Basic Procedures for Applied Biology Experiments **(Ver. 1.5)**

– For Carrying Out Experiments Safely and Properly –

1. Introduction

1.1 Experiments Are Essentially Dangerous

If you do not take sufficient precautions when carrying out experiments, accidents resulting in injury or fire and explosion may occur. In particular, when using the following, you must have sufficient background knowledge and should take sufficient precautions.

- Dangerous reagents and samples (e.g., flammable, explosive, carcinogenic and pathogenic reagents)
- Machines exposed to high temperature (consider that all electric apparatuses can cause fire or burn)
- Machines exposed to high pressure (e.g., autoclave and evaporator)
- Machines having high kinetic energy (e.g., centrifuge and shaking incubator)
- Fragile items (e.g., glassware)

If you cause an accident, you may be injured and people around may also be injured. If you cause a fire accident, you may lose not only expensive research equipment but also very precious samples and data. (If this situation occurs, you might cause much trouble to other persons that you might regret for the rest of your life.) If you misuse or fail to maintain equipment, not only does the lifetime of expensive equipment shorten but also the experiment fails or the accuracy of equipment markedly decreases.

1.2 Being professional

Apparatuses used for research are manufactured for professionals. Because many apparatuses are different from home electronic equipment, they are very dangerous when used carelessly. A few years ago, there was a small fire at the Suita campus, which was caused by a student who was not expert in machine operation. Excuses, such as "I did not know the right operational procedure," "I have not learned how to use the equipment yet," "it was too much bother to study the operational procedure preliminarily," and "it was troublesome to follow the procedure even though I knew it" are never acceptable. In the dictionary (Kojien), "professional" means "technical, occupational." You are a professional because you conduct a study technically and responsibly as a researcher. You must be a professional, and **you must study the proper handling of the experiment and reagents to be used before starting the experiment to avoid oversight (seniors and instructors are not always right)**. It is one of the basic duties of a researcher to use an apparatus properly, improve the efficiency of the experiment and check the accuracy of the apparatus.

Most accidents are a result of "human error," which everybody commonly makes. **However, leaving the scene of an accident unattended or neglecting to report an accident is not "human error." It will not be tolerated**. In addition, it is a criminal act to leave the scene of an accident unattended knowing that it may injure other people.

In this text, the following summarizes the general procedures for biochemical experiments.

(1) Precautions for safe experimental operation
(2) Precautions for preventing shortening in lifetime of experimental apparatuses
(3) Procedures for carrying out experiments efficiently and maintaining good accuracy

When using special apparatuses, which are not mentioned in this text, receive sufficient instruction from the principal investigator or instructor, and carefully read the user instruction manual.

2. Carrying Out Safe Experiments

2.1 Daily Life

2.1.1 Working time

As a rule, carry out experiments with an instructor present. Without an instructor, you cannot get proper advice or properly respond to an emergency. There are many accidents that can be avoided (that can be recovered) with just one piece of advice from the instructor. The following accidents in Japanese Universities occurred when the instructor was absent.

- Explosion of vacuum evaporation system resulting in the death of two graduate students
- Electric shock from the Gene Pulsar (electroporation machine) resulting in the death of one graduate student
- Explosion during ether distillation resulting in the blinding of one undergraduate student
- Explosion of vial container for freeze-drying resulting in a face laceration in one graduate student

2.1.2 Abnormal odors or sounds

Abnormal odors and sounds are warnings of an accident. If abnormal reaction, overheating of an apparatus or a cable, abrasion of a driving part and gas leakage are left untreated, there is a high risk of a serious accident. Immediately inform the instructor of the situation (even at midnight) and receive instruction on how to proceed.

2.1.3 Headache or bad condition

If one develops a headache or does not feel well, such as lassitude, during the experiment, **stop** the experiment immediately and check the surroundings. It is possible that an odorless harmful gas, such as carbon monoxide, has been generated. Even if the headache comes from a cold or fatigue, stop the experiment at once. Continuing the experiment when tired may lead to a serious accident.

2.1.4 Apparatuses requiring electricity

(1) Do not touch an apparatus with wet hands. Otherwise, you may get an electric shock.

(2) Ground the apparatus. In the case of electrical leakage, there is a risk of an electric shock.

(3) Do not use an extension cord carelessly. For example, the use of an extension cord with a capacity of only 6 A for a 1500-W incubator (15 A of current passes) will cause fire (refer to 2.5.1(4)).

2.1.5 Precautions before leaving laboratory

(1) Cleaning up

Clean the instruments and equipment used in the experiment, as well as the laboratory table. Inform the person staying in the laboratory about your leaving.

(2) Points to be checked by the last person leaving the laboratory

1) Gas valves and water heaters are turned off.
→ to prevent fire

2) Apparatuses that have not been marked for all-night running are turned off.
→ to prevent fire and save energy

3) Air conditioners and stoves are turned off.
→ to prevent fire and save energy

4) Doorways and windows are locked.
→ to prevent theft

5) Lights are switched off.
→ to save energy

(3) Consideration for last person leaving laboratory

The second to the last person leaving the laboratory has to inform the last person that he/she will be the last person in the laboratory. Be sure to help him/her if he/she wants.

2.1.6 All-night running

(1) Indicate user's name and scheduled termination date and time

When running an apparatus all night, be sure to indicate the user's name and scheduled termination date and time next to the apparatus. The last person leaving the laboratory has to **turn off** any apparatuses that have not been clearly indicated for all-night running. Even if the experiment fails as a result of the apparatus being turned off, the person who did not indicate the necessary information is responsible for the failure.

(2) Check steady operation

When running an apparatus all night, do not go out until it becomes steady. Start running an apparatus one hour before going out, as a rough measure, and be sure to check that it is running in the steady state, that

is, current (voltage) or temperature has reached the preset value. If you must go out unavoidably, ask someone staying in the laboratory to check the above. This is important not only for security but also for preventing experiment failure.

2.1.7 Stove and gas burner

(1) Keep flammable materials away from a stove or a gas burner. Never place any containers with organic solvents near an open flame.

(2) Switch off the stove or gas burner when leaving the laboratory.

(3) **Even when just "holding" or "picking up" an organic solvent bottle, put out all open flames including that in the stove or gas burner**. At a certain university, an organic solvent bottle was dropped and subsequently broke, resulting in one laboratory catching fire owing to a stove that had left on and being totally burned down. Some students did not graduate in that year because all the precious experimental data and research samples were burned to ashes. No flames are permitted not only when "using" organic solvent but also when "holding" bottles containing organic solvent.

2.1.8 Food, beverages, and smoking

Food, beverages, smoking, and cosmetics are prohibited in the laboratory, for the following reasons.

(1) Poisonous and deleterious substances, carcinogens and pathogenic microorganisms are used in the laboratory, and are very dangerous when consumed. These harmful substances may be extensively spread on the skin by cosmetics.

(2) Spilled food or leftovers become a breeding ground for bacteria and cause contamination.

2.1.9 Cigarette butt

Smoking is not allowed in the laboratory or the corridors. **Be sure to pour water on cigarette butts in an ashtray before disposal.** There has been a case of fire breaking out 18 hours after a cigarette butt was extinguished in an ashtray. **Be sure to dispose of cigarette butts after pouring water even though they look extinguished.**

2.1.10 Energy saving, water saving and water leakage

(1) If you will be away from your desk for a long time, shut down the computer.

(2) Turn off the lights and air conditioner in an unattended laboratory unless there is a special reason.

(3) Do not use the aspirator.
To save water, use the circulation water pump or diaphragm pump if possible.

(4) Do not use cooling water carelessly.
If using cooling water in a distillation apparatus, a rotary evaporator and a jar fermenter, take care not to use too much. Also be careful of increased water pressure because water usage decreases at night. There has been a case of an expensive apparatus becoming unusable because the cooling water hose broke (disconnected from the faucet) and the downstairs was flooded when water pressure increased at night. Be sure to use a clamp when connecting the hose to the faucet.

(5) Adjustment of reverse osmosis water
More than 200 L of tap water is used per 20 L of reverse osmosis water in the reverse osmosis water maker for washing reverse osmosis membranes. Waste of reverse osmosis water is equivalent to a 10-fold waste of tap water.

2.2 Handling and Cleaning of Glassware

Among many types of injury that may occur in the laboratory, laceration (cuts) from glass, in addition to burns, is the most frequent. At worst, tendons and nerves may be cut by glassware, causing interference with everyday activities. **Do not forget that glassware is easily broken with the application of too much force**.

2.2.1 General safety precautions

(1) Dispose of cracked glassware
Cracked glassware should be handled with care. Broken glassware may not only cause injury but also spread dangerous substances, such as organic solvent and poisonous and deleterious substances. Dispose of them upon notice of cracks.

(2) Immediately treat a broken glass container properly
If the brim (rim) of the glass container is chipped, make the chipped area smooth by filing or heating in a burner. If it is left unfixed, the next user may be injured.

(3) If a container with a large volume is necessary, use

Beakers and measuring cylinders with a volume of 1 L or more are expensive and easily broken (refer to Table 1). Plastic containers should be used except under special conditions, such as during the use of organic solvent.

(4) Arrangement and organization

It is not only inefficient but also dangerous to carry out experiments in a small space. Samples may spill, or glassware may collide with other objects, break or fall from the laboratory table. Always keep the laboratory table neat and in order, and ensure sufficient working space.

(5) Prevention of glassware being knocked off tables

Do not place any objects at the corners of laboratory tables. When a person passes by, such objects may be caught, fall down and break. Try to move glassware, which has a possibility of being knocked off by a person passing by, to a safe place.

(6) Avoid directly writing on glassware with permanent markers

If a permanent marker (in particular, a thick marker) is used for writing the sample name on the glassware, it is difficult to erase it even if washed and scrubbing with too much force may cause glassware breakage and injury to the person. Label with a vinyl tape and write the sample name on it. If something has been unavoidably written with a marker, dip the glassware into water overnight to easily remove the ink or erase it with solvent, such as ethanol or acetone, and then wash the glassware.

(7) Placement of magnet bar of stirrer

When putting a magnet bar into a glass container, slide the magnet bar into the tilted container slowly. Because the stirrer contains a strong magnet, if it is put into the glass container after the container is placed on the stirrer, the container will be broken by the strong impact.

Table 1 Regular price of glassware (Yen)

Volume (mL)	Beaker	Conical flask	Measuring cylinder
500	630	860	4140
1000	1200	1580	8820
2000	2950	3780	12100
3000	4830	5150	—
5000	8200	8500	—

2.2.2 Insertion of glass tube into rubber stopper (insertion of pipette into pipetter)

(1) Do not forcibly insert a thick glass tube into a small hole.

(2) Insert the glass tube after putting water (vaseline can be used if circumstances allow) onto it for lubrication. However, water and vaseline should not be used to insert the measuring pipette into the pipetter.

(3) **Hold the glass tube with three fingers, that is, the thumb, index finger and middle finger approximately 2 cm from the tip, and insert the tube carefully and slowly while rotating it**.

In most cases of breakage, the glass tube breaks at the base of the rubber stopper or a few cm from the base. If the glass tube is inserted while being grasped at a position far from the rubber stopper, a strong bending stress is applied to the glass tube around the rubber stopper section as a supporting point. If the glass tube is grasped with five fingers, bending stress is increased by the force applied by the ring finger and little finger, which are further from the supporting point. In addition, the glass tube is pushed up by the thumb and pushed down by the little finger around the rubber stopper section as a supporting point, and then receives sufficient force to break the glass tube at the thumb position. Therefore, grasp the glass tube at a position 2 cm or less from the rubber stopper with three fingers, that is, the thumb, index finger and middle finger and not using the ring finger and little finger, and almost all breakage can be prevented. Do not insert the glass tube with five fingers even though adequate force cannot be applied with three fingers. Difficult insertion with three fingers is due to the hole in the rubber stopper being too small. Therefore, enlarge the hole to an appropriate radius.

2.2.3 Hold large glass container and dangerous reagent bottle with both hands

(1) Do not grab a beaker with a volume of 500 mL or more with one hand. If the beaker with solution is grabbed with one hand, it will have force applied on it by the fingers to support the weight. This force may break the beaker. An acquaintance of the author had a nerve in his finger cut, which required nearly a year for rehabilitation.

(2) Hold a gallon bottle (3 L) with both hands. If the bottle is carried by grasping only the handle on the neck of the bottle, the handle may break off owing to the bottle's own weight. Consider that the handle is attached not for carrying but for tilting the bottle when

pouring the contents.

2.2.4 If lid of glass container cannot be opened

Take particular caution when handling thin-glass containers, such as vial containers and screw-top test tubes. Do not forget that **the glass is easily broken by too much force.** Think of a smart way to open the lid naturally, for example, cool the glass part of the container and warm the lid. Although common household jam jars have sufficient thickness so that they cannot be broken by force, vial containers and screw-top test tubes are made of thin glass that can be easily broken by force.

2.2.5 Opening of ampoule

There are several possible reasons for storing a reagent in an ampoule. For example, the reagent is very sensitive to oxygen or moisture, it has an extremely bad odor, or its vapor is extremely poisonous. First, know the reason why the reagent is stored in an ampoule and be sure to make adequate preparations for its use.

(1) Prepare a sealable container with adequate volume if the reagent in the opened ampoule is not used up completely.
(2) In the case of a volatile substance, sufficiently cool its ampoule and wipe the moisture well.
(3) Scratch the ampoule with a file.
(4) Open the ampoule taking care not to cut one's hand when breaking it. Grasp the ampoule with the left hand and break the ampoule with the right hand as if holding the ampoule and the left hand together (for a left-hander, reverse the hand). It is also better to break the ampoule wrapped in a dry cloth.

When opening an ampoule with a volume of 5 mL or more or that with dangerous reagents (e.g., poisonous, special flammable and explosive substances), be sure to follow the instructions of a well-experienced instructor.

2.2.6 Accidental Fall --- Cleanup of broken glassware

When glassware breaks, avoid cleaning the broken glass chips with bare hands as much as possible; use the broom or vacuum cleaner. Completely collect the broken glass chips because they are very dangerous. If glassware containing solution is broken, wipe the spilled solution with paper towel. Never use floor cloth. If a floor cloth is used, minute fine chips will remain on it that may cut the user's hand when squeezing the cloth.

2.2.7 Other precautions when handling glassware

(1) Ground glassware

1) The bore and pitch of the body of a ground glassware must correspond to those of the lid. The body and lid have a number, which must be the same for sealing.
2) Do not rotate the ground part under dry condition. Otherwise, the glass will be cut and cannot be sealed.
3) When storing it, keep the reed-shaped paper between the body and lid after drying it (otherwise, the lid cannot be removed from the body).
4) Do not use it for storing alkali solutions. The glass will dissolve in the alkali solution.

