (glass or plastic), put them in a clear polyethylene bag (write the department name on it), and take it to the designated place. (Attention: Do not mix with the general unburnable.)

Ⅷ. RADIOACTIVE MATERIALS NOT UNDER THE DIRECT CONTROL

Although radioactive materials are not supposed to exist in ordinary laboratories, there have been cases seen nationwide where radioactive materials that were used in the past have been left unattended, even though they are not being used now. For example, those materials that were used more than 20-30 years ago have been found in the back of the cupboard, in a storage that nobody goes in, in a safe that has not been opened recently, etc, so be careful when cleaning the old goods. Radioactive materials are usually labeled with a radioactivity mark. If any radioactive materials are found, do not touch or move it unnecessarily, report to the instructor immediately.

Table 14. Symbols and Indications of Reagent Risks and Their Toxicities

Symbols	Risks	Applicable Materials by Related National Laws and Regulations
 Exprosive	Explode on impact, friction, heat, etc.	Explosives Control Law Article 2(1) High Pressure Gas Safety Law Article 2
 Extremely Inflammable	Extremely inflammable liquids Flash point < -20°C Boiling point ≦ 40°C Kindling point ≦ 100°C	Fire Services Act Class 4 Special Inflammables
 Inflammable	Inflammable liquids Flash point less than 70°C	Fire Services Act Class 4 No 1 Petroleum, Alchol No2 Petroleum
 Combustible	Solids that are easily ignitionable by flame, solids and gases that are inflammable at low temperature	Fire Services Act Class 2 Inflammable Solids Industrial Safety and Health Act Annex Table 1 (5)
 Pyrophoric	It ignites automatically in the air.	Fire Service Act Class 3 Pyrophoric Solids Dangerous Goods Regulations Annex Table 6 (excludes autoreactible and other pryophorics)
 Water Reactive	Ignite when it contacts with water, or generate a combustible gas	Fire Service Act Class 3 Water Reactive Substances Dangerous Good Regulations Annex Table 6 Other Inflammable substances
 Oxidizable	Burn or explode when it is mixed with combustibles	Fire Service Act Class 1 Oxidizing Solid and Class 6 Oxidizing liquids. Dangerous Good Regulations Annex Table 7
 Autoreactible	Generate heat by impact or heat, or react explosively	Fire Service Act Class 5 Autoreactible Substances
 Extremely Poisonous	It is extremely poisonous when swallowed, inhaled, or it touches the skin, and might lead to death. Reference: LD_{50}: less than 30mg/kg (rat, oral)	Poisonous and Deleterious Substances Control Law Substances that are not subjected to the Poisonous Law, but on the Dangerous Good Regulations Annex Table 4, that are extremely poisonous
 Toxic	It is poisonous when swallowed, inhaled, or it touches the skin. Reference: LD_{50} 30~300mg/kg (rat, oral)	Poisonous and Deleterious Substances Control Law Substances that are not subjected to the Poisonous Law, but on the Dangerous Good Regulations Annex Table 4, that are toxic
 Noxious	It might be poisonous when swallowed, inhaled, or it touches the skin. Reference: LD_{50} : 200~2000mg/kg (rat, oral)	Substances that are not subjected to the Poisonous Law, but on the Dangerous Good Regulations Annex Table 4, that are noxious. Existing chemical substances that the published mutegenicity was recognized by the Labor Standards Bureau Notification No.51 on 2/10/1992. New chemical substances that the published mutegenicity was recognized by the Labor Standards Bureau Notification No.414 (3) on 6/25/1991. The Class 2 Specified Chemical Substances and Designated Substances that are prescribed in the Chemical Substance Control Law Article 2.
 Corrosive	It corrodes skins or devices.	Dangerous Goods Regulations Annex Table 3 (excluding other corrosives)
 Stimulative	It might stimulate and damage skins, eyes, or respiratory system.	No related laws and regulations

Reference: Japan Reagent Association

Table 15. Main Poisonous Substances

This table shows the main general chemical substances from the poisonous substances that would be regulated by the Poisonous and Deleterious Substance Control and the Industrial Safety and Health Law.

Symbols ● : Poisonous × : Prohibition of manufacturing
○ : Deleterious ■ : Permission for manufacturing

(Industrial Safety and Health Law)

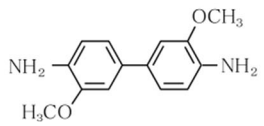
Substance	Chemical Formula	Group	Substance	Chemical Formula	Group
Potassium nitrite	KNO_2	○	Yellow phosphorus	P_4	●
Silver nitrite	$AgNO_2$	○	Hydrogen peroxide	H_2O_2	○
Sodium nitrite	$NaNO_2$	○	Sodium peroxide	Na_2O_2	○
Sodium selenite	Na_2SeO_3	●	Barium peroxide	BaO_2	○
Arsenous Acid	As_2O_3	●	Cadmium compound	Cd	○
Potassium arsenite	K_3AsO_3	●	Potassium	K	○
Calcium arsenite	$Ca(AsO)_2$	●	Chloroauric acid	$HAuCl_4$	○
Sodium arsenite	$NaAsO_2$	●	Gold potassium chloride	$KAuCl_4$	○
Lead arsenite	$Pb(AsO_4)_2$	●	Sodium chloraurate	$NaAuCl_4$	○
Lead antimonate	$Pb(SbO_3)_2$	○	Chlorosulfonic acid	HSO_3Cl	○
Ammonia	NH_3	○	Zinc chromate	$ZnCrO_4$	○
Arsenic sulfide	As_4S_4	●	Potassium chromate	K_2CrO_4	○
Lead monoxide	PbO	○	Calcium chromate	$CaCrO_4$	○
Sodium uranate	NaU_2O_7	○	Silver chromate	Ag_2CrO_4	○
Zinc chloride	$ZnCl_2$	○	Bismuth chromate	$Bi_2(CrO_4)_3$	○
Cadmium chloride	$CdCl_2$	○	Sodium chromate	Na_2CrO_4	○
Hydrogen chloride	HCl	○	Lead chromate	$PbCrO_4$	○
Mercury chloride (I)	HgCl	○	Hydrofluorosilicic acid	H_2SiF_4	○
Tin(II) chloride	$SnCl_2$	○	Potassium silicofluoride	K_2SiF_6	○
Copper chloride (I)	CuCl	○	Sodium silicofluoride	Na_2SiF_6	○
Lead chloride (II)	$PbCl_2$	○	Barium silicofluoride	$BaSiF_6$	○
Gold chloride (III)	$AuCl_3$	○	biarsenic pentoxide	As_2O_5	●
Mercury chloride	$HgCl_2$	●	Vanadium pentoxide	V_2O_5	○
Tin(IV) chloride	$SnCl_4$	○	Antimony trichloride	$SbCl_3$	○
Copper chloride ammonia	$CuCl_2$	○	Mercury (II) oxide	HgO	●
Copper chloride potassium	$CuCl_2 \cdot 2NH_4Cl$	○	Barium oxide	BaO	○
Barium chloride	$CuCl_2 \cdot 2KCl$	○	Antimony trioxide	Sb_2O_3	○
Thionyl chloride	$BaCl_2$	○	Arsenic trioxide	As_2O_3	●
Copper oxychloride	$SOCl_2$	○	Potassium pyroantimonate acid	$K_2HSb_2O_7$	○
Basic lead chromate	$CuCl_2 \cdot 3Cu(OH)_2$	○	Phosphorus pentachloride	PCl_5	●
Basic copper carbonate	$PbCrO_4 \cdot PbO$	○	Phosphorus oxychloride	$POCl_3$	●
Hydrochloric acid	$CuCO_3 \cdot Cu(OH)_2$	○	Phosphorus trisulphide	P_4S_3	●
Potassium chlorate	HCl	○	Cadmium cyanide	$Cd(CN)_2$	●
Cobalt chlorate	$KClO_3$	○	Potassium cyanide	KCN	●
Sodium chlorate	$Co(ClO_3)_2$	○	Calcium cyanide	$Ca(CN)_2$	●
Barium chlorate	$NaClO_3$	○	Silver cyanide	AgCN	●
	$Ba(ClO_3)_2$	○	Hydrogen cyanide	HCN	●

