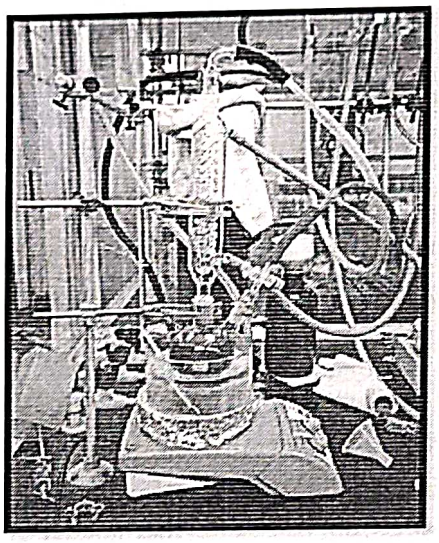
Basic concept in Laboratory Techniques



M.Sc. 2nd Semester

❖ **Chemical Use Guideline**

1) Never use a product that doesn't have a label to reference.
2) Don't mix chemicals without specific authorization from the formulator.
3) Always use personal protective equipments.
4) When pouring chemicals, pour concentrates into the water and not vice-versa.
5) Never pour chemicals into an empty, unlabeled container.
6) Don't store flammable chemicals near a source of heat.
7) Pesticides, fungicides, etc. always must be stored in a safe and elevated position.

❖ **Basically, there are four types of chemicals**

- **Toxic chemicals**: These are chemicals that are poisonous to you, and can act upon the body very rapidly. Hydrogen sulfide and cyanide are examples of toxic agents.
- **Corrosives**: This type of chemical is usually an irritant. Corrosives can damage your body by burning, scalding or inflaming body tissues. Examples are chlorine and HCl acid.
- **Flammables**: Flammables are the chemicals that burn readily. They may explode or burn if sparks, flames or other ignition sources are present. Examples are gasoline, benzene and ethyl ether.
- **Reactive**: Reactive chemicals are those that require stability and careful handling. Some of them can explode or react violently if the container is dropped or hit. Nitroglycerine is an example.

❖ **Basic Tips of Safe Chemical Handling**

1. Read the label
2. Dress the part
3. Follow directions
4. Know emergency procedures
5. Be careful!
6. Report any suspected problems
7. Keep your work area neat, clean and organized
8. Store everything properly

❖ **Basic Rules of Chemical Safety**

Be Aware !..... Be Alert !..... Be Alive!.....

1. Don't buy or store chemicals you do not need
2. Store chemicals in their original container

Original container was designed to hold the chemical without degrading. The original container will have an accurate label, Serious injury can result when people try to identify chemicals with missing or uncertain labels by smelling, tasting or touching.

3. Always wear appropriate cloths and work in a safe environment
4. Always dispose of chemicals safely

❖ **Safety rules of Chemistry Laboratory**

1. Protect Your Eyes and wear appropriate protective clothing
2. Do not apply cosmetics, eat, or drink in the laboratory
3. Pour from large containers to smaller ones (Always ADD ACID to water)
4. Work with volatile chemicals under a fume hood
5. Do not smell any chemicals directly and do not pipet solutions by mouth
6. Know the safety about equipments

❖ **Basic Laboratory Procedures**

- Weighing: Requires careful weighing of all component
- Measuring Liquids: Calibrated glassware, Pipettes should be filled with a hand-operated device called a pipettor etc.

❖ Cleaning Glassware: Follow proper method of cleaning

❖ Sterilization: Sterilizing glassware and Instruments, Sterilizing Nutrient Media, Sterilizing, Plant Materials, Sterile Culture Techniques

❖ **Acids: Strong acids** are very corrosive. They react with metals and can cause severe burns on the skin. Strong acids are Hydrochloric (HCl), Nitric (HNO_3), Sulfuric (H_2SO_4) and Hydrobromic (HBr).

❖ **Weak acids** are often organic acids contain a –COOH group. Week acids are Formic acid (HCOOH), Acetic acid (CH_3COOH), Salicylic acid ($C_6H_4(OH)COOH$) and Citric acid ($C_5H_7O_5COOH$).