(2) When writing something with marker pen

Do not write anything on the part printed with white characters or the fogged glass part as a rule. Otherwise, it cannot be erased.

(3) Be sure to remove the label and marker ink

If glassware with a label and marker ink is placed into a dry heater or dryer, it will become difficult to remove them. If the label is forcibly removed, it may cause the glassware to break.

(4) Accuracy of scale

Except for glassware used for measuring, such as measuring cylinders and measuring flasks, it is common for the scale to have an error of 5% or more. Many calibrated test tubes have an error to this extent. In experiments requiring accuracy, measure the weight of the glassware with a certain amount of water or check the accuracy by measuring a certain amount of water in a measuring cylinder or measuring pipette before use.

2.2.8 Caution in cleaning

(1) Secure work space.

Secure sufficient work space when washing. Start washing after making at least half of the sink open (putting some of the instruments to be washed in a tray and taking them out from the sink).

(2) Do not leave unwashed instruments in the sink

A professional housekeeper immediately washes the dishes used. This is because much labor is required to remove stains that have dried up on dishes. If for any reason you cannot immediately wash glassware,

soak it in water in a tray and **place it on your own laboratory table**. If it is left in the common sink, you will inconvenience the next users for the following reasons.

1) It cannot be determined whether the glassware was used with a dangerous reagent.
2) Sufficient space cannot be secured.
3) Necessary glassware is not available when needed.

(3) Lay down the measuring cylinder when placing it on the sink.

The measuring cylinder, the center of gravity of which is at a high position, can easily fall down and break. Be sure to lay it down in the sink.

(4) Be sure to remove marker ink and tape.

If glassware with marker ink and tape is put in a dryer (dry heater), the label cannot be removed after. Even if it can be removed, much force is required which may break the glassware.

(5) Do not use cleansers

Do not use a cleanser when removing marker ink or engrained stains. Even cream cleansers may cause fine cracks on the glass surface. In such a case, not only will the glassware become easily breakable but also the stain on the crack will be difficult to remove, influencing experiment accuracy.

(6) Brush the instrument sufficiently

Glassware cannot be completely cleaned by merely brushing it a few times. Check that the brush shape is appropriate for the glassware to be cleaned and check that the glassware is sufficiently brushed (in particular, that the bottom corners are properly brushed). Use a sponge for open-mouthed containers, such as a beaker. It is desirable to use special glassware for phosphorus determination, reducing sugar determination by the phenol-sulfuric acid method, and fluorometric microanalysis. For example, glassware can be thoroughly cleaned when it is placed in a large stainless-steel container, dipped in approximately 0.5% mild detergent (not alkali detergent) and then autoclaved.

(7) Precautions for alkali detergent use

When using alkali detergent, wear protective glasses and gloves. Glass can be dissolved by a strong acid. If glassware is dipped into an alkali detergent or unnecessarily heated, the glass surface will melt and fine cracks will appear; therefore, exercise caution.

(8) Wash not only the inside of glassware but also the outside

Wash the outside and rim of glassware with a sponge. It is highly possible that a solution is dried up and solute is engrained around the rim. Unless stains on the outside are removed, the glassware rim will be contaminated by moisture rolling down the outside when it is dried upside down.

(9) Rinsing

Rinsing is more important than brushing. If the glassware is insufficiently rinsed, "it will be contaminated with detergent." It is desirable to rinse it at least five times, in particular, at least 10 times for a test tube.

(10) Rinse the glassware with pure water

When preparing a reagent with pure water, there is no sense in using glassware rinsed with only tap water. Rinse the outside of the glassware as well as the inside with pure water after rinsing off the detergent with tap water.

(11) If water is spilled on the floor, wipe it immediately

Wet floors are slippery and very dangerous. Immediately wipe the floor with a mop.

Accidents will happen if you forget the common sense that "glass cannot withstand too much force" and "broken glass is an edged weapon." For example:

(1) When inserting glass tubes (e.g., pipette) into rubber stopper (safety pipetter) (refer to 2.2.2).
(2) When grabbing beakers by their top (refer to 2.2.3).
(3) When forcibly opening lids of vial containers (refer to 2.2.4).
(4) When scrubbing sample names written with a marker pen to remove it (refer to 2.2.7(5)).
(5) When leaving broken glassware unattended (refer to 2.2.1(2),(3) and 2.2.6).

2.3 Autoclave

2.3.1 Autoclave structure

Among the many possible injuries that can occur in the laboratory, a burn caused by the use of an autoclave is common as well as laceration from glass. Use the autoclave bearing in mind that its operation has risks.

Figure 1 shows the structure of the most common autoclave in the field of biochemistry. The time to maintain the obtained temperature after the temperature reaches the set value (the range indicated by the arrow in Figs. 2(A) and (B)) is set on the timer and the standard time is 10 – 20 minutes at 121°C.

2.3.2 Operation

(1) Check that the autoclave is filled with water up to the bottom plate level.

(2) Check that the drain valve is closed.

(3) Fasten the lid moderately.
If the lid is fasten insufficiently, the vapor will leak, and if fastened too tightly, the packing will deteriorate fast, also causing vapor leakage. It is better, as a rule, to turn the handle using the forefinger to the end, and then fasten it by turning the handle by 90 degrees with both hands.

(4) Set the time and start the sterilization.

(5) Wait until the temperature decreases to at least 70°C (refer to 2.3.3(2)-(3)).

(6) Check that the temperature is 70°C or less and the pressure is zero. Actually, there was a case in which the thermometer was broken and the spewing vapor caused a heavy burn on the upper body of the victim. Be sure to check both values.

(7) In addition, open the drain valve and check that no vapor spews, and then open the lid (some autoclaves have no drain valve or automatic open and close system).

2.3.3 Safety precautions

Do not leave the laboratory unattended until the timer cuts out. This is because no-water burning will be caused if the vapor leaks caused by the aged deterioration of the packing. If vapor leaks, the sample will continue to be heated up because the temperature cannot reach the set value and the timer cannot start. Finally, all of the water will be exhausted, causing no-water burning (refer to Fig. 2(C)).

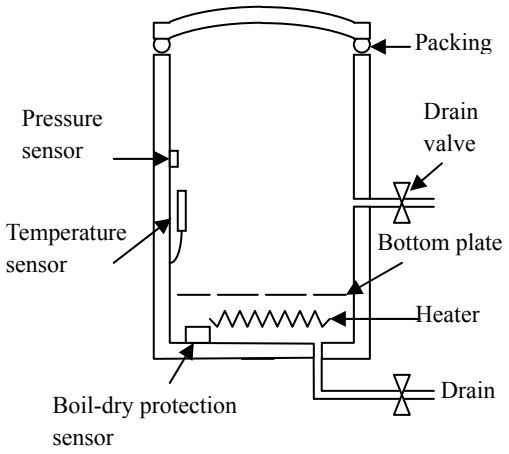

Fig. 1 Structure of autoclave

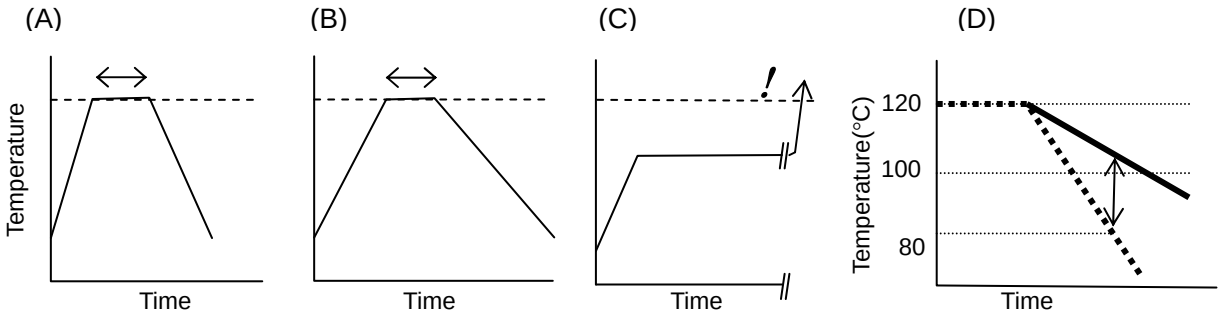

Fig. 2 Change in temperature in autoclave with time

(A) A small amount of medium is sterilized, (B) a large amount of medium is sterilized, (C) when the vapor leaks, and (D) the deviation of the real temperature of difficultly cooled liquid (solid line) from the value indicated on the thermometer (dashed line)

(1) **Do not leave the laboratory before the timer is shut off**. **This rule should be strictly applied when everyone leaves the laboratory for a magazine meeting or seminar meeting**. **Do not place too much confidence on no-water burning protection sensor because it is not assured to always correctly function and it cannot be checked whether the sensor correctly functions**.

(2) There is a deviation (difference) of the real temperature of the autoclaved liquid from the value indicated on the thermometer (the thermometer part is cooled earlier than the liquid because the outside of the autoclave is first cooled). **The real temperature of agar medium, antiforming agent, highly viscous liquids, such as sugar at high concentration and glycerin, and a large amount of solution may be more than 100°C even though the thermometer indicates a temperature less than 80°C** (Fig. 2 (D)). When taking out such a sample, sudden boiling may occur, causing a burn. When it is autoclaved, wait until the value indicated on the thermometer decreases to 60 - 70°C, take it out to avoid sudden boiling, and **never shake up the solution just after it is taken out**.

(3) Do not put agar medium into a container exceeding half of its volume. For example, when using a conical flask with a volume of 500 mL, the agar medium limit is 250 mL. If more agar medium is used, the risk of sudden boiling increases, causing problems when mixing after autoclaving (the concentration of agar around the bottom after autoclaving is high because agar sinks). If agar is mixed just after autoclaving, sudden boiling may occur. Leave it to cool at room temperature for a few minutes.

(4) Do not autoclave a sample with a low boiling point, such as organic solvents. To do so may cause fire or explosion; in addition, the bad smell produced will disturb others. The concentration of the sample will markedly change because of evaporation.

2.3.4 Other precautions

(1) If the apparatus is to be autoclaved, be sure to check that it can be autoclaved by referring to the catalogue and other information sources. For example, the Pipetman of Gilson cannot be autoclaved. Many plastic apparatuses cannot be autoclaved.

(2) A volatile strong acid (hydrochloric acid, nitric acid) can damage the autoclave. Sterilize it using a filter made of appropriate material.

(3) If a strong alkali is autoclaved, it will dissolve glass. If a glass container is repeatedly autoclaved many times, the glass will progressively become thinner, causing breakage (pay attention because glass components dissolve in alkalis).

(4) If the jar fermenter is autoclaved, do not open the lid until the temperature decreases to 60°C or less as a rule. It is difficult to cool a large amount of medium and it not only has a risk of sudden boiling, as shown in Fig. 2 (C), but also may damage the pH sensor and dissolved oxygen (DO) concentration sensor owing to the change in its pressure.

(5) Be sure to loosen the lid of the container to be autoclaved. If sealed, the pressure outside the container increases prior to the inside as temperature increases, and decreases as temperature decreases. This pressure difference may cause the breakage of a glass container or the deformation of a plastic container.

(6) If a plastic container, such as a centrifuge tube, is autoclaved, the lid will be pressed closely against the body when the container is cooled having a negative pressure after autoclaving, and will be deformed when the container is further cooled having a lower inner pressure. Therefore, sterilize the container with the lid removed, or wrap the container with aluminum foil while the lid is being sufficiently loosened so that the lid cannot be pressed closely against the body.

(7) An uncracked medium bottle resistant to approximately one atmospheric pressure can be sealed and autoclaved. However, it cannot be sterilized unless a drop of distilled water is put in the bottle (it takes at least three hours at 160°C to sterilize the sample under a dry condition).

(8) Overheating may transform the sample. If compounds with amino groups and reducing sugars, such as yeast extract and glucose, are autoclaved at the same time, the Maillard reaction occurs; therefore, the medium turns brownish and the sample strain may be prevented from growing. The longer the heating time and the higher the pH (pH>6), the more marked this reaction. Moreover, this reaction occurs more markedly when a large amount of medium is autoclaved (Fig. 2 (B)) than when a small amount of medium is autoclaved (Fig. 2 (A)).

(9) If the sample is spilled (boiled over) in the autoclave,

Examples of accidents caused by using the autoclave are as follows.

- A bottle, 90% filled with agar medium, was autoclaved with the lid closed. When it was taken out at 90°C or less, sudden boiling occurred and the flying lid caused a burn on the face of the victim.
- When a jar fermenter was taken out at 90°C or more, sudden boiling occurred, causing a heavy burn on the upper body of the victim (a keloid remains).
- An antiforming agent was autoclaved in a conical flask. Although the antiforming agent filled a quarter of the flask, sudden boiling occurred when it was taken out at nearly 90°C, causing a burn that required a month for recovery.

(1) Do not leave the laboratory unattended until the timer shuts off.
(2) Do not open the lid until the temperature decreases to 70°C or less.
(3) A sample with a low heat conduction (high viscosity or a large amount) has a risk of sudden boiling even at 70°C or less.
(4) No organic solvent, strong acid and strong alkali should be autoclaved.

2.4 Centrifuge

First, please answer the following questions (the answers are on the next page).

Q1. Is it permitted to use a combination of differently shaped centrifuge tubes if they are balanced with the scale?
Q2. When there is only one sample, can the sample be balanced with another centrifuge tube of the same shape filled with water?
Q3. When there is only one sample, can the sample be balanced with another centrifuge tube of the same shape filled with a solution, such as sucrose, that has the same specific gravity as the sample?
Q4. Is it unnecessary to switch on the cooler when centrifuging at room temperature?
Q5. Why is the moisture wiped before balancing when the outside of the centrifuge is wet?
Q6. What might happen when a sample leaks from a tube broken during centrifugation?
Q7. Why should the centrifuge rotor be left upside-down after use?
Q8. Why is the cover of the chamber closed during cooling condition and open after use?

Did you answer all the questions correctly? The centrifuge is rotated at a high speed and its kinetic energy is high. If used in a wrong way, a serious accident may result and the lifetime of the machine may be markedly decreased. Know the correct method of use and understand the reasons why such a method is used.

A1. No (There is no balance of moment.)
A2. No (There is no balance of moment.)
A3. No (If separated, the sample will have an imbalance of moment.)
A4. No (There is an increase in temperature due to the heat generated by the motor and the friction between the rotor and air.)
A5. Water on the outside of the centrifuge tube is transferred to the bottom of the rotor by centrifugation, causing an imbalance of moment.
A6. (1) Machine damage (spindle, motor and rotor) due to imbalance.
(2) Biological contamination, chemical contamination, corrosion and fire (explosion) caused by wet sample.
A7. To avoid imbalance owing to dust dropping on the rotor and precipitating water.
A8. Frozen ice in the chamber falls off by the wind pressure generated during centrifugation and damages the rotor.