Substance	Chemical Formula	Group	Substance	Chemical Formula	Group
Gold cyanide (I) potassium	$KAu(CN)_2$	●	Lead dioxide	PbO_2	○
Mercuric cyanide (II)	$Hg(CN)_2$	●	Selenium dioxide	SeO_2	●
Copper cyanide	$Cu_2(CN)_2$	●	Nickel carbonyl	$Ni(CO)_4$	●
Sodium cyanide	NaCN	●	Carbon disulfide	CS_2	○
Lead cyanide	$Pb(CN)_2$	●	Oleum	$H_2SO_4+SO_3$	○
Silver barium cyanide	BaPtCN	●	Arsine	AsH_3	●
Sodium cyanate	NaOCN	○	Arsenic acid	H_3AsO_4	●
Carbon tetrachloride	CCl_4	○	Zinc arsenate	$Zn_3(AsO_4)_2$	●
Hydrobromic acid	HBr	○	Potassium arsenate	K_3AsO_4	●
Bromine	Br_2	○	Calcium arsenate	$Ca_3(AsO_4)_2$	●
Bichromic acid	$H_2Cr_2O_7$		Silver arsenate	$FeAsO_4$	●
Ammonium dichromate	$(NH_4)_2Cr_2C$	○	Copper arsenate	$Cu_3(AsO_4)_2$	●
Potassium dichromate	$K_2Cr_2O_7$	○	Sodium arsenate	Na_3AsO_4	●
Sodium dichromate	$Na_2Cr_2O_7$	○	Manganese arsenate	$Pb_3(AsO_4)_2$	●
Nitric acid	HNO_3	○	Arsenic	$Mn_3(AsO_4)_2$	●
Zinc nitrate	$Zn(HNO_3)_2$	○	Hydrogen fluoride	As	●
Uranyl nitrate	$UO_2(NO_3)_2$	○	Barium fluoride	HF	●
Silver nitrate	$AgNO_3$	○	Calcium arsenate fluoride	BaF_2	○
Mercury nitrate (I)	$Hg_2(NO_3)_2$	●	Beryllium (compound)	Ca_2FAsO_4	○
Mercury nitrate (II)	$Hg(NO_3)_2$	●	Fluoroboric acid	Be	■×
Thallium nitrate	$TlNO_3$	○	Sodium metaantimonate	HBF_4	○
Copper nitrate	$Cu(NO_3)_2$	○	Chromic anhydride	$NaSbO_3$	○
Lead nitrate	$Pb(NO_3)_2$	○	Mercuric iodide (II)	CrO_3	○
Barium nitrate	$Ba(NO_3)_2$	○	Lead iodide	HgI_2	●
Mercury	Hg	●	Iodine	PbI_2	○
Potassium hydroxide	KOH	○	Cadmium sulfide	I_2	○
Sodium hydroxide	NaOH	○	Arsenic sulfide (III)	CdS	●
Barium hydroxide	$Ba(OH)_2$	○	Barium sulfide	As_2S_3	●
Asbestos	—		Phosphorus sulfide	BaS	○
Crocidolite		×	Sulfuric acid	P_4S_{10}	●
Serpentine asbestos		(×)	Zinc sulfate	H_2SO_4	○
Amosite		×	Cadmium sulfate	$ZnSO_4$	○
Selenium	Se	●	Thallium sulfate	$CdSO_4$	○
Selenic acid	H_2SeO_4	●	Copper sulfate	Tl_2SO_4	○
Lead carbonate	$PbCO_3$	○	Zinc phosphide	$CuSO_4$	○
Barium carbonate	$BaCO_3$	○		Zn_3P_2	○
Sodium	Na	○			

1) $NaFe_5Si_8O_{22}(OH)_2$, 2) $Mg_2Si_2O_5(OH)_4$, 3) $(Fe \cdot Mg)_7Si_8O_{22}(OH)_2$

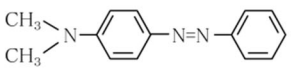
Substance	Chemical Formula	Group	Substance	Chemical Formula	Group
Organic Compounds					
Acrylamide	$CH_2=CHCONH_2$		Lead acetate	$Pb(CH_3CO_2)_2$	○
Acrylic acid	$CH_2=CH-CH_2-CO_2H$	○	Barium acetate	$Ba(CH_3CO_2)_2$	○
Acrylic nitrile	$CH_2=CHCN$	○	Dianisidine	$C_{14}H_{16}N_2O_2$ [1]	■
Acrolein	$CH_2=CHCHO$	○	Tetraethyl lead	$(C_2H_5)_4Pb$	●
Adiponitrile	$NC(CH_2)_4CN$	○	Dimethylmercury	$(CH_3)_2Hg$	
Acetylenedicarboxamide	$NH_2COC\equiv CCONH_2$	○	Dichloroacetic acid	$CHCl_2CO_2H$	○
Acetonitrile	CH_3CN	○	Dichlorobutyne	$CHCl_2C=CCH_2Cl$	○
Aniline	$C_6H_5NH_2$	○	Dichlorobenzidine	$C_{12}H_{10}N_2Cl_2$	■
Allylamine	$CH_2=CH-CH_2-NH_2$	○	1,2-Dibromoethane	$BrCH_2CH_2Br$	○
Allyl alcohol	$CH_2=CH-CH_2-OH$	○	Tetramethyllead	$(CH_3)_4Pb$	●
4-aminobiphenyl	$C_6H_5C_6H_4NH_2$	×	Methyl bromide	CH_3Br	
Isobutyronitrile	$(CH_3)_2CHCN$	○	Oxalic acid	$C_2H_2O_4$	○
Ethylene chlorohydrin	$Cl(CH_2)_2OH$	○	Zinc oxalate	ZnC_2O_4	○
Ethylene cyanhydrin	$CN(CH_2)_2OH$	○	Ammonium oxalate	$(NH_4)_2C_2O_4$	○
Vinyl chloride	$CH_2=CH-Cl$		Potassium oxalate	$K_2C_2O_4$	○
Chlorinated biphenyl	$C_6H_{5-n}Cl_nC_6H_{5-m}Cl_m$	■	Calcium oxalate	CaC_2O_4	○
Aniline hydrochloride	$C_6H_5NH_2\cdot HCl$	○	Silver(II) oxalate	FeC_2O_4	○
Hydroxylamine hydrochloride	$NH_2OH\cdot HCl$	○	Tin oxalate	SnC_2O_4	○
Urea peroxide	$NH_2CONH_2\cdot H_2O_2$	○	Sodium iron oxalate	$Na_3Fe(C_2O_4)_3$	○
Formic acid	HCO_2H	○	Titanium oxalate	$Ti_2(C_2O_4)_3$	○
Cresol	$CH_3C_6H_4OH$	○	Titanium potassium oxalate	$TiO(CO_2\cdot COOK)_2$	○
Chloroethyl	C_2H_5Cl	○	Silver ammonium oxalate	$NH_4Fe(C_2O_4)_2$	○
1-Chloro-1, 2- dibromoethane			Thorium oxalate	$Th(C_2O_4)_2$	○
	$CHClBrCH_2Br$	○	Sodium oxalate	$Na_2C_2O_4$	○
Chloropicrin	CCl_3NO_2	○	Manganese oxalate	MnC_2O_4	○
Chloromethyl	CH_3Cl	○	Ammonium Ion	$(NH_4)HC_2O_4$	○
Chloroform	$CHCl_3$	○	Potassium antimonyltartrate	$C_2H_4O_2CO_2KCO_2SbO$	○
Uranyl acetate	$UO_2(CH_3CO_2)_2$	○	Thiosemicarbazide	$H_2NCSNHNH_2$	○
Ethyl acetate	$CH_3CO_2C_2H_5$	○	Trichloroacetic acid	CCl_3CO_2H	○
Cadmium acetate	$Cd(CH_3CO_2)_2$	○	o-Tolidine	$C_{14}H_{16}N_2$ [3]	■
Mercury (I) acetate	$HgCH_3CO_2$	●	Tolylene diisocyanate	$C_9H_6N_2O_2$ [4]	
Mercury (II) acetate	$Hg(CH_3CO_2)_2$	●	Toluidine (Salt)	$CH_3C_6H_4NH_2$	○
Thallium acetate	CH_3CO_2Tl	○	Toluidine	$CH_3C_6H_4NH_2$	○