❖ **Bases**: Bases are ionic compounds containing metal ions and hydroxide ions. Bases release hydroxide ions in water solutions. **Common Bases** are Sodium hydroxide (NaOH), potassium hydroxide (KOH), Calcium hydroxide $Ca(OH)_2$, Magnesium hydroxide ($Mg(OH)_2$) and Ammonium hydroxide (NH_4OH)

❖ **Salts:** In general, salts are ionic compounds composed of metallic ions and nonmetallic ions. Salts dissociate in water. Salt solutions are generally electrolytes. An electrolyte is a substance that ionizes or dissociates into ions when it dissolves in water.

❖ **pH:** The pH scale is a measure of the hydrogen ion concentration. A pH of 7 indicates a neutral solution while, acids are less than 7 and bases are greater than 7.

❖ **Know the safety equipments in laboratory**

(1) Eye wash fountain (2) Safety shower (3) Fire extinguisher (4) Emergency exits

❖ **Personal Safety while handling Pesticides**

1. Avoid contact with the pesticides
2. Wear all designated safety equipments
3. Be careful of drips and spills
4. Keep hands away from eyes and mouth
5. Wash your hands before Smoking, Eating, Bathroom breaks
6. Use Designated safety equipments (Based on the WPS statement on the label & Regional requirements 1) Long sleeved shirt & long pants of tightly woven material 2) Waterproofed boots 3) Goggles 4) Hard hat 5) Unlined nitrile gloves)

❖ **List of Instruments in meteorology**

(1) Sunshine Recorder	(2) Anemometer	(3) Wind Vane Instruments
(4) Pyranometer	(5) Stevenson Screen	(6) Hygrometer
(7) Ordinary Rain Gauge	(8) Self Recording Rain Gauge	(9) Thermometer & Barometer

❖ **List of Instruments in Microbiology**

(1) Microscope	(2) Balance/scale	(3) Centrifuge
(4) Laminar airflow	(5) Spectrophotometer	(6) Refrigerator
(7) Freezer	(8) Autoclave	(9) Hot air oven
(10) Incubator	(11) pH Meter	(12) Water bath

❖ **List of Instruments in Biochemistry/Chemistry**

(1) Spectrophotometer	(2) Balance/scale	(3) Centrifuge
(4) Stirrer	(5) pH meter	(6) Refrigerator
(7) Freezer	(8) Flame photometer	(9) Hot air oven
(10) Water bath	(11) EC Meter	(12) Atomic absorption Spectrophotometer

♣ Basic Laboratory Procedures:

The majority of laboratory operations utilized in the in vitro propagation of plants can be easily learned. One needs to concentrate mainly on accuracy, cleanliness, and strict adherence to details when performing in vitro techniques.

⊣ Weighing

- The preparation of media requires careful weighing of all components. Even if a commercially prepared medium is used, care must be taken in preparing it and any stock solutions that are required.
- Because of the diversity of laboratory balances in use, it is impossible to review the details of their operation. The manufacturer's instructions should be consulted before using any balance. The types of balances most often encountered in the laboratory include top-loading single-pan balance, triple-beam balance, double-pan torsion balance, analytical single-pan balance, and top-loading electronic balance. The last type has become quite popular in recent years due to its accuracy, ease of use, and durability. With certain models of top-loading electronic balances, milligram accuracy is possible. Such accuracy previously required the use of analytical balances.
- Several common precautions must be observed to obtain accurate weights. First, the balance should be located on a hard, stable, level surface which is free of vibrations and excessive air drafts. The balance or weigh area should always be kept clean. Most importantly, the balance should never be overloaded (see manufacturer's specification). It is advisable to use a lightweight weighing container or paper rather than placing the material to be weighted directly on the pan surface.

⊣ Measuring Liquids

- Calibrated glassware (e.g., beakers, flasks, and pipettes) are required for the preparation of culture media. Graduated cylinders of 10-, 25-, 100-, and 1000-ml capacities are used for many measuring operations, but volumetric flasks and pipettes are required for more precise measurements. Measurement of solutions with pipettes or graduated cylinders is only accurate when the bottom of the curved air-liquid interface is aligned with the measuring mark.
- Pipettes should be filled with a hand-operated device, called a pipettor, which eliminated the hazards of pipetting by mouth. Never pipette by mouth!! Three types of pipettors are commonly used. The first is a bulb-type pipettor, which is controlled by a series of valves. The second type of pipettor is operated simply by rotating a small wheel on the side of the handle. Rotating the wheel upward creates a suction bringing the liquid into the pipette; rotating the wheel in the opposite direction releases the

liquid. A third type of pipettor utilizes an electric air pump. Liquid is drawn into the pipette by pressing the top button and released by pressing the lower button.