2.4.1 Preautions concerning maximum rotating speed

Use a centrifuge at 80% or less of the maximum allowable rotating speed as a rule. Because each rotor of the centrifuge has a specified maximum allowable rotating speed, be sure to check the speed before use. **Never use the centrifuge at a speed exceeding the maximum allowable rotating speed for any reason.** The maximum allowable rotating speed indicates the allowable rotating speed of the rotor, which has no contamination or scratches, used correctly with regular maintenance. Therefore, normally use the centrifuge within 80% of the maximum allowable rotating speed of the rotor.

If the rotating speed is decreased by 20%, an almost equivalent centrifuging effect can be obtained by increasing the running time by 50%.

2.4.2 Precautions concerning centrifuge tube

(1) Check the allowable centrifugal force and proof-solvent performance

Be sure to check to what extent the centrifuge tube to be used can withstand centrifugal force. If solvent or a strong acid is centrifuged, also check whether the material of the centrifuge tube can withstand them. Refer to Table 2 and the web sites below (it is advisable to bookmark them). The allowable maximum centrifugal force is the value obtained when the rotor shape and centrifuge tube shape are properly fitted. For example, a centrifuge tube with sharp edge is used with a round-bottom rotor, the centrifuge tube may be broken even with centrifugal force within the allowable range.

http://www.hitachi-koki.co.jp/himac/support/m-tube.htm: endurances of centrifuge tubes made by several companies (in Japanese)

http://www.nalgenunc.co.jp/html/info.shtml: endurance, proof-solvent performance, cleaning method, sterilization method (in Japanese)

http://www.assist-sar.co.jp/: endurances of several plastic products (in Japanese)

(2) Do not use a deformed or cracked centrifuge tube

The centrifuge tube inevitably deteriorates as it is used. Do not use a deformed or cracked centrifuge tube. Note that some centrifuge tubes may be deformed when a small amount of sample is centrifuged even at less than the allowable centrifugal force, as is the case with Teflon centrifuge tubes (80 - 90% of the centrifuge tube must be filled with solvent). Note that a disposable centrifuge tube is not designed for repeated use, as its name suggests.

2.4.3 Precautions when balancing

For centrifugation, not weight balance but moment balance (weight× turning radius of center of gravity) is required. This can be easily understood by comparing the case of a piece of clay attached to the center of a string (a) with that of a piece of clay with the same weight on the end of a string (b) shown in Fig. 3. It is clear that the force given to the arm when swinging the string is higher in (b).

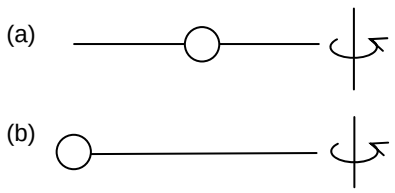

Fig. 3 Gravity balance and moment balance.

Table 2. Maximum allowable centrifugal force of disposable centrifuge tube

Manufacturer	Volume (mL)	Material	Maximum centrifugal force (g)
Assist	50	Polypropylene	4,000
		Polystyrene	4,000
	15, 13	Polypropylene	4,100
		Polystyrene	1,800
Corning	50	Improved polystyrene (Cat.No.430304)	1,800
		Polypropylene (Cat.No. 430290, 430291, 430828, 430829, 430522)	6,000
	15	Improved polystyrene (Cat.No.430053, 430055, 430788, 430789)	1,800
		Polypropylene (Cat.No. 430052,430766, 430790, 430791, 430630)	6,000

(1) There is no balance of moment between centrifuge tubes with different shapes (different centers of gravity).

(2) There is a balance of weight but no balance of moment between a solution with a specific gravity of 1.2 and water with 1.2-fold volume (specific gravity: 1.0) (the distance from the center of gravity to the rotating axis is different).

(3) For example, when a 50% suspension of fungus with a specific gravity of 1.2 (gravity as a suspension: 1.1) is balanced using saline with a specific gravity of 1.1, there is a balance of moment before centrifugation. After centrifugation, however, the fungus with a specific gravity of 1.2 will be concentrated on the bottom of the tube (the center of gravity moves away from the rotating axis), meaning that there is no balance of moment.

(4) If the centrifuge tube is balanced with the moisture on the outside of the tube or with water precipitated in the rotor, there is no balance, either.

Correct balancing involves **equally dividing a sample into centrifuge tubes of the same shape (material), the lid of which should also be of the same shape**. The moment imbalance caused by the specific gravity is practically allowable to some extent (for example, the case that *Escherichia coli* culture solution is balanced with water). However, if a sample is centrifuged at the maximum allowable rotating speed, and ammonium sulfate, sucrose and glycerine solution at high concentration or a fungal (cell) suspension at high concentration are centrifuged, the same sample is equally divided for balancing.

2.4.4 Precautions concerning sample leakage

In the case of an angle rotor, the liquid surface becomes vertical (parallel to the rotational axis) during centrifugation (Fig. 4(A)). If the liquid surface is over the edge of the centrifuge tube, the sample will leak with poor sealing between the centrifuge tube and the lid. If the sample leaks, imbalance will result, which will not only damage the machine and rotor but also cause biological contamination (strain harmful to the human body and phage harmful to other experiments), chemical contamination (for example, carcinogens, and poisonous and deleterious substances), corrosion (for example, ammonium sulfate, if left, heavily corroding aluminum alloy rotors), and at worst, fire and explosion (in the case of organic solvent). Therefore, considering the case of the incomplete sealing of the lid, **do not put a sample into a centrifuge tube so that the liquid surface during centrifugation is over the edge of the centrifuge tube. In particular, when a sample containing solvent with a low flash point (for example, ethanol) is centrifuged, never completely fill the tube to a level where the liquid surface during centrifugation (rotation) is above the edge of the centrifuge tube. In addition, never use a cracked or deformed centrifuge tube. If a dangerous reagent is spilled, be sure to report it to the instructor and then, treat the spill**. Even if the sample does not leak when the closed centrifuge tube with the sample is pressed by the finger, there is no assurance that the lid has been sealed completely. Assume that the liquid surface is 1 cm nearer the side of the rotational axis from the edge of the centrifuge tube in Fig. 4(B). The pressure of a 10 m water column is 1 atmosphere under the condition of 1 *g*. In the case of centrifugation under the condition of 10000 ×*g*, the pressure of a 1 cm water column corresponds to 10 atmosphere.

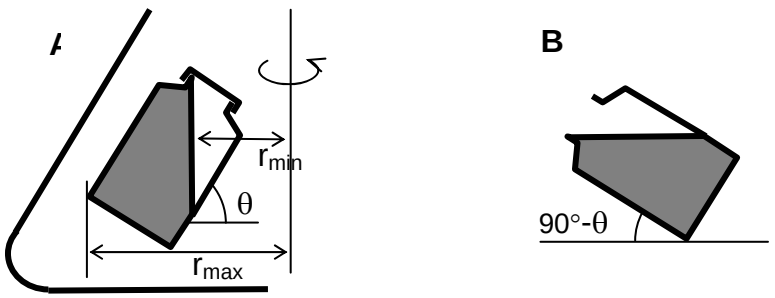

Fig. 4. Liquid surface during rotation (A) and calculation of safe amount of liquid (B).

(1) Examine the angle θ of the rotor to be used.

(2) Tilt the centrifuge tube full of water by 90 - θ.

(3) The remaining amount of liquid is the maximum allowable.

2.4.5 Precautions during operation

(1) Attach the rotor completely

Check the meshing direction of the tab on the bottom of the rotor with that of the rotating axis, and then set the rotor completely (some centrifuges have no tabs). Take care not to bend or break the tab. After setting, check that the rotor is completely set by slightly turning it by hand. If you remember how many turns the lid of the rotor requires for fastening it, you can find the incorrect setting when you turn the lid less than usual.

(2) Check the attachment of packing

Set the packing between the rotor and lid. The screw of the rotor lid is cut in the direction in which the screw is fastened by acceleration. Conversely, it is loosened when the rotor is decelerated (the rotor is decelerated, but the lid tries to rotate by inertia force). If packing is not correctly attached, the screw cannot be sufficiently fastened and the lid may become loose and fall off during deceleration. If the lid flies off during rotation, it is generally very expensive (more than a few hundred thousand yen) to repair it. There were three accidents presumed to result from such a default previously in our department to our knowledge.

(3) Monitor the centrifuge until the running state becomes steady.

Do not leave the centrifuge unattended until the rotating speed increases up to the preset value and becomes steady. Otherwise, appropriate action cannot be taken if there has been a mistake in setting the centrifuge tube or in balancing, or if breakage of the centrifuge tube should occur. If something abnormal (abnormal sound) occurs, immediately push the stop button or set the timer to zero. Take refuge and keep anyone away from the centrifuge until it completely stops.

(4) Never open the cover during running.

Do not open the cover carelessly during running even if there is an abnormal sound. If the centrifuge tube has been broken, the broken piece will scatter at a high speed and cause serious injury, such as blindness.

(5) Never stop the rotor by hand.

The hand may get caught in the rotating rotor, causing a serious accident, such as a fracture. The rotational axis may be bent and the lifetime of the centrifuge may be decreased markedly. “Because I am in a hurry” is not a valid excuse. Be patient and wait until the centrifuge stops completely.

(6) Always keep the cooler switched on

The temperature of the centrifuge will be increased by the heat from the motor and from the friction between the rotor and air. Therefore, keep the cooler switched on even during centrifugation at room temperature.

(7) Cover of the chamber

While the cooler is switched on, keep the lid of the centrifuge closed to prevent condensation and freezing in the chamber. Inversely, when switching off the cooler, open the lid to dry the inside of the chamber. If a large amount of ice is generated, ice crystals may fall off with draft pressure during centrifugation and scatter, damaging the rotor and chamber.

(8) Rotor after use

Remove the rotor and check that there is no contamination by possible sample leakage. Lay the removed rotor downward to prevent losing balance the next time centrifugation is carried out, owing to falling dust and condensed water collecting in the rotor. If the sample leaks, rinse the rotor immediately. The rotor contamination causes not only imbalance but also corrosion of the rotor and breakage. **If a dangerous sample (recombined organism, carcinogen, poisonous and deleterious substances, corrosive substance, and flammable substance) leaks, be sure to report it to the instructor and then, treat the leakage properly.**

2.4.6 Precautions concerning ultracentrifuge use

(1) Use the ultracentrifuge in the presence of an instructor

The ultracentrifuge rotates at an ultrahigh speed of around 100,000 rpm at maximum. Take special care as the rotor may fly out and cause a serious accident if balancing is forgotten or the sample leaks. If the rotor crashes through the chamber, the rotor will fly around the laboratory at a high speed and destroy the laboratory. If we consider the normal centrifuge as analogous to a normal car, the ultracentrifuge would be a racing car (F1 car). Even a small mistake may directly lead to a serious accident. **Use the ultracentrifuge in the presence of an instructor until you acquaint yourself with its use sufficiently. Do not use it alone until you are recognized to be**

sufficiently familiar with its use by the instructor.

(2) Precautions concerning plasmid purification

When purifying a plasmid DNA by cesium chloride – ethidium bromide equilibrium density-gradient centrifugation, **take special care of the temperature setting**. If a density gradient is formed, the concentration of cesium chloride at the bottom of the centrifuge tube will increase. Therefore, if the temperature is wrongly set at '**4°C**,' the concentration of cesium chloride will exceed the saturated concentration and its crystal will precipitate. Because the density of the crystal is much higher than that of the solution, **the moment balance will be lost and the rotor will fly out. Strictly observe the setting of temperature and time designated in the protocol**.

Ethidium bromide is a carcinogen and usually used at high concentration for equilibrium density-gradient centrifugation (consider that it is more dangerous than RI). **If it is spilled, be sure to report it to the instructor and conduct proper decontamination**. In particular, the Beckman ultracentrifuge in the common equipment room in the basement can become easily contaminated at the time of heat sealing the centrifuge tube. Check whether there is contamination using a built-in ultraviolet (UV) lamp before and after sealing. Under short-wavelength ultraviolet irradiation in a dark room, an orange glow indicates contamination. Of course, short-wavelength ultraviolet must not be irradiated directly onto the skin or looked at with the naked eye (refer to 2.7).

(3) Heat sealing

When sealing the centrifuge tube by melting its rim through heating, any solution adhering to the rim will cause incomplete sealing (increase in inner pressure by the centrifugal force → liquid leakage → imbalance → very serious accident). **Carefully read the instruction manual** and pay attention to the following when sealing.

1) Pour a sample up to the line shown in Figure 5. If a larger amount is poured, the tube will tend to be sealed incompletely, and if smaller, the centrifuge tube will be deformed by the pressure due to the centrifugal force, also causing imbalance.
2) Completely wipe the sample solution adhering to the rim of the tube with Kimwipe. Because the temperature at the part of the tube with the attached solution will not increase even when heated by a heater (the tube will not melt), the tube will be incompletely sealed. When the solution adhering to the rim dries up, the solute dissolved in the solution will remain in a dried-up state, also causing incomplete sealing.

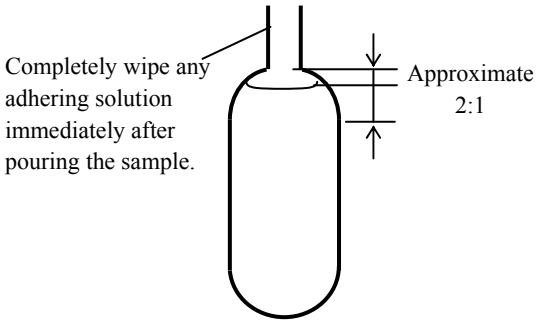

Fig. 5 Appropriate sample volume

2.4.7 Precautions concerning swing-type centrifuge (clinical centrifuge)

When running a swing-type centrifuge, in which the test tubes can be centrifuged, mount the same assemblies in all four arms. Even if centrifuging one sample (two samples for balancing), mount four identical assemblies. In general, when using an assembly in which eight test tubes can be placed, the maximum rotating speed is 2,000 rpm (be sure to check the value yourself because it depends on the type of centrifuge).