1)



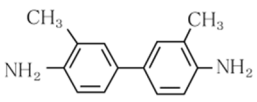
Dianisidine

2)



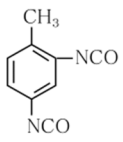
p−Dimethylaminoazobenzene

3)



o−Tridine

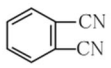
4)



Tolylene diisocyanate

Substance	Chemical Formula	Group	Substance	Chemical Formula	Group
Toluylenediamine	$CH_3C_6H_3(NH_2)_2$	○	Benzidine(salt)	$(C_6H_4NH_2)_2$	×
Toluene	$C_6H_5CH_3$	○	Benzotrichloride	$C_7H_5Cl_3$	■
α-naphthylamine(salt)	$C_{10}H_7NH_2$	■	Benzonitrile	C_6H_5CN	○
β-naphthalidine(salt)	$C_{10}H_7NH_2$	×	Pentachlorophenol	C_6OHCl_5	○
β-naphthol	$C_{10}H_7OH$	○	Pentachlorophenol	(Na 塩)	○
4-nitrodiphenylamine	$C_6H_5C_6H_4NO_2$	×	Formaldehyde	HCHO	○
Nitrobenzene	$C_6H_5NO_2$	○	Methacrylic acid	$CH_2=C(CH_3)-CO_2H$	○
Picric acid	$C_6H_2OH(NO_2)_3$	○	Methanol	CH_3OH	○
Bis-chloro methyl ether	$(CH_2Cl)_2O$	×	N-methylaniline	$C_6H_5NHCH_3$	○
Hydroxylamine	NH_2OH	○	Methylamine	CH_3NH_2	○
Phenylenediamine	$C_6H_4(NH_2)_2$	○	Methyl ethyl ketone	$CH_3COC_2H_5$	○
Phenol	C_6H_5OH	○	Monochloroacetate	CH_2C1CO_2H	○
o -Phthalonitrile	$C_8H_4N_2$ [1)]	○	Sodium monofluoroacetate	CH_2FCO_2Na	●
Bromacetone	CH_3COCH_2Br	○	Fluoroacetamide	CH_2FCONH_2	●
Bromoethyl	C_2H_5Br	○	Methyl iodide	CH_3I	○
Bromomethyl	CH_3Br	○	Dimethyl sulfate	$(CH_3)_2SO_4$	○
Benzene	C_6H_6	×			

1)



o -Phthalonitrile

2)