- **Cleaning Glassware**
  - The conventional method of washing glassware involves soaking glass in a chromic acid-sulfuric acid bath followed by tap water rinses, distilled water rinses, and finally double-distilled water rinses. Due to the corrosive nature of chromic acid, the use of this procedure has been eliminated except for highly contaminated or soiled glassware. Adequate cleaning of most glassware for tissue culture purposes can be achieved by washing in hot water (70°C+) with commercial detergents, rinsing with hot tap water (70°C+), and finally rinsing with distilled and double-distilled water. However, highly contaminated glassware should be cleaned in a chromic acid-sulfuric acid bath or by some other proven method such as (1) ultrasonic cleaning, (2) washing with sodium pyrophosphate, or (3) boiling in metaphosphate (alconox), rinsing then boiling in a dilute hydrochloric acid solution, and then finally re-rinsing. Cleaned glassware should be inspected, dried at 150°C in a drying oven, capped with aluminum foil, and stored in a closed cabinet.

The following general procedure is recommended for cleaning glassware that contains media and cultures after all data has been collected:

— Autoclave all glassware with media and cultures still in it. This kills any contaminating microorganisms that may be presents.

— After the autoclaved media has cooled, but while it is still in a liquid state, pour it into bio-hazard plastic bags or thick plastic bags, seal, then discard.

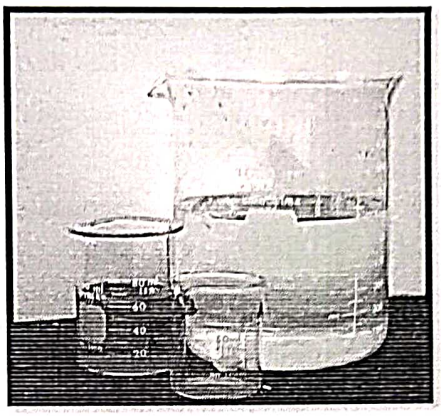
— Wash all glassware in hot soapy water using a suitable bottle brush to clean the internal parts of the glassware. Any glassware that is stained should be soaked in a concentrated sulfuric acid-potassium dichromate acid bath for 4 hr, then rinsed 10 times before washing it with soapy water.

— All glassware should be rinsed three times in tap water, three times in deionized water, three times in double-distilled water, dried, and stored in a clean place.

— Wash all instruments and new glassware in a similar manner.

GLASSWARE AND GUIDE TO CLEANING GLASSWARE

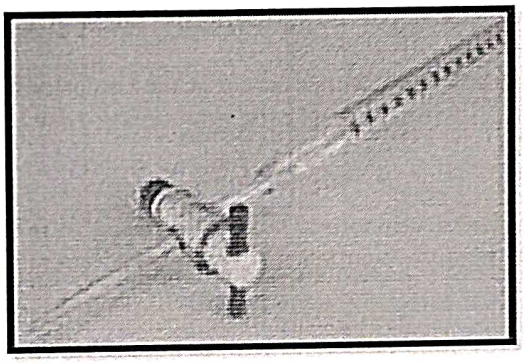
1. Beaker



A beaker is a simple container for stirring, mixing and heating liquids commonly used in many laboratories. Beakers are generally cylindrical in shape, with a flat bottom and a lip for pouring. Many also have a small spout to aid pouring as shown in the picture. Beakers are available in a wide range of sizes, from one millilitre up to several litres. Beakers are commonly made of glass (today usually borosilicate glass), but can also be in metal (such as stainless steel or aluminium) or certain plastics (notably polythene, polypropylene, PTFE).

Beakers are often graduated, that is, marked on the side with lines indicating the volume contained. For instance, a 250 mL beaker might be marked with lines to indicate 50, 100, 150, 200, and 250 mL of volume. These marks are not intended for obtaining a precise measurement of volume.