2.4.8 Know-how for centrifuging

(1) Points for deriving the centrifugation effect from the theoretical formula

Assuming that the Stokes radius and specific gravity of the particle to be centrifuged are R and ρ, respectively, the rotation speed is ω, the specific gravity and viscosity of the solvent are ρ_0 and η, the distance between the rotational axis and the liquid surface is r_{min}, and the distance between the rotational axis and the bottom of the centrifuge tube is r_{max}, the time for the deposition of the objective particle is given as

$$t = \frac{9}{2}\frac{\eta}{\omega^2 R^2(\rho - \rho_0)}\ln\frac{r_{max}}{r_{min}}.$$

Therefore, the following apply.

1) The centrifugation effect is proportional to the product of centrifugal force (rotating speed squared) and time. Therefore, if the rotating speed increases by 1.2-fold, 1.44-fold the effect is obtained (here, strictly observe the maximum

allowable rotating speed). If the rotating speed cannot be increased, rotation time can be increased.

2) When particles in a solution of salt or sugar at high concentration are centrifuged, it takes a long time to centrifuge because $\rho-\rho_0$ is low. It is desirable to centrifuge such solutions after diluting with water or buffer solution if possible. For example, when a sample with a specific gravity of 1.21 in a solution with a specific gravity of 1.20 is centrifuged, the difference in specific gravity between the two is 0.01. However, if a solution is diluted to a specific gravity of 1.10 using an equivalent amount of water (specific gravity: 1.00), the difference in specific gravity will be 0.11 and the sample will be centrifuged for a time ten-fold shorter than the original. Moreover, dilution has an effect of decreasing the viscosity of a solution.
3) The viscosity of a solution decreases as its temperature increases. When increasing preset temperature or diluting a solution, the viscosity of the solution will decrease, which enables shortening of the centrifuging time.
4) Using an angle-type rotor with a low r_{max}/r_{min}, a sample is centrifuged more rapidly than when using a swing-type rotor with a high r_{max}/r_{min}.

(2) If there are problems with suspension of precipitate

In most cases, the objective is not centrifugation at a given rotating speed but precipitate collection; therefore, it is desirable to decrease rotating speed. Moreover, when resuspending a precipitate, loosening (softening) the precipitate to which the solution has not been added yet using a vortex mixer will facilitate suspension.

(3) Precooling of rotor

In principle, mount the rotor, set the temperature and precool it 0.5 – 1 hour before use. If the rotor is required to cool rapidly, it is desirable to properly mount the rotor and lid and then to idle the rotor at 1,000 – 2,000 rpm for 5 – 10 min.

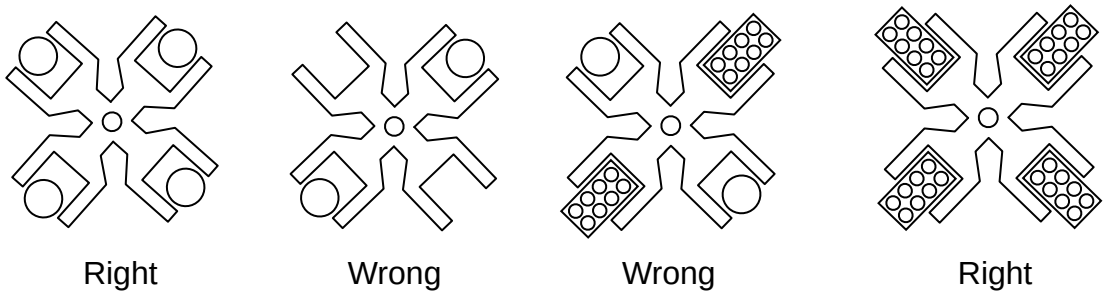

Fig. 6 Right and wrong mounting of assemblies for swing-type centrifuge

About normal centrifuge:

(1) Balance the centrifuge with respect to moment not weight.
(2) Strictly observe the maximum rotating speed and usually use the centrifuge at 80% or less.
(3) Check that the centrifuge tube withstands the given centrifugal force and solvent.
(4) Sample leakage = risk (imbalance, biological contamination, chemical contamination, explosion and fire).
(5) Be sure to use a cooling device if equipped.
(6) Mount the same assemblies to all the arms of a swing-type rotor.
(7) Supervise the rotor until its rotation becomes stable.
(8) Do not open the cover of the chamber during rotation.

(9) Never stop the rotor by hand.

(10) Report sample leaks to the instructor.

<u>About ultracentrifuge</u>:

(1) Use an ultracentrifuge in the presence of an instructor until sufficiently acquainted with its use.

(2) Strictly observe temperature during equilibrium density-gradient centrifugation.

(3) Take special precautions not to contaminate the sample with ethidium bromide.

2.5 Thermostat Bath (Incubator)

The thermostat bath is generally used without special care but has a risk of causing large-scale accidents such as fires. In our department, there have been small fires caused by boiling the water bath dry and by heating of the oil bath, and foul odor emissions of melting plastic on the verge of fire in the dryer. An incorrect operation of the thermostat may cause inaccurate control of temperature, resulting in the failure of the experiment even though no accident is caused.

2.5.1 Basic precautions

(1) Display the user name and scheduled termination date and time for all-night running

When running the thermostat bath overnight, be sure to display the user name and scheduled termination date and time without fail. A bath left on without a display of user name and scheduled termination date and time must be turned off by the last person leaving the laboratory. Even if the experiment fails because the bath was turned off, the person who neglected to display the necessary sign must take full responsibility for the failure.

(2) Precautions concerning unattended (overnight) running

Unattended (overnight) running without a safety device is prohibited. It is not guaranteed that the temperature controller will always function correctly even though it has a safety device**. Do not leave until you confirm that the temperature has reached the preset value and that the bath is in the steady state**. If you must leave, ask someone staying in the laboratory to perform the check in your place.

(3) Precautions concerning power source capacity

The thermostat bath has a large power consumption (12 – 20 A, 12 – 20 kW). Check that the power source has sufficient capacity. The use of socket multipliers is prohibited.

(4) Use of extension cord

If an extension cord with insufficient capacity is used, heat will be generated, which may cause fire. As a rule, extension cords should not be used for the thermostat bath. Because many thermostat baths require 15 A of current or more, if an extension cord must be used, ensure that it has sufficient capacity to accommodate such a current (for your reference, some household extension cords have only 6 A capacity).

(5) Do not wet the control unit

There is a risk of fire and electric shock. Moreover, the circuit may short out and the thermostat bath may be broken. If the control unit becomes wet, disconnect the plug of the power source (do not use wet hands), wipe up the water as much as possible, and request the manufacturer to inspect it.

(6) Base heater and control heater

Some thermostat baths are equipped with a base heater, in which current always flows, in addition to a control heater (the heater turns off when the temperature exceeds the preset value and turns on when below the preset value). The base heater is used only when a rapid increase in temperature is required and when the capacity of the control heater is insufficient to obtain a high preset value. Therefore, if the base heater is used during normal running, the temperature continuously increases, causing not only experiment failure but also a risk of fire.

(7) Thermometer accuracy

Some thermometers have an error of 2 – 3^{o}C. In

general, a chemical reaction (enzyme reaction) progresses 6% more rapidly as the temperature increases by 1°C. In an experiment in which temperature is an important factor, check the accuracy of the thermometer to be employed using a standard thermometer. Use the standard thermometer only for the calibration of the experiment thermometer and never in the experiment because it is expensive. Make sure that the end of the thermometer is not in contact with the wall or bottom of the thermostat bath, or the temperature will not be accurately measured.

2.5.2 Water bath incubator

(1) Caution concerning overnight running and unattended running

Never run a water bath overnight without the boil-dry protection device. Even in the daytime, unattended running is prohibited. At each use, check that the boil-dry protection device is functioning correctly before carrying out the experiment. In the case of the boil-dry protection device, which stops the current flow when the float descends to a certain level due to a decrease in water level, if water residue accumulates on the float, it will not move and will fail to function properly when the water level descends. Do not neglect the maintenance of the bath to prevent residue accumulation around the float.

(2) Water supplement

Water in the incubator (thermostat bath) evaporates. Be sure to maintain a sufficient amount of water in the bath. Because there is a large difference between room temperature and water temperature and a low air humidity in winter, pay particular attention to increases in evaporation. When running the bath all night, devise an automatic water supply system or prevent evaporation by covering the bath with aluminum foil. In particular, when using the bath at a high temperature, examine the water level decrease per unit time and check whether a safe water level can be maintained until your arrival at the laboratory the next day. It is dangerous to place plastic balls or foam polystyrene on the water surface. They may cause fire if they come into contact with the heater.

(3) Check of water level

The last person leaving the laboratory must check the water level of a water bath left to run overnight and add water if necessary even if it is not their own experiment.

(4) Do not overly rely on the safety device

In the user instruction of the water bath equipped with the boil-dry protection device, there is a note "check the operation of the safety device at each use." The operation is not assured unless checked properly. **If the safety device is activated, the power will be shut off and the temperature will not be maintained, resulting in the failure of the experiment**. The presence of a safety device is not an excuse to neglect the addition of water even for a water bath equipped with the safety device.

(5) Caution concerning shaking

When using the water bath with a shaking apparatus, adjust the water level and shaking speed so that water is not scattered during shaking. In particular, when a sample requiring sterilization is shaken, devise a way to keep dirty water (more than 10^7 bacteria/mL is not unusual) away from the cotton stopper and silicon stopper.

2.5.3 Incubator, dryer and dry heat sterilizer

(1) The incubator, dryer and dry heat sterilizer have no explosion-proof construction.

Never put organic solvent or flammable gas into them.

(2) Do not put any flammable materials into the dry heat sterilizer

Do not put combustible material, such as plastic and paper, into the dry heat sterilizer. The only exception is the cotton plug.

(3) Fix the temperature setting dial with tape

If the thermostat bath has a temperature setting dial, fix the dial with tape so that the preset temperature cannot be shifted by contact with other materials. In our department, there has been a case in which a plastic instrument melted due to an inadvertent shift of the temperature dial (fortunately, fire was prevented because of detection of a foul odor).

(4) Check that any instrument used in the dryer or dry heat sterilizer can withstand the preset temperature

In general, plastic instruments (including the Pipetman) cannot withstand the temperature in the dry heat sterilizer (160°C). If a chip, Eppendorf tube or plastic beaker is dry-heat-sterilized, it will melt and flow into the high-temperature heater, causing a fire.

(5) Do not block air circulation

Do not place the sample or instruments on the outlet of the fan. Do not place large objects in the machines and do not fill the machines with too many objects. In general, the temperature sensor is installed in the upper part of the machines and the heater in the lower part. If the air circulation (convection) is prevented, the temperature cannot be controlled. The heater continues working until the temperature in the upper part reached the preset value, which causes the temperature in the lower part to become too high. There has been an incident of fire due to a plastic instrument melting in the high-temperature heater and flowing into the heater part.

(6) Do not remove the bottom plate

The board at the bottom of the machines separates the bottom plate, the temperature of which will become high, and the sample to ensure air circulation. Do not remove the bottom plate even when attempting to put in a tall sample. Otherwise, fire may result.

(7) When the sample is spilled

If the sample is spilled into the incubator, wipe it off immediately. Otherwise, it may corrode the incubator and cause contamination.

Basic precautions

(1) Display the user name and scheduled termination time for overnight running.

(2) Unattended (overnight) running of the machines without the safety device is prohibited.

(3) Check that the temperature has attained the steady state.

(4) Never use a socket multiplier.

(5) Pay attention to the electric capacity when using an extension cord.

(6) The control unit must be kept dry.

Water bath

(1) Is the amount of water sufficient?
(No water + nonfunctional safety device = fire)

(2) At each use, check that the boil-dry protection device functions correctly.

(3) The last person leaving the laboratory must check the water level regardless of whose experiment it is.

Incubator, dryer and dry heat sterilizer

(1) Do not use organic solvent or flammable gas.

(2) Do not block the air circulation in the machines.

(3) Do not place combustible material in the dry heat sterilizer.

2.6 Reducing Pressure

2.6.1 Cautions concerning apparatuses

(1) Do not draw up benzene or halogen-containing solvent directly with the water-jet pump.

Do not draw up benzene or halogen-containing solvent (chloroform, dichloromethane and chloroethylene) with the water-jet pump. Otherwise, halogen-containing solvent will be discharged into the sewage and cause environmental contamination. Neither should they be drawn up with the circulation water-jet pump (when replacing water in the circulation water-jet pump, water with the halogen-containing solvent will be disposed of

into the sewage).

(2) Do not draw up acidic gas directly with the vacuum pump.

For example, hydrochloric acid, acetic acid and trifluoroacetic acid will corrode the inside of the pump and considerably reduce its lifetime. Use a special Teflon-coated pump or a pump with a trap containing sodium hydroxide or other chemicals.

(3) Do not draw up organic solvent or solution directly with the hydraulic vacuum pump.

Organic solvent and solution are less detrimental than acidic gas, but also reduce the lifetime of the pump. In addition, a sufficient vacuum level cannot be attained because of the vapor pressure of the drawn solvent or water. Draw them up through a cold trap cooled with liquid nitrogen or dry ice - ethanol (methanol). If drawing up a solution, the silica gel column can be used alternatively. In this case, replace the silica gel when its color changes (the gel becomes tinged with red).

(4) Break the vacuum condition before stopping the pump

Before stopping the pump, break the vacuum condition by using the three-way cock or detaching the hose. Some vacuum pumps have no backflow valve. If such a pump is stopped before breaking the vacuum, oil will flow back and ruin the sample. (Cleanup is very difficult.) The water-jet pump normally has no backflow prevention valve. If it is operated incorrectly, water will flow back and ruin the valuable sample. In particular, for drying at a low pressure with a desiccator, backflowing water will react strongly with the drying agent (phosphorus pentoxide, calcium chloride, concentrated sulfuric acid, etc; silica gel is no exception), causing an accident. In fact, the use of the water-jet pump for drying at a low pressure is incorrect (the sample cannot be dried completely because of the vapor from the water flow).

2.6.2 Notes on operation for reducing pressure

(1) A container exposed to a low pressure must be resistant to a low pressure

Use only thick containers designed for use under a low pressure, such as a pressure-reduction bottle, an aspiration bottle and a desiccator. Before each use, check that the bottle is not cracked or chipped. Be aware that a conical flask may break when the pressure is reduced. The broken pieces of the flask and contents scatter violently, which is very dangerous.

(2) Be ready for quick response to sudden 'boiling'

When reducing the pressure of the solvent, attach a three-way stop cock to the pump **to enable immediate cessation of pressure reduction in the event of sudden boiling**. Sudden boiling tends to occur particularly just after starting pressure reduction, when starting heating of the sample, and just before the sample solvent is completely exhausted. Therefore, always monitor the pump and be prepared to take immediate action, such as by keeping the hand on the stop cock, in the event of sudden boiling. Never leave the pump unattended just after starting pressure reduction.