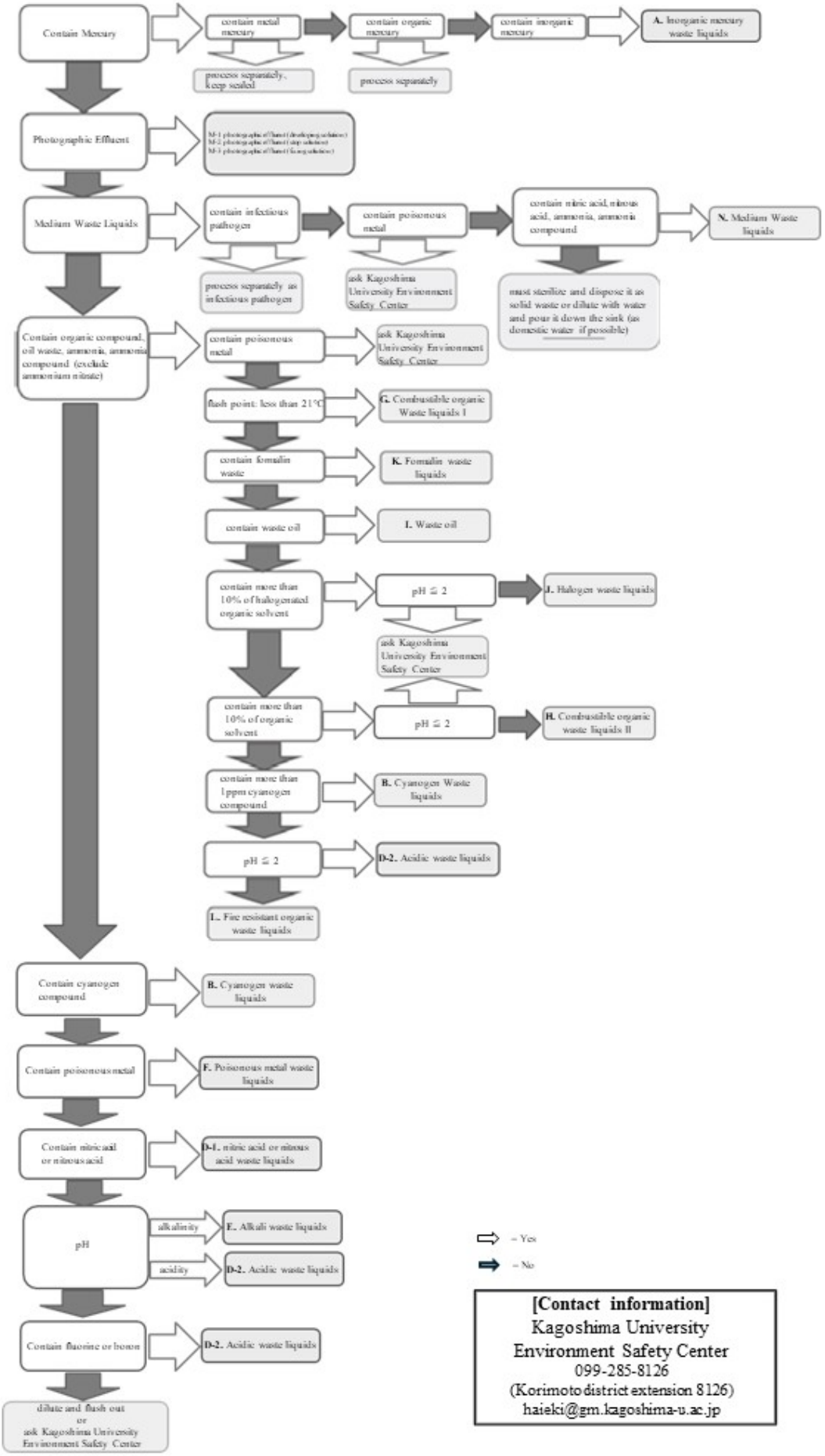
Propiolactone

Table 16. Classification Table of Experiment waste liquids Kagoshima University Environment Safety Center, March, 2020

	Group	Type	Subject	Notes	Disposal Method	Container *1
Inorganic	A	Inorganic Mercury Waste Liquids	Solutions of inorganic mercury compounds	* Do not mix the metal mercury and organic mercury. (Contact the Environment Safety Center for collecting waste liquids including metal mercury and organic mercury) * Indicate the contents (name of substance, concentration, etc.) * If it includes cyanogen, indicate that. * if other poisonous metals are included, indicate its composition.	Neutralization/Coagulative precipitation (Sulfide Method)	20L or 10L Plastic Container
	D	Acidic Waste Liquids	1. Nitric acid, Nitrous acid, and solutions of those inorganic compounds 2. Inorganic acid waste liquids such as hydrochloric acid, sulfuric acid, phosphoric acid 3. Fluorine and fluorine compounds waste liquid with pH≦7 4. Boron and boron compounds waste liquid with pH≦7	* Indicate the contents (name of substance, concentration,etc.) * If it includes Nitric acid and Nitrous acid, the entire amount should be disposed as waste liquids after being diluted or neutralized without releasing. * Waste liquids that the contents of hydrochloric acid and sulfuric acid are 5% or less and do not include poisonous substances should be neutralized with alkali (such as sodium bicarbonate), diluted and released at laboratories. * If it includes hydrocyanic acid, it should be in Category B (Do not mix with acid waste liquid). *Chromic acid and other poisonous metal should be in Category F. * If it includes organic phosphorous compounds, it should be in Category L. * If it includes organic acid, it should be in Category L.	Neutralization/ Coagulative precipitation	
	E	Alkali Waste Liquids	1. Waste liquids such as sodium hydroxide, Potassium hydroxide, and sodium carbonate 2. Fluorine and fluorine compounds waste liquid pH > 8 3. Boron and boron compounds waste liquid pH > 8	* Indicate the contents (name of substance, concentration,etc.) * Waste liquids that the contents of potassium compounds are 5% or less and do not include poisonous substances should be neutralized with acid (dilute hydrochloric acid, dilute sulphuric acid,etc.), diluted and released at laboratories. * Containing amine and ammonia solutions are in Category L.		
	F	Poisonous Metal Waste Liquids	Waste liquids that include poisonous metal such as Cd, Pb, As, Se, Cu, Zn, Fe, Mn	* Indicate the contents (name of substance, concentration,etc.) * Do not put radionuclide or any that are contaminated by it. * Ferricyanide and ferrocyanide are in Category B. * Metal chelate such as organic liands (EDTA etc.) are in Category L.		
	M	Photographic Effluent	1. Developing solution 2. Stop solution 3. Fixing solution	* Store them by categories (clearly specify when they are mixed)	Electrolyze → Collect silver	
Organic	G	Combustible Organic Waste Liquids I (Flash point: less than 21℃)	1. Flammable organic waste liquids that does not include water (toluene, ethyl acetate, benzene, acetone, acetonitrile etc.) 2. Alcohol that the water content is less than 40% (methanol, ethanol,etc.)	* Indicate the contents (name of the substance, concentration, etc.,) * Explosive contents (N-O bond, acetylenes, etc.) are to be detoxified separately. * Alcohols, which water content is 40 - 90% are to be in Category H. * Alcohols, which water content is more than 90% are to be in Category L.	Incineration	10L Plastic Container *2
	H	Combustible Organic Waste Liquids II (Flash point: less than 21°C) (Water content: less than 90%)	Hydrocarbon Alcohol (Water content:40 - 90%) Ketone Phenol	* indicate the contents (name of the substance, concentration, etc.) * Explosive contents (N-O bond, acetylenes, etc.) are to be detoxified separately. * Alcohols, which water content is more than 90% are to be in Category L. * If it contains halogen compounds of 10% and over, it goes to Category J.		20L*2 or 10L Plastic Container
	I	Waste Oil	1. Kerosene,diesel,tenpin oil, etc. 2. Heavy oil, creosol oil, spindle oil 3. Turbine oil, transformer oil, etc. 4. Gear oil, motor oil, etc. 5. Animal and vegetable oil waste liquids	* Indicate the contents (name of the substance, concentration, etc.) * Do not put anything that contains PCB. * Consult about the carrying container.		
	J	Halogen Waste Liquids	1. Halogenated organic solvent (chloroform, methyl chloride, dichloromethane, carbon tetrachloride, trichloroacetic acid etc.) 2. Combustible organic waste liquids that include more than 10% of halogenated organic solvent	* Indicate the contents (name of the substance, concentration, etc.)		
	K	Formalin Waste Liquids	Formalin Waste Liquids	* Remove any solids.		
	L	Fire Resistant Organic Waste Liquids (Water content: more than 90%)	1. Organic waste liquids that contain less than 10% of hydrocarbon, halogen compound, organic acid, nitrite ester, amines 2.Organic metal (chelate, etc.) waste liquids 3. contain less than 1 ppm cyanogen compound 4. contain ammonia and ammonium compounds (exclude ammonium nitrate)	* Indicate the contents (name of the substance, concentration, etc.) * Indicate the pH. * Do not put anything that contains PCB. * Waste liquids that contain mercury are to be in Category A. * Waste liquids that are less than pH2 are to be in Category D. * Waste liquids that contain ammonium nitrate are to be in Category D-1.		
	B	Cyanogen Waste Liquids	1. Free cyanide waste liquids 2. Refractory cyano complex, organic cyanide compounds (cyan concentration 1 ppm or more)	* Indicate the contents (name of the substance, concentration, etc.) * Indicate the pH. * Store at more than pH10.5.		
	N	Medium Waste Liquids	nitric acid compound, nitrous acid compound, ammonia and medium waste liquids containing ammonia compound	* Waste liquids containing infectious pathogen are to be processed separately as infectious pathogen waste. * Remove solid material. * Dispose waste liquids after sterilizing with an autoclave etc. * Do not add highly flammable chemical such as alcohol for the purpose of sterilization. * Specify "Medium Waste Liquids" on a request form. If it contains highly flammable chemical such as alcohol, indicate that . * Sterilize, absorb into a rag etc. and dispose it as solid waste if it is a small quantity.		10L Plastic Container, Centrifuge tube, etc. *3

*1 All collected containers are to be disposed.
*2 A 18-liter square can can be used only when there is no possibility of metallic corrosion.
*3 A countainer which is free from risk of leaking or breakage and disposed by incineration can be also used. When disposing in a centrifuge tube, put it into an outer container such as a cardboard box.