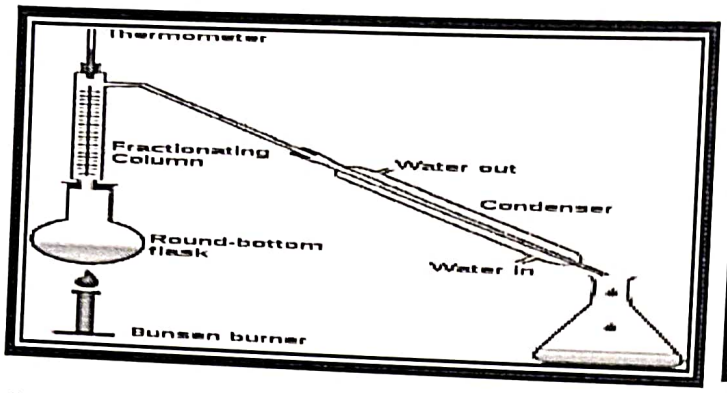
2. Burette (also Buret)

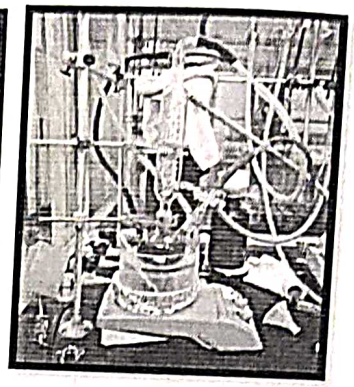


It is a vertical cylindrical piece of laboratory glassware with a volumetric graduation on its full length and a precision tap, or stopcock, on the bottom. It is used to dispense known amounts of a liquid reagent in experiments for which such precision is necessary, such as a titration experiment. Burettes measure from the top since they are used to measure liquids dispensed out the bottom. The difference between starting and final volume is the amount dispensed.

Check the tip of the burette for an air bubble. If an air bubble is present during a titration, volume readings may be in error. Rinse the tip of the burette with water from a wash bottle and dry it carefully. After a minute, check for solution on the tip to see if your burette is leaking. The tip should be clean and dry before you take an initial volume reading. When your burette is conditioned and filled, with no air bubbles or leaks, take an initial volume reading. Read the *bottom* of the meniscus.

3. Condenser



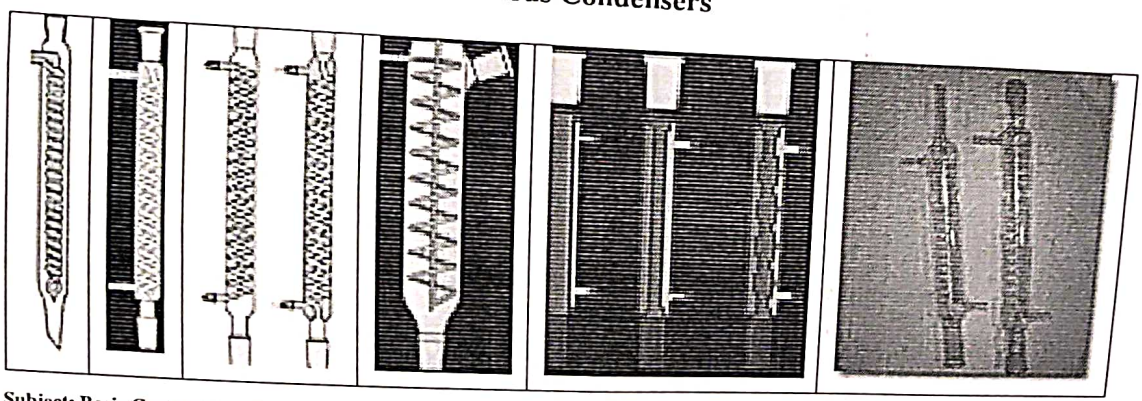


In a laboratory a condenser is a piece of laboratory glassware used to cool hot vapors or liquids. A condenser usually consists of a large glass tube containing a smaller glass tube running its entire length, within which the hot fluids pass. The ends of the inner glass tube are usually fitted with ground glass joints which are easily fitted with other glassware. The upper end is usually left open to the atmosphere. The outer glass tube usually has two hose connections, and a coolant (usually tap water or chilled water/anti-freeze mixture) is passed through it. For maximum efficiency, and to maintain a smooth and correctly directed thermal gradient so as to minimize the risk of thermal shock to adjacent glassware, the coolant usually enters through the lower fitting, and exits through the higher fitting. Maintaining a correct thermal gradient (i.e. entering coolant at the cooler point) is the critical factor. Multiple condensers may be connected in series.