2.6.3 Rotary evaporator

(1) Start the exhaust slowly and carefully

Start the exhaust, keeping the hand on the stop cock to immediately break the vacuum condition in the event of bubbling or sudden boiling. Never leave the pump unattended just after starting pressure reduction.

(2) Do not regularly use the vacuum pump for pressure reduction

Generally use the water-jet pump, not the vacuum pump. If using the vacuum pump, draw up solutions through the trap cooled with liquid nitrogen or dry ice – ethanol (methanol), as noted above.

(3) Do not heat until the pressure has become sufficiently low and stable

Start heating after a sufficiently steady low pressure is obtained and no bubbling or sudden boiling occurs.

(4) Stopping the flow of cooling water may cause fire

Check that sufficient cooling water flows, 5 minutes after starting pressure reduction and at the time of leaving the evaporator unattended. The solvent may be not collected but exhausted, which may cause explosion and fire.

(1) Use a container designed for use under a low pressure.
(2) Stopping the flow of cooling water in the rotary evaporator causes explosion and fire.
(3) Drying agent + backflow water = danger (stop the pump after all operations)
(4) The use of benzene or halogen-containing solvent with the water-jet pump is prohibited.
(5) Attach the proper trap to the vacuum pump.

2.7 Ultraviolet Lamp

Ultraviolet radiation, in particular, short-wavelength ultraviolet radiation, is harmful to the human body. When staining and observing DNA, short-wavelength ultraviolet radiation will damage the DNA, for example, by causing thymine dimer formation and DNA breakage.

(1) When turning on the lamp, even for a short time, always wear protective glasses.
(2) For operations taking more than a few seconds, such as collecting DNA fragments from agarose gel, wear long-sleeved clothes (lab coat), gloves and a full-face mask.
(3) The ultraviolet lamp attached to the clean bench emits harmful short-wavelength (normally 245 – 254 nm) ultraviolet radiation. Although ultraviolet radiation can be blocked by glass or acrylic plates, direct exposure of the skin or direct viewing with the naked eye must be prevented. Furthermore, it differs from the long-wavelength lamp (generally 312 – 365 nm) used in the transilluminator. Care must be taken not to mistake them when exchanging the lamp.
(4) When using the transilluminator, the wavelength of which can be varied, set the switch to the long wavelength for DNA excision (pay attention to H and L on the selector switch, which refer not to the output power but to the wavelength).

2.8 Handling Reagent

When using reagent, always wear protective glasses. If you get dangerous reagent (solution) in the eye, immediately (within a few seconds) wash the eye with generous amounts of water for more than 15 minutes, and consult a physician. In particular, if you get alkali or organic solvent in the eye, wash the eye vigorously without delay. Every second you delay the higher the risk of blindness. It is more important to sufficiently wash the eye than to quickly consult a physician.

Before using reagent in your experiment, understand the handling instruction for all reagents. Refer to a textbook, such as "*Carrying Out Experiments Safely*" (KAGAKU-DOJIN) to acquire general basic knowledge about "what substances are dangerous in what ways," and "what should be done in the event of an accident." In addition, consult the Merck Index and make sure to acquire sufficient background knowledge for each reagent to be used with respect to, for example, the following points:

(1) How harmful is the reagent to the human body?
(2) Are there any risks of fire or explosion?
(3) What procedure should be followed when the reagent is spilled?
(4) Proper method of treating waste liquid
(5) Proper storage method (refrigerating, freezing, protecting from light, nitrogen substitution, etc.)

2.8.1 Examples of reagents to handle with care

Some reagents, which are normally used without special care, may be unexpectedly dangerous. Some familiar examples and reagents which are rarely used but should be handled with special care are given below.

(1) Special flammable substances

Ether is an example. According to the Fire Defense Law, its danger level is ranked the highest, and therefore, all open flames in the room must be extinguished before it can be handled. Here,

"handling" includes "holding" the solvent bottle (refer to 2.1.2). Ether is flammable because of its extremely low ignition temperature. Once the ether catches fire, the fire expands explosively, which is difficult to extinguish, and therefore, utmost caution is required. **The centrifugation of these solvents is strictly prohibited** (a special explosion-proof centrifuge, which is unavailable in our department, is required).

(2) Highly flammable substances

Ethanol, methanol, acetonitrile, hexane and acetone are examples. Similar to special flammable substances, extinguish all open flames, such as that in a stove, before "holding" the bottle, in case the bottle is dropped and broken. It is dangerous to dispose of these solvents in a sink even though they are soluble. Because the amount of solvent vapor increases with liquid surface area, these solvents may catch fire from the water heater or a spark from the motor in the centrifuge and explode.

(3) Poisonous and deleterious substances

Potassium cyanide, sodium cyanide, sodium azide, mercury compounds and arsenic compounds are examples. Phenol, chloroform, acrylamide, sulfuric acid, hydrochloric acid, sodium hydroxide, potassium hydroxide and acetonitrile are also examples. Store them in their designated lockable storage cabinet and manage their mass by preparing a log. **Upon recognizing a lost or incomplete form in the log, immediately report it to the instructor**.

When the ordered poisonous and deleterious substances are delivered, be sure to record them in the log. When borrowing or lending such substances between laboratories, be sure to obtain approval from the instructor.

(4) Other familiar dangerous substances

- Hydrogen peroxide solution

  If metal is mixed into this solution, it may react explosively (recall the science experiment to generate oxygen from hydrogen peroxide solution using potassium permanganate as a catalyst, performed in elementary and junior high schools). There has been a case of explosion due to the contamination of a small amount of rust. **Never use a metallic container or injector**.

- Ammonium persulfate

  This is used as a polymerization initiator of polyacrylamide gel. **Because it occasionally may react explosively with metal, do not use a metallic medical spoon when weighing the ammonium persulfate**. Use a plastic or bamboo spoon. When using the ammonium persulfate for the preparation of polyacrylamide gel, the following procedure is recommended (10% ammonium persulfate solution for electrophoresis, although slightly more expensive, is commercially available):

  1) Purchase ammonium persulfate in units of 1 g.
  2) Add 3–5 mL of pure water directly into the reagent bottle and dissolve the ammonium persulfate.
  3) Transfer the solution into a disposable tube with a volume of 15 mL and dilute to 10 mL.
  4) Dispense an amount suitable for one-time use in the range of 100 – 150 μL into several Eppendorf tubes.
  5) Store it in the freezer below –20°C.

  In most protocols, ammonium persulfate should be prepared at the time of use. However, there is no problem with the use of ammonium persulfate dispensed from a stock kept in the freezer. The error in the preparation by this method does not cause any problem when using ammonium persulfate as a polymerization initiator of acrylamide gel.

- Acrylamide

  This is neurotoxic. If it comes in contact with the skin, no problem arises if the skin is washed immediately, but it may cause paralysis if the skin is left untreated.

- Phenol

  This is a corrosive substance. If it comes in contact with the skin, it causes burns. Precautions during the extraction of nucleic acid are given in 2.8.6.

- Chloroform

  This is deleterious and corrosive and is designated as a specified toxic substance. If it comes in contact with the skin, although less harmful than phenol, it may still cause burns. Chloroform is considered to cause liver cancer if inhaled over a long period. Be sure to use it within the draft chamber. Precautions during the extraction of nucleic acid are given in 2.8.6.

- Ethidium bromide, nitrosoguanidine, ethyl (methyl)

These are highly carcinogenic agents. Consider them more dangerous than isotopes. Use them under the instructor's guidance until you acquire full handling expertise. Ensure full knowledge on the treatment for spills and the disposal method. Refer to 2.8.4 and 2.8.5.

- Phosphorus pentoxide, quicklime (calcium oxide), concentrated sulfuric acid, silica gel

  These are also used as drying agents and reagents of these four substances react violently with water. Even silica gel is dangerous because it bursts upon contact with water. Exercise sufficient caution when disposing of the contents of a desiccator.

2.8.2 Cautions in purchasing

(1) Acquire proper knowledge about the reagent to be purchased

Be sure to acquire knowledge about the reagent to be used, using information sources, such as the Merck Index. It is necessary not only to obtain the proper knowledge concerning toxicity and explosiveness but also not to fail in the experiment. For example, useful information, such as the preparation method of the solution of a difficult-to-dissolve reagent (shifting its pH slightly or dissolving it initially in another solvent) and the stability of the reagent, can be obtained.

(2) Purchase the reagent only in the smallest necessary amount

It is not uncommon for a reagent disposal fee to be tens or hundreds of times more expensive than the purchasing cost. Recognize that the remains of excessive purchases are not only wasteful but require additional disposal fees.

Many reagents deteriorate in storage. Purchase reagents requiring special storage methods (hygroscopic reagent, reagent in ampoule and reagents to be protected from light, stored under nitrogen, refrigerated or frozen) in only the smallest necessary amounts. For example, 1-g bottles and 10-g bottles are commercially available; if 2 g of the reagent is required, it is often less expensive to purchase two 1-g bottles than one 10-g bottle.

2.8.3 Precautions for use

(1) Label check

Check the label three times so as not to use an incorrect reagent. First check: when retrieving the reagent bottle from the reagent cabinet (always check the label). Second check: when measuring the reagent (if placing numerous reagent bottles together near the balance, always check the label before measuring the reagent). Third check: when returning the bottle to the reagent cabinet (always check the label when replacing the bottle back on the shelf). The following are examples of commonly confused reagents.

1) Polyvalent anions, such as phosphoric acid, citric acid, EDTA and ATP

For example, tetravalent EDTA has five types of salt, namely, free acid and mono-, di-, tri- and tetra sodium salt. Phosphoric acid has several salts, such as sodium salt, potassium salt and ammonium salt, and each salt has three types.

2) Number of bonded water molecules

For example, Na_2HPO_4 has three types of salt, namely, anhydride, heptahydrate and dodecahydrate. For mol concentration, the amount to be measured can be calculated from each molecular weight. However, note that the weight concentration is different depending on the type of salt used (the number of bonded water molecules in the reagent used must be accurately recorded in the experiment note).

(2) If reagent is spilled

If the reagent is spilled during measurement, be sure to clean it up. Otherwise, the spilled reagent will come into contact with the back of the powder paper and contaminate the next reagent being measured. **Only the person who spilled the reagent knows its identity. If left untreated, it cannot be properly cleaned up**.

(3) Medical spoon

Before using the medical spoon to measure reagent, ensure that the spoon is washed well, rinsed with pure water and dried completely. If a wet medical spoon is used, the reagent may deteriorate due to moisture, and will not be measured accurately. It is not uncommon for experiments to fail because of a very slight amount of impurity contamination. Note that a metallic medical spoon should not be used for some reagents, such as ammonium persulfate (refer to 2.8.1(4)).

(4) Be sure to return the reagent

Be sure to immediately return the reagent used

with the label facing forward. If the label is peeling off or worn away, take proper steps, such as reattachment or rewriting of the name. It is highly expensive to dispose of reagents containing unknown substances.

(5) Opening container of refrigerated or frozen reagent

When using a reagent stored in a refrigerator or a freezer, **be sure to bring it to room temperature before opening the lid.** If the lid of a cold bottle is opened, vapor in the air will condense, making the reagent wet. When wet, the reagent may deteriorate and will no longer be measured accurately. The deterioration of a cold reagent, caused by exposure to room temperature for a short time, is negligible compared with that caused by storing under a wet condition at a low temperature for a long time. However, (6) and (7) are exceptions.

(6) Ammonium water, hydrogen peroxide, corrosive carbohydrate solution

The pressure in a bottle of ammonium water and hydrogen peroxide may be high. If the temperature in the bottle is high, cool it with ice water before opening the lid. When a medium containing sugar corrodes in a sealed bottle and the pressure within a bottle filled with carbon dioxide is high, be aware that the contents may scatter explosively if the lid is carelessly loosened. **Always wear protective glasses without fail.**

(7) Opening an ampoule

Open an ampoule after cooling it with ice water. If the total amount of reagent is not used in the experiment, prepare a container of suitable volume and material and which can be sealed, before opening the ampoule. Because there is a reason for sealing the reagent in an ampoule (for example, extreme sensitivity to oxygen or moisture, or a foul odor), it must again be sealed for storage after opening. Note that Parafilm cannot be used to seal an ampoule, because of its breathability.

2.8.4 Handling of carcinogens

Ethidium bromide, nitrosoguanidine, ethyl (methyl) methane sulfonate are highly carcinogenic and should even a slight amount of these substances be spilled and left untreated, all those present, not just the experimenter, will be exposed to long-term risk of disease. **Consider these substances more dangerous than isotopes because spilled substances are difficult to detect** (ethidium bromide can be detected by ultraviolet irradiation). When using these substances, as with the case of isotopes (or more than with isotopes), exercise particular caution, as described below. **Be sure to use them under the instructor's guidance** because procedures differ depending on the reagent and the experimental aim**.**

(1) **Always wear protective glasses and gloves**. Never treat these substances with bare hands. **Consider used gloves to be contaminated, and do not carelessly touch other materials when wearing gloves**.

(2) **Request the presence of the instructor when measuring these substances**. Take sufficient care not to spill any amount of the substance.

(3) Store the reagent bottle in a transparent plastic bag with the top closed.

(4) Dispose of the old bag used to store the reagent by the method described in (8).

(5) Place polyethylene-coated filter paper of sufficient size on the laboratory table to be used.

(6) If the substance is spilled outside the area of the filter paper, report to the instructor and immediately neutralize it properly and adequately.

(7) Be sure to perform neutralization for all the instruments used (containers such as test tubes, chips, medical spoons and powder paper).

(8) Dispose of the used glove, filter paper on the laboratory table and reagent bag, keeping them away from people (not only those in the laboratoy but also those involved in waste disposal). For example, hold the bag to be disposed of while wearing gloves, then take the gloves off inside out such that they envelop the bag and then bind them at the top. Moreover, put the entirety into another bag, bind the top of the bag and dispose. Be sure not to include any sharp objects, such as a tip, which may tear the bag. Dispose of the tips after separate neutralization.

2.8.5 Treatment of carcinogens

The following are general precautions and not to be considered complete. Study and devise proper suitable treatments by yourself.

(1) Ethidium bromide

This is often treated with sodium hypochlorite solution, however, its carcinogenicity cannot be completely neutralized, and it is also said that its toxicity may in fact be increased. The oxidation treatment with potassium permanganate described in

"Molecular Cloning (2nd Ed., E8)" is also incomplete. Therefore, hire a professional disposer to dispose of dilute solution, such as gel stain solution, and concentrated solution used for equilibrium density-gradient centrifugation, treating them both as halogen-containing waste liquid. The dilute waste should be treated by adsorption into activated carbon or Amberlite XAD-16 (refer to "Molecular Cloning (2nd Ed., E9)"). Here, the activated carbon or Amberlite XAD-16 used must be properly disposed of.