Classification diagram of experiment waste liquids Kagoshima University Environment Safety Center, March, 2020



[Contact information]
Kagoshima University
Environment Safety Center
099-285-8126
(Korimoto district extension 8126)
haieki@gm.kagoshima-u.ac.jp

A request form template for waste liquids disposal

All the waste liquids containers for waste collection must have “Request form for waste liquids disposal” stickers on. Waste liquids are to be processed at disposal factories according to the request forms. Improper disposal and unexpected accidents could occur due to incorrect information or lack of information. Fill out a request form with accurate information referring to the example bellow.

<table>
<tr><td colspan="3">Kagoshima University
Request form for
waste liquids disposal</td><td colspan="2">Name of department
Science</td></tr>
<tr><td>Group</td><td>F</td><td colspan="3">Details of contents
(chemical substances or chemical formulae and concentration)</td></tr>
<tr><td>Volume</td><td>18 L</td><td colspan="3" rowspan="2">0.1M-Na_2HAsO_4 0.3L,
4M-HNO_3 1.5L,
0.5M-$(NH_4)MoO_4$ 1.5L,
4M-NaOH 0.1L,
Water 14.6L</td></tr>
<tr><td>pH</td><td>Fill out when waste liquids contain B cyan system and H, J, L water containing organic waste liquids.</td></tr>
<tr><td>Class,
Major</td><td></td><td>Name of
Laboratory</td><td colspan="2"></td></tr>
<tr><td>Name</td><td></td><td>Phone
number</td><td colspan="2">8126</td></tr>
</table>

Waste liquids classified G (Combustible Organic Waste Liquids I), H (Combustible Organic Waste Liquids II), and I (Waste Oil) that are applicable to “Category IV, inflammable liquids” in the Fire Service Act, must have the “total fire ban” stickers on their respective containers. Special request forms for inflammable waste liquids printed “total fire ban” are available. Please contact staffs at a department in charge or Kagoshima University Environment Safety Cener for more details.

BIOLOGY EXPERIMENTS

Ⅰ. GENERAL SAFETY TIPS

Accidents during experiments are likely to happen from carelessness and lack of knowledge. It is important to plan and prepare well in advance and never overdo it. A first experiment should be conducted under the directions of the instructor. Recognize the risk of the experiment, and prepare in advance for possible accidents.

The experimenter has to not only protect himself/herself, but also think about the safety and health of others. Therefore, pay attention to the surroundings during the experiment, and after the experiment, follow the directions for cleaning, organizing, and maintaining any tools, devices, and drugs that are used.

Ⅱ. MAINTENANCE OF EXPERIMENTAL ENVIRONMENT

Laboratories and work tables should be always cleaned and organized. In order to conduct experiments safely, it is desirable to separate the laboratory area and the residential space if possible. It is better not to put any materials on the floor for safety reasons.

1) Always clean and organize the laboratory.
2) Do not eat, drink, and smoke in the laboratory.
3) Do not use a cellphone in the laboratory.
4) Always wear a lab coat in the laboratory.

Ⅲ. HANDLING EXPERIMENTAL DEVICES

Consider the safety and the smooth work flows when installing and arranging any experimental devices. Read the user's manual, and follow the directions of the instructor when using any devices for the first time. Wear protective clothes and gear as needed when using high-risk devices such as high and low temperature device, high pressure device, large equipment, high energy device, and X-rays device.

When an abnormality occurs during use, stop the use and report to the instructor. Do not leave the abnormalities unattended. Especially, devices such as centrifuges, dry heat sterilizers, autoclaves, and shaking incubators could lead into a serious accident. Be careful when handling sharp knives such as the blades of microtome, razors, scalpels, and dissection scissors.

Separate the medical wastes such as reagent bottles, syringes, and needles from the general wastes.

Be careful when handling the optical precision apparatus such as a biological microscope and a stereoscopic microscope, not to drop them carelessly or accidentally. Also, be careful not to stain the surface of the lenses by touching them. Make sure to turn off the microscope when leaving the seat. Wipe the device with Kimwipes after using it, and put it back to the designated spot.

Ⅳ. STORAGE AND HANDLING OF MEDICINES

Medicines used in experiments are mostly dangerous and poisonous. When using any medicines for the first time, make sure to read precautions on the label and the technical books to recognize its risk, and handle them properly. It is good to know how to handle the situation in case of the accidents. At Kagoshima

University, it is required that all medicines have to be registered and the records have to be entered and kept in the medicine management system with a history of their use. After every use, the record has to be put into the system, return it to the storage, and if any toxic medicines are used, it is advised to gaggle and wash hands.

Any poisonous and deleterious substances should be stored in a lockable medicine cabinet with the necessary information entered in the logbook for record. For example, strong acids such as sulfuric acid, hydrochloric acid, nitric acid, hydrogen peroxide, trichloroacetic acid and picric acid, strong base such as sodium hydroxide, cyan compound, mercury, heavy metal salt, phenol, and formalin are included in these.

When using a reagent, make sure not to spill on hand, clothes or work tables. Make sure to seal the lid after using it and put it back to the designated storage spot.

When handling volatile organic solvents that are easily inflammable such as alcohol, ether, acetone, and xylene, be careful with fire and ventilation (it is out of question to be smoking). These dangerous materials should be stored in lower area of the storage room as much as possible.

When using formalin, alcohols, and handling its specimen immersed in formalin or alcohol, make sure to ventilate and wash the specimen. Especially, formalin has to be handled in the exhaust equipment. Be careful it does not touch skins or eyes, and if it does, immediately wash with water. Go see a doctor as needed.

Ⅴ. WASTE LIQUIDS AND WASTE DISPOSAL

Waste liquids from experiments are collected separately. They are separated into inorganic and organic waste liquids, then outsourced to off-campus contractors for disposal. Inorganic and organic waste liquids from each laboratory are to be stored in the designated containers separately according to the instructor’s direction.

Any wastes from experiments are to be separated into burnable and non-burnable, and taken to the designated trash spot.

Ⅵ. USAGE OF ELECTROCITY AND GAS

Accidents associated with usage of electricity are electronic shocks, fire and explosives. Make sure to ground devices with high voltage and large current devices, and always take care of and maintain the device for not causing the electric leakage. When using an extension cord, be careful not to exceed the allowable current and avoid plugging many cords into one outlet so-called "octopus" wiring.