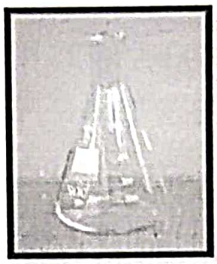
❖ Applications

Condensers are often used in reflux, where the hot solvent vapors of a liquid being heated are cooled and allowed to drip back. This reduces the loss of solvent allowing the mixture to be heated for extended periods. Condensers are used in distillation to cool the hot vapors, condensing them into liquid for separate collection.

Various Condensers

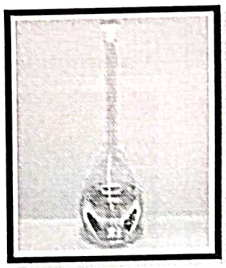


4. **Erlenmeyer/ Conical flask**



An Erlenmeyer, commonly known as a conical or E-Spot, is a widely used type of laboratory flask which features a flat, conical body, and a cylindrical neck. It's named after the German chemist Emil Erlenmeyer, who created it in 1861. The Erlenmeyer is usually marked on the side (*graduated*) to indicate the approximate volume of contents, and has a spot of ground glass where it can be labeled with a pencil. It differs from the beaker in its tapered body and narrow neck. The opening usually has slight rounded lips so that the Erlenmeyer can be easily stopper using a piece of cotton wool. The conical shape allows the contents to be swirled or stirred during an experiment, either by hand or by a shaker; the narrow neck keeps the contents from spilling out. The smaller neck also slows evaporative loss better than a bigger neck. Erlenmeyers are used in chemistry labs for titration. Erlenmeyers are also used in microbiology for the preparation of microbial cultures. Plastic Erlenmeyer flasks used in cell culture.

5. **Volumetric flask**



Volumetric flask is a piece of laboratory glassware that is used to measure a very accurate and precise amount of a liquid.. It has a flatter bottom and long neck. The long and narrow neck is marked, at a very accurate measurement. The volume marks are usually made by machine, so it can be more assuredly accurate than hand-made marks. Volumetric flasks come with a stopper or cap. The stoppers are made of glass and are used for capping the opening at the top of the neck. When a glass stopper is used, the opening at top of the neck has an outer tapered ground glass joint and the glass stopper has a matching tapered inner ground glass joint surface, but often only of stopper quality.

— **Uses of Volumetric Flask**: The volumetric flask is used in two major ways. In one way a solute of known mass is placed in the flask and dissolved. The other technique involves placing an aliquot (sample of precisely known volume) of a solution of known molarity in the flask, then diluting to the mark with solvent. A volumetric flask is used either for making solutions or diluting a liquid to the size of the flask. A volumetric flask is used as a container used to measure the volume of a liquid with extremely high accuracy. Each volumetric flask is designed to measure one particular volume. That's why they come in a variety of sizes, such as 5 ml, 10 ml, 25 ml, 50 ml, 100 ml, 250 ml, 500 ml, 1000 ml, etc.

GUIDE TO CLEANING GLASSWARE

Clean glassware is essential in chemistry. The problem is that the tolerance for contaminants varies with the work you are doing, and sometimes a chemist does not know how important clean glassware is to an experiment until it has failed.

There are two broad degrees of clean in chemistry; quantitative and normal cleaning

- **Quantitatively clean glassware** is required for the most demanding applications where a quantity is being measured at high precision, such in analytical or physical chemistry. At this level of cleanliness there are no residues (e. g., grease) or other impurities on the glassware.
- **Normal clean glassware** is free of most contamination but some contaminates (e. g., grease) is tolerated. Glassware that has been cleaned normally is used where a high degree of precision is not required, such as in a synthesis.
  - — Clean glassware is essential in chemistry.
  - — Quantitative and normal washing.
  - — Quantitatively clean glassware is required for high precision, such in analytical or physical chemistry.
  - — Glassware at this level of cleanliness has no residues Normal clean glassware is used where high degrees of precision are not required.