A spot contaminated with ethidium bromide shows orange luminescence when ultraviolet light is irradiated in the dark. Although the detection sensitivity is higher when using short-wavelength ultraviolet light, such light is itself carcinogenic. If ethidium bromide comes into contact with the skin (or there is a possibility of it having come into contact with the skin), check for orange luminescence using the long-wavelength ultraviolet lamp and wash the skin with a brush and soap until the orange luminescence is no longer seen.

(2) Nitrosoguanidine

Nitrosoguanidine is decomposed upon heating in an autoclave as an acid or alkali solution. Take special caution never to spill this solution in a space where heat treatment is prohibited.

(3) Ethylmethane sulfonate (EMS)

Neutralize this solution with sodium hypochlorite or sodium thiosulfate solution. According to "Microorganism Genetics Experiment Method," 9.8 mL of 6% sodium hypochlorite solution is used to neutralize 0.2 mL of 3% EMS. If EMS is spilled, wipe it thoroughly with sodium hypochlorite solution wearing gloves.

2.8.6 Extraction of nucleic acid with phenol/chloroform

(1) Wear protective glasses and gloves when treating phenol/chloroform

When extracting nucleic acid with phenol/chloroform, use a tube made by a manufacturer with a reliable sealing level. Depending on the manufacturer, some tubes that are insufficiently sealed and cause sample leakage may be included in the provided supply, which will require special attention when mixing tubes using a vortex mixer. **Be sure to wear protective glasses and gloves**.

(2) When phenol or chloroform comes into contact with the eye or the skin

If it enters the eye, immediately (within a few seconds) flush the eye with water for more than 15 minutes, and then, see a doctor. **If it comes in contact with the skin, never wipe it with ethanol**. Skin oil will be removed by the solvent, which will cause an even worse burn. Immediately wash it away with water, and then with soap.

(3) Collect the waste liquid

Be sure to collect waste liquid and hire the proper waste disposal firm for its disposal. Do not dispose of it in the tube. Tubes are disposed of as nonflammable substances, which are usually used as landfill. Therefore, tubes with waste liquid will contaminate the environment and pose a danger to the worker.

2.9 Handling of Liquid Gas and Gas Cylinder

It is easily understood that combustible gases, such as hydrogen and oxygen, must be treated with caution to avoid explosion and fire. Nitrogen, argon and carbon dioxide, which may cause fatal suffocation, must also be treated with care.

2.9.1 If using the elevator for delivery

Do not ride the elevator with a liquid gas container or cylinder during delivery. The valve of the cylinder may sometimes break and cause gas blowout, leading to a high probability of death by suffocation to any person in the elevator. If liquid gas is spilled in an elevator, vaporized gas may cause suffocation. If the elevator stops because of a power outage, vaporized gas may cause suffocation even if liquid gas is not spilled. When a liquid gas container or cylinder is transported by elevator, press the button for the desired floor and leave the elevator unmanned; persons must take the stairs. When the door of an elevator opens, do not get on if there is a liquid gas container or cylinder.

2.9.2 When handling liquid gas

Only persons who have attended the lecture at the

Low Temperature Center and have carefully read the manual concerning handling, which is distributed at the lecture, are permitted to treat liquefied gas unassisted.

When treating liquid gas, such as liquid nitrogen, **open the window even in winter. Never use it in a room that cannot be ventilated.** In several universities and companies, there have been fatal suffocation accidents caused by the use of liquid nitrogen in unventilated rooms. With a lack of oxygen, a person will become groggy and probably be unable to escape or call for help.

2.9.3 When handling a gas cylinder

Only persons who have attended the lecture concerning safe handling of high-pressure gas are permitted to handle the gas cylinders unassisted.

(1) Be sure to take precautions to prevent falling

There is a direct risk of injury being caused by being trapped under a fallen cylinder. In addition, if the valve breaks and the gas blows out, the cylinder may fly like a rocket, causing an explosion and fire or fatal suffocation. Install a special cylinder rack and fix it securely with a chain or other measures.

(2) Check for leakage

After attaching the regulator, be sure to check for leakage by applying soapy water to it. Leakage of combustible gas or toxic gas is dangerous, as is the leakage of a large amount of oxygen because it may ignite (cotton will spontaneously combust, and heavy sparks may be generated when metal parts come in contact with each other even lightly).

(3) Opening the main valve of the cylinder is a dangerous operation

When opening the main valve of the cylinder, do not stand on the side at which the regulator is attached. If the regulator is not completely attached, it may fly off at high speed. When opening the main valve, leave the side of the regulator unattended and keep instruments, which may create a hazard when broken, away from that side.

3. Carrying Out Proper Experiments

3.1 Storage of Reagent and Sample

3.1.1 Diluted solution is unstable.

Diluted reagents and samples are generally unstable. They should be stored at high concentration.

(1) Enzymatic Degradation

A sample degrades when mixed with even a small amount of nuclease or protease (contamination means incorporation of nuclease or protease). Therefore, a sample at high concentration is mostly free from degradation.

(2) Container Adsorption

Plastic or glass adsorbs approximately 0.1 – 1 μg of nucleic acid or protein per 1 cm^2. The loss caused by container adsorption can be considerably prevented by siliconizing the container in the case of nucleic acid solution (refer to "Molecular Cloning E1 – E2") and by adding approximately 0.1% of bovine serum albumin (BSA) in the case of protein solution.

(3) Oxidation

Pure water at 30°C contains 7.4 mg/L (= 0.23 mM) of oxygen molecules at maximum, and oxygen at higher concentration is soluble at lower temperatures. For example, 4 monovalent thiol molecules are oxidized by one oxygen molecule and therefore, a solution of 1 mM or less is oxidized by dissolved oxygen even if kept in a closed container.

3.1.2 Precautions when refrigerating or freezing "perishables"

Do you eat *sushi* that has been refrigerated for several days? *Sushi* (raw fish) loses flavor when refrigerated even for one day, which means that its components have changed. A cell always contains nuclease and protease. Samples extracted from a cell, such as protein and nucleic acid, are degraded by nuclease or protease unless highly purified. Therefore, an experiment using such samples must be carried out at as low a temperature as possible and completed quickly. Even a frozen sample is not necessarily stable. Because deterioration by freezing is a matter of degree (there is no storage method that completely prevents deterioration), one need not be excessively cautious but should take care with the following.

(1) When frozen, a cell is heavily damaged by the physical force generated by the freezing of water within the cell (when water is frozen, its volume increases; large ice crystals that damage the cell grow easily, particularly at approximately –20°C). Large protein molecules may also be deactivated when frozen. An appropriate protective agent, such as glycerol, must be added.

(2) If the freezing temperature is approximately -20°C, protease and nuclease are active significantly in a nonfrozen solution (a roughly purified sample extracted from cells always contains protease and nuclease).

(3) When the sample is frozen, its pH may change markedly, depending on the solubility of the salt contained in the sample. For example, a sodium phosphate buffer of pH 7 is made by mixing NaH_2PO_4 with Na_2HPO_4, and when cooled, water first turns to ice and the concentration of sodium phosphate within the nonfrozen solution increases. Because the solubility of Na_2HPO_4 is lower than that of NaH_2PO_4, Na_2HPO_4 is precipitated first. Because the pH of the remaining NaH_2PO_4 is low, that of the nonfrozen solution decreases to 4 and in some cases protein may be deactivated. (Protein at high concentration is free from such an extreme pH change because of its own buffering capacity. The pH change can be considerably reduced also by adding approximately 10% (w/v) glycerol.)

(4) It is generally better to freeze an unstable sample at -80°C than at -20°C (care should be taken as some samples may be deactivated by freezing).

3.1.3 Storage Method

(1) Protein

Each protein has an optimal storage method to keep the protein stable. In general, proteins are stable when stored as a 2 - 3 M ammonium sulfate suspension or 50% glycerol solution at a low temperature (each protein has its own optimal temperature for storage). It is desirable to add an appropriate protease inhibitors.

(2) Nucleic Acid

Nucleic acid is considered to remain most stable when stored in a dried state or in TE solution with an equivalent amount of ethanol added, kept in a liquid state at –20°C.

3.2 Balance

Although some electronic balances can display a value to a 5- or 6-digit accuracy, buoyancy varies depending on pressure or humidity and weight varies depending on latitude or altitude (gravity) except when measured with a scale balance. In addition, a reagent absorbs moisture to some degree. Therefore, the balance should be properly calibrated and it is considered that a value beyond four significant digits is not reliable unless humidity is well taken into account. Because most scaled reagents are used as solutions, except when a dry weight is measured, errors of the measuring cylinder and measuring flask (approximately 0.5% and 0.1%, respectively) should be considered.

3.2.1 Careful use

(1) Check levelness

A precision balance is equipped with a level. Make the balance horizontal using the level (if there is an inclination of θ degrees, a value sinθ lower is displayed).

(2) Calibrate balance

Because the buoyancy varies depending on the pressure, as mentioned above, use the balance after calibration if accuracy is required. Because each balance has its proper calibration method, refer to the respective instruction manual.

3.2.2 If reagent is spilled

Wipe a spilled reagent immediately, otherwise, the following problems will arise.

(1) A spilled reagent will corrode and corrupt the balance, causing microbial contamination (microbial contamination = nuclease + protease).

(2) Each reagent has a specific method of treatment when spilled. If the method is unknown, follow the instructions of the instructor.

(3) A spilled reagent will adhere to the back of the powder paper to be used in the next measurement and will contaminate the next reagent.

Only the person who spilled the reagent knows its true identity. If you spill a reagent, you are obliged to treat it appropriately immediately. Keep in mind that the spilled reagent may cause harm to other persons involved or may ruin their experiments.

3.3 Measuring Pipette

3.3.1 Safety Pipetter

When measuring a solution other than that recognized to be safe, use a safety pipetter. When inserting a measuring pipette into the safety pipetter, exercise caution, as instructed in 2.2.2. If a solution is accidentally drawn up into the safety pipetter, rinse it immediately as described below. Otherwise, it may cause harm to the next user (the solution drawn up may spatter when exhausted).

If the solution is accidentally drawn up, draw up and drain the distilled water. Repeat this operation ten times or more. Then, pinch the pipetter valve with a binder clip, and ventilate and dry the safety pipetter using a compressor, while keeping the valve open.

3.3.2 Error

(1) Error factor

The labeled amount of a measuring pipette or whole pipette indicates the amount of flow for free-falling water at 20°C after being drawn up to the gauge line. Therefore, an accurate volume of the solution cannot be measured with these pipettes in the

following cases. Moreover, some disposable pipettes have not been tested and some have an error as high as nearly 10%.

1) When the solution is blown out with the mouth
   → measured volume tends to be small
2) When moisture is left on the inner wall of the pipette because of insufficient rinsing
   → smaller volume
3) When the solution is attached to the outer wall of the pipette
   → greater volume
4) When the solution is a high-temperature solution
   → greater volume
5) When the solution is a highly viscous solution
   → smaller volume

(2) Handling of highly viscous solution

A highly viscous solution (e.g., sugar at high concentration, glycerin, protein, and surfactant solution) cannot be measured accurately because an appreciable amount of solution is left on the inner wall of the pipette. The following measures should be taken: 1) measure the weight (examine the specific gravity in indices, such as "Merck Index" and then, calculate the volume), 2) use an injector.

3.4 Pipetman

3.4.1 Basic operation

(1) Be sure to attach a chip. Dip only the smallest necessary portion of the tip of the chip into the solution.

(2) Hold the Pipetman vertically and draw up the solution **slowly**.

(3) Drain the solution **slowly**. Wait until the solution on the inner wall of the chip descends to the tip of the chip, then push the piston into the pipette completely.

(4) Check that no solution remains inside the chip.

3.4.2 Precautions

(1) Do not draw up a volatile strong acid solution.

Vapor from hydrochloric acid or trifluoroacetic acid corrodes the stainless-steel piston in the Pipetman. If these acids are used, the Pipetman will become unusable. In such a case, disassemble the Pipetman and wash it immediately, as instructed in 3.4.4.

(2) Beware of a small amount of solution remaining on the tip of the chip.

Before drawing up the solution, the piston must first be pushed into the pipette. At this time, air is forced out of the tip of the chip. A small amount of solution often remains in the tip of a previously used chip. **Such a solution will be ejected as fine droplets** by pushing the piston. When using dangerous solutions (carcinogens, poisonous and deleterious substances, microorganisms, strong acid, strong alkali, etc.), exercise particular caution. To avoid accidents, take sufficient precautions as follows.

1) Take time to slowly drain the solution from the tip of the chip completely.
2) Do not reuse a chip.
3) Place the tip of the chip in a container when pushing the piston fully.

(3) Operate the Pipetman slowly.

If the solution is drawn up suddenly, it may penetrate the body of the Pipetman (in such a case, disassemble it and wash it immediately as instructed in 3.4.4.), and accurate measurement of the volume will not be possible. When the solution is drained suddenly, a small amount of solution remains on the inner wall of the chip, again causing inaccurate measurement.

(4) Do not set the scale beyond the limit.

For example, do not set the scale at 25 μL for P-20, as it may cause inaccuracy or breakdown.

3.4.3 Error

When maintained sufficiently well, used with a new chip and operated properly, the Pipetman has good reproducibility. However, the measured amount may have an error of 2–3% compared with the absolute amount. When using the Pipetman in an experiment in which this absolute error becomes a problem, calibrate it by measuring the volume of pure water (relative density = 1.00) using a balance. It is considered that the following solutions cannot be measured accurately.

1) Highly viscous solution (glycerin at high concentration, sugar, surfactant solution, protein solution at high concentration, etc.)
2) High-temperature solution (this should be measured after cooling to room temperature)
3) Volatile solution (chloroform, acetone, etc.)

Fig. 7 Calibration of Pipetman

Place a small beaker on a balance and measure the volume of pure eater.

3.4.4 Maintenance

If a test solution is drawn into the body of the Pipetman or corrosive acid is used, disassemble and wash the Pipetman. Otherwise, the stainless-steel piston will be corroded, which cannot be easily repaired. If a medium component or culture solution is drawn into the body, bacteria may propagate inside the Pipetman, causing microorganism or nuclease contamination. The disassembly and washing procedure is as follows.

1) Detach the remover.
2) Remove the nose and wash it (rinse finally with pure water).
3) Remove the O-ring and Teflon-coated seal attached on the piston.
4) Wipe the piston well using Kimwipe soaked with pure water or ethanol. If heavily contaminated, scrub it using a soft sponge with detergent. Rust can be wiped off using 0.1–1 M dithiothreitol solution (old solution is sufficient).
5) After drying the piston and nose completely, reassemble them.
6) Measure the volume of water using a balance and check the reproducibility of the Pipetman and for any solution leakage (whether or not solution drips from the chip). If reproducibility is poor or solution leakage is detected, disassemble the Pipetman again and replace the O-ring and Teflon-coated seal with new ones. Always have a supply of O-rings and Teflon-coated seals in stock.