It is important to make sure that the device is prepared so that water does not splash on the connections, when conducting an experiment with water.

To avoid accidents involving gas leakage, when leaving the room, make sure to close the gas tap. Any unused gas stopcock should be covered with rubber caps. Pay attention to any scratches and cracks on the gas pipes.

Ⅶ. FIRE HANDLINGS

Avoid any combustibles when using stove, gas burner, and heater. Keep the area around combustibles strictly no fire when using any flammable chemicals/medicines. Turn off the possible source of fire when leaving the room (Smoking is prohibited on University's premises).

It is important to prepare in advance how to handle/what to do when fire occurs. Always know where the fire extinguisher and the fire alarms are, and how to put the fire out and the escape routes on a daily basis.

Ⅷ. RESPONSE TO THE ACCIDENT

Report any accidents (even the small ones) to the instructor in the event of accidents, even a minor one,.

CHEMICAL EXPERIMENTS

Chemical experiments use dangerous medicines, and operation errors can lead to explosions or fires, so utmost care should be paid. Listen carefully and follow the instructor's directions well at the beginning of the experiment.

Do not conduct an experiment alone in weekends or at night. Always conduct an experiment under the direction of the instructor. "In Order to Conduct an Experiment Safely" and "In Order to Conduct an Experiment Safely Sequel" (published by Kagakudojin) are very useful and informative reference books. They are available at the Central Library, and it is desirable to read them.

Ⅰ. GENERAL PRECAUTIONS

(1) Do not eat, drink, and smoke in a laboratory since there are a lot of deleterious, poisonous, and inflammable medicines.
(2) Always organize the working tables. Handle any tools with care to avoid any injuries.
(3) Conduct any experiments that might produce poisonous or irritating gases in the draft chamber.
(4) Wear a protective mask when conducting any experiments that might explode. Even in any general experiments, wear protective glasses to avoid any reactants from getting into eyes.
(5) Wipe the floor to avoid slipping. When the floor is wet after using the aspirator or after washing tools, make sure to wipe the floor.
(6) Do not look into or peek the mouth and inside of containers during the reactions. There are possibilities that the contents splash from the sudden/rapid reaction and cause injuries or intoxication if it gets into mouth.
(7) Chemical experiments often use the city gas for heating. After each experiment, always make sure to close the tap. If there is a gas leak, it is dangerous to carelessly turn on the electronic device such as exhaust fan, since it might explode. Open the windows, ventilate the room, then turn on the switch. Report to the instructor if you notice any gas leakages.

Ⅱ. HEATING

(1) Before heating, make sure that the reactor is not a closed system.
(2) Do not heat large glassware and combustible organic solvents by direct fire.

(3) As solutions may bump while heating, so do not point the injection port at the face nor look into it.
(4) Heat gradually.
(5) Do not put any combustible organic solvents (such as ether) close.

Ⅲ. DISTILATION

(1) To avoid bumping, a boiling tip should be put before heating. Once the boiling stops, wait until the solution cools down to put the new boiling tip.
(2) When distilling ethers, if peroxides are mixed in, it might explode in the end, so leave the residues and do not get them dried and hardened.

Ⅳ. AUTOCLAVE

(1) Check the normal pressure and the maximum operating temperature of the device and use it within the range.
(2) Regularly inspect the safety valve and other safety device. If the pipe clogs, there is a danger of explosion.
(3) Do not stock the ingredient more than 1/3 of the capacity of the container.
(4) When opening an autoclave lid, wait until it goes back to the room temperature and the normal pressure.

Ⅴ. HANDLING OF DANGEROUS TOOLS

(1) When handling any electric device, avoid electric shocks.
(2) When the electric device is broken, make sure to pull out the cord before fixing it.
(3) The rotor of the centrifugal devices should be always kept clean. To balance the centrifuge tubes, the solutions have to be less than 70% of the capacity. Do not try to stop the rotor's rotations by hand.

Ⅵ. ACCIDENTS AND EXAMPLES OF ACCIDENT RESPONSE

(1) The small amount of sample in the testing tube was mixed with a concentrated sulfuric acid. It generated heat, boiled, and splashed onto eyes and face [Instantly washed with a lot of water, so it did not leave any injuries].
(2) Sulfuric acid got spilled on the handler's lower leg, but the handler was hesitant to take off the hose, so the handler got a slight burn.
(3) When sucking a sulfuric acid with a whole pipette, the handler was spoken to by a person next to him/her, and ended up breathing it in [Fortunately, the concentration was weak, and the handler rinsed with water and there was no damage].
(4) Hydrogen peroxide (purity 30%) was left on the handler's hand and he/she got burned. The handler thought it was same as an Oxidol (hydrogen peroxide 3%) and did not think it was serious.

(5) The handler did not notice that hydrofluoric acid was on his/her hand during the experiment. He/she realized the seriousness of burn only after the handler felt a pain [The skin was cut open and there was no damage].
(6) During distilling the ethanol, the handler put the boiling tip after heating had started. It suddenly got bumping, and the ethanol boiled over. Fortunately, the handler was using the mantle heater, and it avoided catching fire. There are some other examples of putting the boiling tip in the middle of heating and got burned.
(7) During the concentration of ether in water bath, the flame of the gas burner got ignited and burned the handler. It was not the appropriate way to handle any inflammable liquids.
(8) While making the sodium hydroxide solution, the handler was mixing it near his/her eyes and it spilled to his/her eyes [The handler instantly washed his/her eyes with running water, saw a doctor, and there was no damage].
(9) The handler weighed sodium hydroxide, used it in the experiment. The handler wrapped the left over sodium hydroxide with the medicine wrapping paper, put it in his/her pocket, went home and got burned. This is the example of not understanding the risk of sodium hydroxide being deliquescence.
(10) While distilling acetonitrile, there was not enough cooling water, and the sparks from the variable transformer ignited the steam that was filled near the refrigerator. Fortunately, it was in the draft chamber, so there was no serious damage.
(11) The handler put metal tin to nitrobenzene and was adding hydrochloric acid. It suddenly generated heat and burned the handler. It was due to too fast dripping speed of hydrochloric acid and insufficient mixing.
(12) The handler got burned on skin from phenol solution. It would have been dangerous if it gets into eyes. Wear protective glasses.
(13) The handler was trying to move the water bath that was filled with boiling water, slipped on the wet floor, and the hot water was poured on the handler. The handler got burned and got hospitalized for a few weeks. When conducting experiments, it is necessary to pay attention to clothing, footwear, and tidiness around the experimental table.
(14) The handler poured the waste liquids of sodium cyanide into the waste liquid storage solution containing acid, and it generated hydrogen cyanide (hydrocyanic acid gas). [Store the compounds that generate hydrogen cyanide in the container that made to alkaline (pH10)].
(15) The container of piranha liquids (a mixture of concentrated sulfuric acid and hydrogen peroxide water) for washing glass tools were sealed, the generated gas were filled inside and exploded. When storing any substances that generate gas, make sure to secure the exhaust route.

EMERGENCY PROCEDURES

This paragraph describes emergency procedures that must be implemented immediately when unfortunate human accidents happen in laboratories or outside.

I . INTOXICATION BY MEDICINES

(Check each medicine's Material Safety Data Sheet (MSDS))

1. General Emergency Measures

Report the type and amount of the chemicals, the condition of intoxication (swallowed, inhaled, on skins, etc.), and the exact time of the accident to the Health Service Center and the Ambulance Service.

2. Accidentally Swallowed

If the person falls into a fit of convulsions or unconscious, perform CPR. Follow the followings in cases other than oil products, corrosive chemicals, acids, and alkalis.

(1) Put a finger or spoon to a throat or the back of tongue to help the person vomit. (Do not force it)
(2) Give water or milk to lower the concentration of the chemicals in the stomach, delay the internal absorption and protect mucous membrane. (Do not force it).
(3) Warm the body with blanket. Avoid heating externally.

3. Accidentally Inhaled

Inhalation of gas and steam: carbon monoxide, hydrogen cyanide, phosgene, hydrogen phosphide, hydrogen arsenide, sulfur dioxide, nitrogen oxide, hydrogen sulfide, hydrogen fluoride, diazomethane, hydrogen azide, mercury, etc.

(1) Move the person where there is a fresh air, and loosen the clothes.
(2) When the person's respiratory function is lowered, perform the artificial respiration.
(3) Call the ambulance and contact the Health Service Center.

4. Accidentally Touched the Skins

Concentrated nitric acid solution, hot concentrated sulfuric acid, chlorosulfonic acid, strong alkaline, phenol, acetic anhydride, picric acid, dichloroacetic acid, hydrofluoric acid, etc.

(1) Wash with water (such as shower) immediately.
(2) Take off clothes and pour enough water to the skin. (Do not forcefully take off the clothes.)

5. Accidentally Got into Eyes

(1) Open eyelids and wash with running water for at least 15 minutes.
(2) Even if there is no pain, go see a doctor or seek instructions from the Health Center.

6. Prevention from Intoxication

(1) When handling any chemicals with high risk, study their characteristics and accident prevention methods from MSDS, and prepare any preventive wears.
(2) Conduct the experiment inside the draft chamber with high ventilation efficiency.
(3) Inform the surrounding people of details on the experiment and prepare for any unexpected accidents.
(4) Always process the tools with resolution or detoxicant and wash them well.
(5) Wash hands after the experiment. Do not touch food before washing hands.

Ⅱ. BURNS

Burns can happen by various causes, but the principle of treatments is same as the chemicals.

1. Determining the Severity of a Burn

It is necessary to determine the severity of the burn to decide the treatments. Base on the following two items and the presence/absence of complications, comprehensively determine the severity of the burn.

(1) Burnt Area

Determining the ratio of burnt area to the whole surface area. In order to easily calculate the ratio of each body part, there is so called "the palm method," where the area of the patient's palm is estimated as 1% of the body surface area. The other method is called "the rule of nines," where the head equals 9% of the body surface area, thoracoabdominal part, back, both arms and legs equal 18%, and the genital area equals 1% of the body surface area (the case of infants is different).

(2) Depth of Burn (skin conditions)

The depth is determined by the intensity of the heat and the reaction time. It is determined based on the appearance and the pain of the skin (Ⅰ-First degree: red, painful, and irritated skin, Ⅱ-Second degree: blistering and strong/severe pain, Ⅲ-Third degree: whitish, or black from being carbonized and not much pain). It is hard to determine the degree of burn, and it might deepen as the time passes.

2. Classification of Burns

(1) Slight burns (outpatient treatments)
15% or less in Ⅱ, 2% or less in Ⅲ, it is rare to cause a shock.

(2) Moderate burns (inpatient treatments)
15~30% in Ⅱ, 10% or less in Ⅲ, all cases have the risk of causing a shock and the patient needs to be hospitalized.

(3) Severe/Serious burns (hospitalized in a general hospital)
30% in Ⅱ, more than 10% in Ⅲ, as well as burns to the face, hands and legs in -Ⅲ, involving respiratory track damage, electric injury, deep chemical burns, damages of soft tissues, broken bones. The patient should be hospitalized in a general hospital within 2~3 hours of injuries. Prioritize the whole body control.

3. Emergency Procedures

(1) Cooling
As a first aid measure, it is important to cool the area immediately. If the clothes are on fire, pour water to extinguish. Wash the area with water, and take off the clothes not to hurt the skin (never forcefully remove clothing). Cool the area for at least 30 mins to 2 hours (appropriate water temperature: 10~15℃). For the face, wrap ice cubes with some wet towels, and apply it onto the area. Keep moving it around not to cool the same area for a long time.

In case of severe burns, cover the wound area with a clean towel or sheet and send the patient immediately to a general hospital, preferably with cooling. Elevate the limb to prevent edema.

(2) Precautions

If any oils or zinc ointment are applied on the area, it can easily to get infected, so never use those. It is out of questions to apply a soy sauce to the area.

Ⅲ. FROSTBITES FROM COOLING DEVICES

Slight frostbites cause redness and discomfort, which recover in a few hours. For moderate frostbites, they become reddish purple and blisters. If it is severe/serious, it can lead to necrosis.

1. Emergency Procedures

Soak the frozen part in warm water at 40℃ (do not use anything higher) for 20 ~ 30 minutes. Even after the regular temperature is reached, elevate the part and keep the part at room temperature. If there is no warm water, or the frostbitten area cannot be soaked in water, such as in the ear, warm the area with warm part of the body (hands, armpits). Take off any wet clothes. Smoking should be prohibited since it can shrink the blood vessels.

N.B.; Never exercise or massage with snow or ice water to warm up.

Ⅳ. EXTERNAL INJURIES

First of all, stop the bleeding. Excessive bleeding can cause a shock.

1. Hemostasis (Stop Bleeding)

It is important to immediately try to stop bleeding. Do not directly touch the blood since it can cause various infections, therefore, the following measures should be taken, such as wearing rubber gloves or alternatives. When the injury is due to pieces of glass, wash with water, and remove the pieces first (Do not overdo it).

2. Pressure Hemostasis

To stop bleeding from capillary vessels, small arteries, or small veins, apply gauze to the wound, press strongly for 5~10 minutes, and bandage tightly around the area. Most bleeding can stop with this method.

3. Artery Hemostasis (Indirect Pressure Hemostasis)

This is a method of temporarily stopping bleeding by strongly pressing on the trunk of the bleeding artery with a finger when the bleeding does not stop with the pressure hemostasis. Press the hemostasis point close to the heart (there are a few points on the body where blood vessels can be pressed to stop bleeding by pressing the blood vessels) with fingers or hands.

4. Tourniquet

(1) If the bleeding does not stop with the methods described above, use it as the last resort, but it is better not to use it unless it’s really necessary.

(2) Use a rubber tourniquet or a triangular bandage to tie tightly.

(3) Record the time in some way.
(4) Encourage the patient not to despond.
(5) Loosen the tourniquet every 15~30 minutes.
(6) Do not perform tourniquet over 2 hours (if it continues, the tissues undergo necrosis).

Ⅴ. ELECTRIC INJURY

Direct current is less dangerous than alternating current. High frequency and voltage interchange (AC) are less dangerous than low frequency and low voltage AC. However, there is a case where low-voltage direct current (DC) such as 3 volts caused burns.