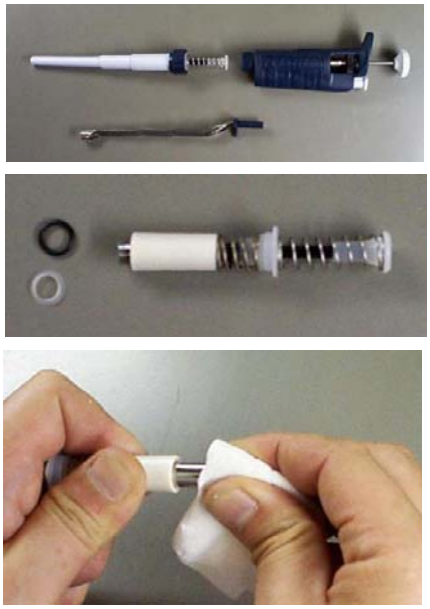

Fig. 8 Maintenance of Pipetman

3.4.5 Volume measurement using Pipetman

Set the scale of the Pipetman at a value slightly less than that needed and draw up the solution to be measured (A). Draw it up completely by turning the knob while the chip is held in the solution (B). Drain the solution from the tip once (C). Then, draw it up again and check that the solution has been drawn up completely. Adjust the scale again if necessary. The scale measured as above can indicate the volume of the solution (D). Use a calibrated Pipetman properly.

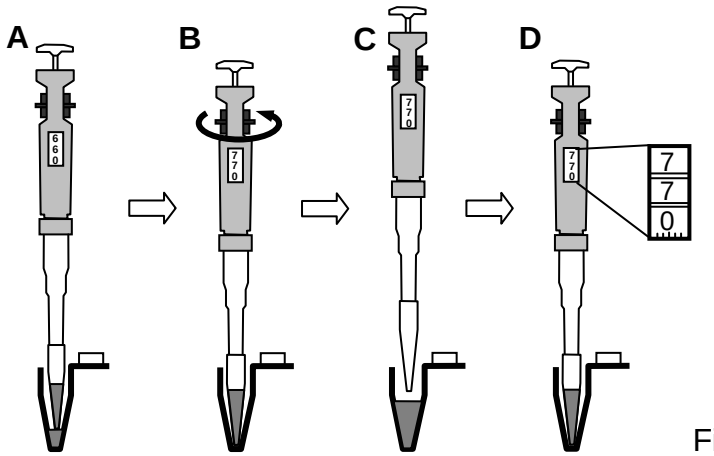

Fig. 9 Volume measurement using

3.5 Stirrer

3.5.1 Putting in the magnet bar

When putting the magnet bar into a glass container, slide the magnet bar into the tilted container. When the container cannot be tilted because it already contains a solution, attach the magnet bar to a magnet placed on the outside of the container and slowly lead the magnet bar to the bottom of the container. Do not drop the magnet bar into the container on the stirrer (refer to 2.2.1.–(7)).

Because the magnet bar is made of metal, do not heat a container with a magnet bar in a microwave.

3.5.2 Stirrer with heating device (hot-plate stirrer)

Do not leave the hot-plate stirrer unattended during heating. The sample may burn dry or other people may touch the apparatus inadvertently and be burned. Use only a glass container, never a plastic one.

3.6 Vortex Mixer

3.6.1 Precautions

(1) Solution may ascend to the position of the supporting hand.

Because the vortex mixer rotates, a small amount of solution may ascend to the supporting point (position of the supporting hand) if a certain amount of solution is placed in the mixer. Conversely, the solution may spill if the orifice of the test tube is held.

(2) Do not set a test tube that is more than one-third full.

In particular, if the solution contains dangerous reagents, a spilled solution will cause an accident. To avoid such an accident, modify the experimental system so that a solution of one-third or less the volume of the test tube is mixed, or mix the solution/shake the test tube upside down with the mouth of the test tube covered with Parafilm.

3.6.2 To mix solution completely

(1) The solution cannot be completely mixed merely by rotating the mixer 2 - 3 times.

Because the solution circulates in the form of a laminar flow (the upper layer of the solution circulates only in the upper layer, and the lower layer circulates in the lower), it cannot be completely mixed unless the rotation – pause cycle is repeated several times to generate a turbulent flow.

(2) The amount of solution should be one-third or less than the volume of the test tube.

If the amount of solution is one-third or more than the volume of the test tube, the solution cannot be completely mixed unless considerable care is taken. It is easier and more reliable to mix the solution upside down several times with the test tube wrapped in Parafilm.

3.7 Clean Bench

3.7.1 Safety precautions

(1) Turn off the gas burner.

When leaving the clean bench, even for a short time, as well as after completing the experiment, turn off the gas burner. Keep the gas burner a sufficient distance from the wall of the clean bench. Do not place it where the wall may be heated.

(2) Take sufficient precautions when using sterilizing ethanol.

When the sample is burned and sterilized using a conradi bar with ethanol, be fully aware that it is dangerous to use solvent and fire together, and take sufficient precautions as follows.

1) Do not bring more ethanol than necessary to the clean bench.
2) **Keep ethanol in a metal container with a lid and keep the lid within reach during operation.** In the case of fire in the container, calmly close the lid to extinguish it.
3) Keep ethanol in a stable container that does not easily fall over when jostled (for example, place the container on a large glass petri dish to increase the surface area of the base).
4) Keep ethanol as far as possible from the gas burner. In particular, do not place ethanol windward of the gas burner.

3.7.2 Sterilizing operation points

(1) Wash your hands

Three factors required for microorganism propagation are water, nutrition and sufficient temperature. Human skin or saliva satisfies all three conditions. Approximately 10^4 – 10^6 bacteria/cm^2 are considered to exist on the human palm. Because

nail scrapings are essentially balls of bacteria, it would not be surprising to find $10^7 - 10^8$ bacteria in them. That is, humans are the major source of contamination in the laboratory.

The number of surviving bacteria N when N_0 bacteria are sterilized by heating for t minutes under certain conditions, is given as $N = N_0e^{-kt}$ (k: death rate constant), and this equation can be applied to most cases of chemical sterilization. As is obvious from the equation, there are two methods of reducing the number of surviving bacteria to a value as close to 0 as possible: increase kt (sterilization for a long time under severer conditions) and decrease N_0. Although most nutritive cells can be sterilized using 70% ethanol, mold spores and bacteria spores are unaffected (in this case, k is nearly 0). On the contrary, N_0 can be decreased by 3 – 4 orders merely by washing one's hands with soap. Because nail scrapings are essentially balls of bacteria, they may contain as many as $10^7 - 10^8$ bacteria in them. It is very difficult to sterilize the entire bacteria ball with agents (do not overestimate the effects of disinfectant ethanol or inverted soap). The most effective method is to scrub the hands and fingers carefully as a doctor does before surgery.

The most effective simplest method of preventing contamination is to wash your hands with soap before performing your experiment.

(2) Close windows

If wind blows in, a clean bench cannot be kept sterile. The same applies for the case of a clean bench exposed to airflow from an air conditioner.

(3) **Consider the placement of apparatus and the operation procedure so as not to needlessly place your hands over** (even when washed, hands are the dirtiest items at the clean bench) **the apparatus that is to be kept sterile.**

1) Place objects to be kept sterile at the back and nonsterile objects at the front.
2) As a rule, place objects handled by the right hand on the right side and those handled by the left hand on the left.
3) Complete operations requiring both hands, such as opening the lid of a bottle, before taking hold of an item.

(4) Do not speak.

Saliva is a major source of bacteria. Consider why a doctor wears a mask during surgery.

(5) Keep the clean bench clean.

If a medium is spilled, wipe it quickly and carefully. Otherwise, it will decay and become a major source of bacteria.

(6) Do not leave unnecessary instruments lying around.

Do not leave unused items, such as petri dishes, medium, Pipetman and mixer lying around. Otherwise, the following problems will arise.

1) The work space will be reduced.
2) Sections in shade will be increased and will not be sterilized by ultraviolet irradiation.
3) Instruments, particularly the plastic parts, will deteriorate and become useless because of irradiation by ultraviolet.

3.7.3 When using pipette or Pipetman

(1) Pipetman

The inside of a Pipetman is not sterile. If a solution (in particular, medium and culture) is drawn up into the Pipetman body, rinse it immediately. Otherwise, bacteria will propagate and cause contamination. Because the Pipetman cannot be autoclaved, use a chip with a filter or a bench mate that can be autoclaved. The outside of the Pipetman is also unsterile. Wipe it with inverted soap (benzalkonium chloride solution) before use.

(2) Pipette

When a solution is drawn up into a pipette manually by sucking, the risk of contamination is increased. The inside of the pipette is also not sterile. Hence, some precautions should be taken, such as the use of disposable pipettes with a filter, measuring pipettes filled with cotton, or an electric pipetter with a built-in filter.

3.8 pH Meter

3.8.1 Measurement procedure

(1) Switch on the power and remove the rubber cap at the top of the sensor.

(2) Check that the surface of the liquid in the comparison electrode is sufficiently above the drainage point. If insufficient, add more liquid.

(3) Calibrate the meter.

(4) Rinse the sensor with pure water and wipe it with

(5) Agitate the solution using a stirrer and wait until the rotation of the magnet bar is steady. The proper pH cannot be measured unless the pH is measured while the solution is being agitated.

(6) Dip the sensor into the solution until the drainage point (a hole in the side of the sensor tip) is dipped. The pH cannot be accurately measured unless the sensor is dipped into the solution with the drainage point below the surface of the solution. Also exercise caution to avoid contact of the sensor with the rotating magnet bar. In the case of a small amount of solution, take measures to keep the sensor away from the magnet bar, for example, replace the container with a thinner one.

(7) Read the value displayed. Under normal measurement operation, the 3rd digit of the displayed value is unreliable. In case of an experiment requiring 3 significant digits, use a clean sensor (a pH sensor contaminated due to the measurement of impure solution, such as a natural medium, is inapplicable) and carefully monitor the temperature, ionic strength, and absorption of carbon dioxide gas in air and ammonia (for details, refer to books, such as "*pH Theory and Measurement*," Yasushi Masuko, Tokyo Chemistry Dojin (1967)).

(8) Return the rubber cap to the top of the sensor. If the sensor is kept open, moisture will evaporate from the interior liquid and potassium chloride salt will precipitate. If salt is deposited, the interior liquid cannot be replaced, causing inaccurate pH measurement.

(9) Rinse the sensor with pure water and imerse it in pure water. Some recent sensors are recommended to be stored not in potassium chloride solution but in pure water. Read the manual and confirm the optimal preservative solution for each sensor. When using a complex-type pH electrode, understand that a small amount of interior liquid (potassium chloride) is mixed into the solution, the pH of which is to be measured because of the structure of the sensor. In the case of an experiment in which the contamination of potassium or chlorine must be avoided, divide the solution into two equal parts and adjust them by adding, to one part, an equivalent amount of the acid (alkali) used for the pH adjustment of the other part.

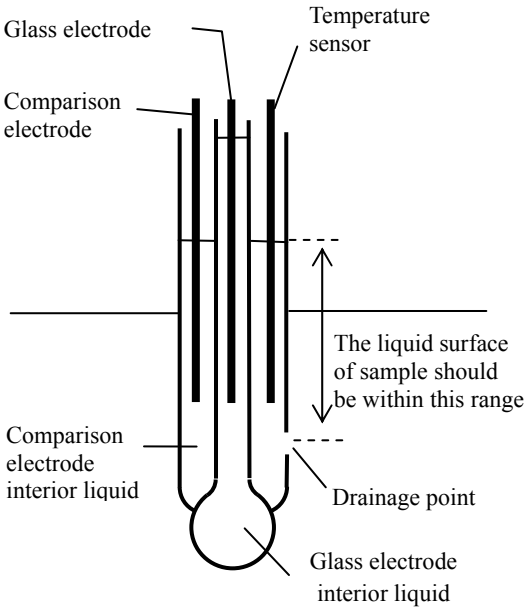

Fig. 10 Structure of pH Sensor

3.8.2 Calibration

(1) Do not mix any solution into the standard buffer solution

Rinse the sensor always with pure water and wipe the moisture with Kimwipe, then dip it in the standard buffer solution.

(2) How to choose the standard buffer solution.

Generally, zero adjustment is carried out using pH 7 standard buffer solution and sensitivity adjustment is carried out using pH 4 standard buffer solution. Although there is pH 9 (pH 10) standard buffer solution, it absorbs carbon dioxide gas in air, by which its pH rapidly changes. Be sure to use a fresh standard buffer solution.

(3) Precautions concerning temperature

When using a manually-calibrated pH meter (by turning a knob), note that the pH of the standard buffer solution itself also varies depending on temperature. That is, the meter should be calibrated to the pH of the standard buffer solution at the temperature at that time. For example, in the case of phosphoric acid standard buffer solution, calibrate the pH meter to 6.86 at 25°C and 6.92 at 15°C. If an accurate pH adjustment is required, use a clean sensor and a fresh standard buffer solution, and calibrate the meter at the temperature at which it is used (refer to 3.8.3).

3.8.3 Temperature and dilution

The pH of solution varies depending on dilution as well as temperature. For example, in the case of Tris buffer solution, pH 8.85 at 0°C decreases to pH 8.08 at 25°C. Refer to Chapter 12 in *Basic Experiment Method Using Protein and Enzyme* (2nd revision, Ed. Takekazu Horio, Nankodo) to see how much the pH of each buffer solution varies with temperature. If ionic strength exceeds 0.2, the effect of the electric potential difference between solutions will gradually become marked, causing difficulty in accurate pH adjustment (for details, refer to books such as "*pH Theory and Measurement*," Yasushi Masuko, Tokyo Chemistry Dojin (1967)). Some effects of temperature and electric potential difference between solutions may greatly exceed the margin of error in some experiments. For example, if 1 M Tris-HCl that **was thought to have been** adjusted to pH 8.0 is diluted 100-fold to a 10 mM solution, the pH may deviate by more than 0.5. Adjust the pH of the buffer solution according to its temperature and concentration.

The procedure for preparing 1 L of buffer solution at 10-fold concentration is as follows.