1. Emergency Procedures

Immediately release the patient from the electric current while being careful not to get electrocuted. For example, turn off the power, cut the wire using an axe with a wooden handle, route the electric current to another circuit, and move the patient/victim away from the wire using a dry cloth or leather. If the pulse has stopped, perform CPR.

Ⅵ. RADIATION EXPOSURE

There is no proper treatment for radiation exposure, and it is necessary to pay close attention to the preventive methods.

1. Emergency Procedures

For the whole body exposure, avoid re-exposure, rest and supplement nutrition. When a radioisotope adheres to the skin, wash it off immediately. If swallowed, try to eject from the body as much as possible. See a specialized doctor.

Ⅶ. ACCIDENTS AT FIELDS

1. Sprain, Dislocation, Fracture

(1) Sprain

A sprain is caused when a joint is twisted or a ligament is stretched suddenly. The area around the joint bleeds internally, gets feverishly swollen and painful.

[Emergency Procedures]

① When the injury is light, apply a cold compress, or bandage with a poultice.
② If swollen badly and very painful, it might be cracked. Go see an orthopedic surgeon.

(2) Dislocation

A joint dislocation is caused when a hand or a leg is suddenly pulled, or strong force is applied. It is very painful, and the local area might deform or bend in an unusual direction.

[Emergency Procedures]

A dislocated joint can be successfully put back in place, but when the area is hit hard, it is hard to differentiate from a fracture. It is better to see an orthopedic surgeon.

(3) Fracture

There are following types of fractures according to the degree and type.

① Infraction fracture: It is not broken but the bone has a crack.

② Complete fracture: The bone is completely broken, and the fracture is separated.

③ Simple fracture: There are no external injuries, just a broken bone.

④ Open fracture: The broken bone fragments stick out through the skin.

⑤ Complex fracture: Not only is the bone broken, but the blood vessel is cut and bleeding, and nerve, muscle, etc. are damaged.

The infraction fracture is sometimes hard to differentiate from a sprain, but other fractures cause severely pain, localized swelling, and the shape of the bone might look deformed.

[Emergency Procedures]

① Do not move hands or legs, and restrain where it is less painful and comfortable.

② When the fracture is accompanied by bleeding, stop the bleeding first.

③ Splint a fracture and go to a hospital for medical treatment.

2. Sunburn and Heatstroke

(1) Sunburn

When the sunlight is strong, even being under the direct sunlight for a short period of time, you might get a severe sunburn. As the sunburn gets worse, the skin reddens and become painful. Fair-skinned people can get blisters.

[Emergency Procedures]

① When it is slightly sunburned, apply an appropriate amount of after-sun lotion or cream.

② When the blisters break open, treat them the same as burns.

(2) Heatstroke

A heatstroke is a disease that caused when there are not enough water and salt in the body due to intense exercise or activities under high temperature. The symptoms start with thirst, fatigue, dizziness, lack of clear vision, nausea, headache and muscle cramps. If the condition gets worse, the symptoms may include vomiting, pallor, cold skin, and sometime a stupor.

[Emergency Procedures]

① Immediately stop exercise or activity and rest in shades.

② Drink cold water that contains some salt and sugar or a sport drink until the thirst stops.

③ When the symptom is serious, go see a doctor or call an ambulance.

3. Frostbites

Frostbites are caused when wet skins are left exposed to a cold air. They are usually seen on the tips of fingers, ears, and nose. When it is minor, it gets red, swollen, and itchy. Once it gets worse, the skin can turn purple and gets blisters.

[Emergency Procedures]

① For minor frostbites, dry the skin and massage the area to improve blood circulation.

② For serious frostbites, dry and warm the skin, then go see a doctor.

4. Drowning

[Emergency Procedures]

① Wipe off any dirt from one's mouth and let the water eject.

② If there is no signs of breathing or no pulse, perform CPR and call an ambulance.

③ If the person is breathing, wrap the body in a blanket or futon for warmth and lay down.

5. Bitten by Dangerous Animals

(1) Bitten by a dog

[Emergency Procedures]

① Wash the wound with soap water for about 10 minutes.

② Leave the wound, and go to the Health Center or a hospital.

(2) Bitten by a snake

[Emergency Procedures]

① Tie the area closest to the body.

② Squeeze out the poison along with blood. Do not suck the blood. Use an aspirator if available.

③ Wash with water and cool the area until the patient goes to a hospital.

④ Do not cover the wound, and go to the Health Center or hospital.

(3) Bitten by other animals

[Emergency Procedures]

Procedures depend on the types of animals. Immediately go to the Health Center or hospital.

6. Sting by Dangerous Animals

(1) Stung by a wasp or a bee

[Emergency Procedures]

① Check the spot that is bitten. If there is a small black needle like spot, do not force to take it off, and go see a doctor. Use an aspirator if available.

② Wash the spot well and cool it to relieve pain and swelling. Do not close the wound.

③ When the symptom is bad, go to the Health Center or hospital.

④ Be careful with any signs of allergies such as difficulty in breathing, wheezing, skin flare and not feeling well.

(2) Stung by other animals

[Emergency Procedures]

Procedures depend on the types of animals. If symptoms are severe, seek immediate medical attention from a surgeon.

7. Rash from Dangerous Plants

[Emergency Procedures]

① When it is a minor rash, remove the toxin and use antihistamines.

② When the symptom is serious, go to the Health Center or hospital.

Major Laws and School Regulations Concerning Safety

1) Order for Enforcement of Industrial Safety and Health Act
https://elaws.e-gov.go.jp/document?lawid=347CO0000000318_20231001_505CO0000000069 (Japanese)

2) Fire Services Act
https://elaws.e-gov.go.jp/document?lawid=323AC1000000186 (Japanese)

3) Poisonous and Deleterious Substances Control Act
https://elaws.e-gov.go.jp/document?lawid=325AC0000000303 (Japanese)

4) Act on the Assessment of Releases of Specified Chemical Substances in the Environment and the Promotion of Management Improvement (PRTR Law)
https://elaws.e-gov.go.jp/document?lawid=411AC0000000086 (Japanese)

5) Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms (Cartagena Protocol on Biosafety)
http://www.lifescience.mext.go.jp/bioethics/anzen.html (Japanese)

6) National University Corporation Kagoshima University Worker Industrial Safety and Health Management Regulations
http://www1.g-reiki.net/kagoshima-u/reiki_honbun/x890RG00000060.html (Japanese)

7) Kagoshima University Radiation Safety Management Regulations
http://www1.g-reiki.net/kagoshima-u/reiki_honbun/x890RG00000096.html (Japanese)

8) Kagoshima University Recombinant DNA Experiments Safety Management Regulations
http://www1.g-reiki.net/kagoshima-u/reiki_honbun/x890RG00000097.html (Japanese)

9) National University Corporation Kagoshima University Waste Liquids Processing Regulations
https://www1.g-reiki.net/kagoshima-u/reiki_honbun/x890RG00001036.html (Japanese)

10) Regulations Relating to Animal Experiments at Kagoshima University
http://www1.g-reiki.net/kagoshima-u/reiki_honbun/x890RG00000584.html (Japanese)