(1) Dissolve an amount of reagent for 10 L of solution, and make it up to 900 mL in total using a measuring cylinder.
(2) Transfer 90 mL from (1) using the measuring cylinder, into a beaker and make it up to 800 – 900 mL in total.
(3) Warm (Cool) to the temperature at which the solution is to be used.
(4) Put an acid (alkali) of appropriate concentration into a suitable container and measure the total weight including that of the container (W_1).
(5) Adjust the pH of (3) to a predetermined pH using acid (alkali) (4) (the solution can be made up to 1 L and used as is).
(6) Measure the weight of the remaining acid or alkali including that of the container (W_2).
(7) Add acid (alkali) of the amount $9\times(W_1 - W_2)$ to the remainig (1) (810 mL) and make it up to 900 mL.

3.9 Spectrophotometer

3.9.1 Problems in measuring turbidity

(1) Turbidity varies depending on the type of spectrophotometer used.

Turbidity is evaluated on the basis of the phenomenon that the light reaching the detector is decreased due to interruption by particles, such as cells. At this time, the light scattered by particles partly reaches the detector. The amount of scattered light reaching the detector increases as the width of the slit at the detector side increases or as distance between the cell and the detector
decreases, giving a low turbidity. Therefore, when using a different type of spectrophotometer or a spectrophotometer made by a different manufacturer, a different turbidity value is obtained even when measuring the same sample. In particular, in the case of large cells, such as yeast, the turbidity may differ 3-fold or more depending on the type of spectrophotometer used.

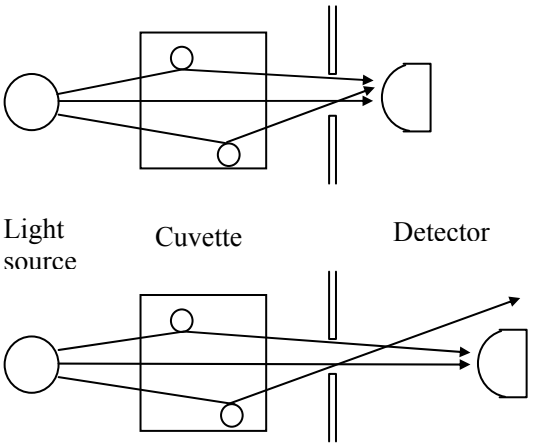

Fig. 11 Effect of detector position on turbidity measurement.

The shorter the distance between the cell and the detector (the larger the slit width), the more scattered light is detected.

(2) Range in which cell concentration is proportional to turbidity

The range in which cell concentration is proportional to turbidity differs depending on the spectrophotometer used, the maintenance condition of the spectrophotometer and the cell of concern. Check the range for each spectrophotometer to be used.

(3) Other factors affecting turbidity
   1) Medium: For example, the optical condition of a cell surface is different between the minimum medium and the synthetic medium, and therefore, turbidity differs, even at the same cell concentration.

2) Osmolarity: Depending on the osmolarity of the medium or diluted water, the cell expands or shrinks, resulting in a different turbidity. When diluting a cell sample, use the same medium or saline at the same osmolarity as that of the medium.
3) Strain: If the strains are different (for example, even of the same *E. coli*), turbidity differs, even at the same cell concentration.
4) Culture phase: The condition of the cell differs depending on its phase, that is, logarithmic phase, stationary phase or extinction phase, and therefore, turbidity differs, even at the same cell concentration.

3.9.2 Cuvette (Cell)

Cuvettes are made of quartz, glass or plastic. A quartz cuvette can be used in the ultraviolet region, but is very expensive. Use the cuvette appropriate for each purpose.

Table 3 Materials and characteristics of cuvette.

Material	Price	Physical durability	Usage in UV region	Heat conduction	Optical accuracy
Quartz	High	Poor	Applicable	Good	Good
Glass	Middle	Poor	Not applicable	Good	Good
Plastic	Low	Good	Fair* or Poor	Poor	Fair or Poor

*Some may be used.

3.10 Electrophoresis

Protein or nucleic acid forms colloid particles when dispersed in a solvent, and has a certain amount of charge Q at constant pH, ionic strength and temperature. These colloid particles move toward the electrode with the sign opposite to their charge with the force $Q \cdot E$ when an electric field, E, is applied to the colloid solution. Here, the friction resistance between the gel-matrix and particles is f and the particle velocity is v, thus, $Q \cdot E = f \cdot v$ holds. The velocity of a particle during electrophoresis depends on voltage and has no direct relationship with current.

3.10.1 Meaning of constant voltage, constant current and constant power

Voltage (V) = Resistance (Ω) × Current (A),

Power (W) = Voltage (V) × Current (A).

(1) Constant voltage

This means that a constant voltage is always being applied. With a lower circuit resistance (the ion concentration of buffer solution is higher), a higher current flows.

(2) Constant current

This means that a constant current is always flowing. With a higher circuit resistance, a higher voltage is applied. When the wire is broken, a tremendously high voltage is applied (for example, if 1 mA of constant current is planned to flow in a 1 MΩ circuit, 1000 V of voltage is required), causing accidents such as discharge or electric shock. Because there are many power supplies without safety devices, exercise sufficient caution.

(3) Constant power

This means controlling voltage or current so that a constant power is always consumed. This is adopted, for example, to maintain heat generation by flowing constant current to maintain the temperature of a gel, such as sequence gel, constant. With a higher circuit resistance, a lower current or a higher voltage is required. Although most power supplies are equipped with a safety device, be sufficiently aware of wire breakage, as in the case of (2).

3.10.2 Procedure of applying current

(1) Check that the voltage (current) adjustment knob on the power supply has been set to minimum before switching on the power.
(2) Check that the circuit is properly connected. When applying constant current, check that the power – electrode – buffer solution – gel – buffer solution – electrode – power are connected. That is,
1) ensure that the buffer solution is in;

2) ensure that the gel or electrode is not exposed due to leakage of the buffer solution;
3) ensure that the seal tube used in gel preparation is removed;
4) ensure that bubbles do not exist at the bottom of the gel.

(3) Switch on the power.

(4) Turn the knob gradually to increase voltage, while monitoring the voltmeter and ammeter.

(5) If current or voltage is different from that during normal operation, immediately turn the knob to minimum and switch off the power. Then, inspect the power supply, referring to the procedure in 3.10.3 (those unfamiliar with the normal value of current or voltage must be accompanied with an experienced person).

3.10.3 Trouble arising when applying current and its cause

It is dangerous to apply constant current. When using a power supply without a voltage limiter function, carry out electrophoresis by applying not a constant current but a constant voltage.

(1) Electrophoresis at constant current

1) The case of voltage higher than normal

In the case of an extremely high voltage, the wire is considered to be broken somewhere. Therefore, inspect it again as described in 3.10.2 (3). Also check for any breakage of the cable or platinum wire in the electrode. There is also the possibility that the buffer solution concentration used for gel preparation and that used for electrophoresis are wrong (lower concentration than predetermined).

2) The case of voltage lower than normal

There may be a short-circuit somewhere. There is also the possibility that the buffer solution concentration used for gel preparation and that used for electrophoresis are wrong (higher concentration than predetermined).

(2) Electrophoresis at constant voltage

1) The case of current higher than normal

There may be a short-circuit somewhere. There is also the possibility that the buffer solution concentration used for gel preparation and that used for electrophoresis are wrong (higher concentration than predetermined).

2) The case of current lower than normal

In the case of an extremely low current, the wire is considered to be broken somewhere. Therefore, inspect it again as described in 3.10.2 (3). Also check for any breakage of the cable or platinum wire in the electrode. There is also the possibility that the buffer solution concentration used for gel preparation and that used for electrophoresis are wrong (lower concentration than predetermined).

3.10.4 Agarose gel electrophoresis (AGE) of nucleic acid

(1) Gel preparation

1) Add agarose to the predetermined buffer solution and dissolve it completely. It is desirable to use an autoclave. Although agarose can be dissolved by heating in a microwave oven (take sufficient precautions against burns caused by sudden boiling), a good electrophoretic pattern cannot be obtained unless the agarose is dissolved completely. Note that the concentration of the buffer solution increases, as does gel concentration, if it is overheated and the amount of moisture evaporation increases.

2) Cool the mixed solution to 50 – 60^{o}C and pour it into the gel maker. If the solution is too hot, it will deform the gel maker (if the gel maker is deformed, a gel with uniform thickness can no longer be prepared). If the solution is too cold, the obtained gel will have an inconsistent meshed structure that causes a disturbed electrophoretic pattern.

3) Solidify the solution on a horizontal surface (uniform electrophoresis cannot be achieved unless the gel thickness is uniform). If the solution is not completely solidified, the electrophoretic pattern will be disturbed, and in most cases, a diffused band will be obtained. It is desirable to store the gel solution covered with polyethylene wrap in the refrigerator for 20 – 30 minutes to solidify it completely. If the gel solution is left without wrapping, the gel concentration of the gel surface increases and a diffused band is obtained (Fig. 12A).

(2) Problems concerning the application of buffer solution and sample

1) If the concentration of the buffer solution to be used for electrophoresis is different from that of the buffer solution used for dissolution of gel, the mobility will be different between the parts closer to and those further from the gel surface, resulting

2) If the temperature increases during electrophoresis (an increase of 40°C or more is dangerous), the meshed structure of the gel will be disturbed, resulting in a diffused band. Because the amount of heat generated is high when using 1×TAE buffer solution, dilute twofold to 1/2×TAE buffer solution for use. Because agarose gel electrophoresis of nucleic acid is normally carried out at a constant voltage, current, as well as the amount of heat generated, decreases by half if the buffer solution is diluted twofold (in this case, prepare a gel using half-diluted buffer solution).

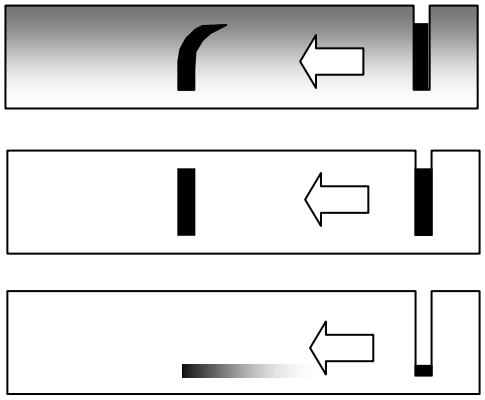

Fig. 12 Cause of disturbed bands obtained in AGE.

A Gel surface is dried,
B Normal electrophoresis,
C A small amount of sample containing a large amount of DNA is applied.

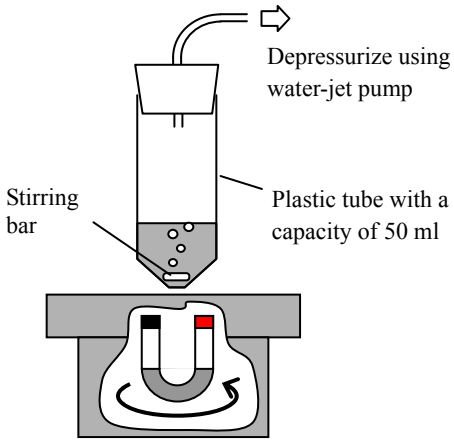

Fig. 13 Degassing of gel solution

3) Even when using the same amount of DNA, if the volume of the sample is small, it is locally overcharged, causing tailing (Fig. 12 (C)).

4) The mobility varies depending on the ionic strength of the sample. It varies particularly markedly in the case of electrophoresis for a short period using Mupid. For example, to precisely compare the mobilities of restriction-enzyme-treated DNA using H buffer and L buffer, add 1/9 volumes each of water and 1 M NaCl to each buffer to adjust the ionic strength of the sample before starting electrophoresis.

3.10.5 Polyacrylamide gel preparation

(1) Main reasons for failure of gel preparation

1) There is a problem with ammonium persulfate.

If adjusted and stored following the method described in 2.8.4(4), ammonium persulfate will present no problem in most cases.

2) The solution is not sufficiently degassed.

Because the dissolved oxygen prevents gel polymerization, the gel solution must be sufficiently degassed (refer to 3.10.2(2)).

3) The temperature of the gel solution is low.

If the temperature of the gel solution is low, the polymerization proceeds slowly because it is a chemical reaction (in general, the reaction rate decreases by half as the temperature decreases by 10°C). Gas solubility increases as the temperature decreases, and the solution becomes insufficiently degassed.

4) The gel is polymerized too early.

If the gel is polymerized too early, a simple solution is to cool the gel solution with ice, before adding ammonium persulfate to the solution, to adjust the polymerization rate after sufficiently degassing the solution (caution: when the solution is first cooled, degassing will become difficult). One can also decrease the amount of ammonium persulfate added.

(2) Simple method for degassing gel solution

1) Put a solution other than SDS, TEMED and ammonium persulfate solutions into an appropriately sized container that is resistant to decompression, and heat it to room temperature (20 - 30°C). The smaller the head space, the more efficiently the gel solution is depressurized and degassed. It is better to degas the solution in a small container that is resistant to decompression

than in a beaker in a desiccator. However, note that the container becomes ineffective for sudden boiling if it is too small.

2) Depressurize and degas the solution, by holding the container in an ultrasonic washing machine or with constant stirring of the gel solution with a small magnet bar placed in the solution.

3) Put SDS, TEMED and ammonium persulfate solutions into the container in this order and close the lid (cover with Parafilm). Then, gently turn the container upside down several times to mix the resulting solution, and pour it into a gel plate.

3.10.6 Polyacrylamide gel electrophoresis of protein

In electrophoresis in a Tris-glycine system, the composition difference among the resolving gel, concentrating gel and electrophoretic buffer solution is effectively used to concentrate the protein band (refer to Chapter 6 in *Basic Experiment Method Using Protein and Enzyme* (2nd revision, Ed. Takekazu Horio, Nankodo)). Therefore, it is ideal for the buffer solution composition of the sample to be the same as that of the concentrating gel. The buffer solution used for the concentrating gel is 62.5 mM Tris-HCl with pH 6.8. If the pH of the sample deviates from that value or the sample contains salt or nucleic acid at high concentration, the protein band cannot be sufficiently concentrated in the concentrating gel. To obtain a good electrophoretic pattern, the following operations are required.

(1) When the pH of the sample is different from that of the concentrating gel

Using pH-test (litmus) paper, adjust the pH of the sample to 6.7 – 6.9 by adding 0.1 M HCl or NaCl.

(2) When the ionic strength of the sample is high

1) Carry out electrophoresis of the sample diluted with water, and stain the gel by a high-sensitivity staining method (for example, silver staining is more sensitive than CBB staining by one order of magnitude or more).

2) Desalt the sample by ultrafiltration or dialysis (pH can be adjusted at the same time). Except when treating low-molecular protein with 10^4 molecules or less, it is simplest to desalt the sample using a commercially available ultrafiltration cartridge with a moderate exclusion limit.