∇ **General Cleaning Tips**

- — Disassemble your apparatus as soon as possible after you are finished with it. Triple rinse with an appropriate solvent.
- — Graduated cylinders, beakers, Erlenmeyer flasks, burettes and pipettes that were only used to dispense reagents generally only need to be triple-rinsed with a solvent followed by tap water and a final DI (deionized) /distilled water rinse. Air dry on a drying rack.
- — Funnels should be rinsed with an appropriate solvent to remove substances that are clinging to them. Follow this by tap water and DI water rinses and air dry.

∇ Health and Safety Considerations

- — A task of washing glassware is simple but hazardous.
- — You must wear eye protection.
- — Gloves are recommended, if glassware contained an irritant, toxic material.
- — Before cleaning be sure that any excess reagent has been disposed of properly.

∇ General Cleaning Procedure

Following steps should be followed for glassware for which a simple solvent rinse is not sufficient.

— More aggressive cleaning methods may be required.

— Degrease your glassware's ground glass joints by wiping them with a paper towel soaked in a small amount of ether, acetone or other solvent.

— CAUTION! Wear appropriate gloves and minimize your exposure to the vapors.

— Place the glassware in a warm concentrated aqueous solution of detergent, and let sit for several minutes.

— Scrub with brush.

— Rinse thoroughly with tap water and give a final rinse with DI water.

— If there is clearly a greasy residue on the glass, more aggressive action must be taken.

∇ More Aggressive Cleaning Methods

— If contaminant is a metal-containing compound, soak glassware in a 6M HCl solution.

— DANGER! This solution can cause severe burns! Wear appropriate gloves.

— Once solid has dissolved, copiously rinse the item with tap water, and then repeat general cleaning steps above.

— This method will also remove some organic residues (not grease).

— If contaminant is organic, submerge item in a base bath (a saturated NaOH or KOH solution in ethanol/methanol).

↓ DANGER! The base bath will dissolve skin and alcohols are flammable! Wear gloves and keep ignition sources away from base bath. Be sure that glassware is completely filled with solution and is sitting upright. After several minutes of soaking, carefully remove the item (it will be slippery), and rinse thoroughly. If glassware is not quantitatively clean at this point, general cleaning steps may be repeated/ a longer soaking time in base bath, be needed.

∇ Even More Aggressive Cleaning Methods

— Sometimes 6 M HCl and a base bath are not sufficient, so, more aggressive methods must be employed. CAUTION! All of these methods will do severe damage to eyes, skin, mucous membranes and lungs. Extreme caution should be exercised when using these methods. Wear gloves, eye protection and a lab coat. Work in the hood.

— Aqua Regia is an extremely powerful oxidizing solution (1 HNO_3: 3 HCl) (1 part H_2O be added if this will be stored to minimize generation of Cl_2). It will dissolve gold and will oxidize everything. Extreme caution must be used because it generates Cl_2 and NO_x gases in addition to causing severe tissue damage. Clean glassware before soaking in aqua regia and rinse with water.

— Acidic Peroxide Solution is prepared by dissolving commercially-available "No Chromix" in conc. H_2SO_4.

— An alternative preparation is to prepare a solution by mixing equal proportions of concentrated H_2SO_4 and aqueous H_2O_2 solutions (remember to add the H_2O_2 to the acid).

— A 3% H_2O_2 solution is sufficient, and under no circumstances should H_2O_2 solutions greater than 10% be used. The H_2O_2/H_2SO_4 solution is both a strong oxidant and a strong reductant, so care must be taken when using it.

— Another acidic peroxide solution for cleaning can be prepared by dissolving 36 g $(NH_4)_2S_2O_8$ (ammonium peroxydisulfate) in 2.2 L of 98% H_2SO_4. Take precautions for their use as per aqua regia.

— Chromic Acid a premeasured mix is available under the name "Chromerge", Take precautions as per aqua regia.

— Because high-valent chromium is carcinogenic, teratogenic and causes severe environmental damage, so the use of chromic acid is not recommended.

— Concentrated Hydrofluoric Acid HF will remove everything from glass and will even etch the surface of glass itself.

— It should not be used on calibrated volumetrics.

— HF causes severe, painful burns that do not heal well, and prolonged or intense exposure can lead to a very slow, painful death.

— It is not to be used by any students under any circumstances.

∇ **Special Cases**

— Cuvettes, you only need to rinse a cuvette in appropriate solvent and wipe outside immediately after use.

— If something has adhered itself to a cuvette, it is best to soak the cuvette in solvent first and gently coax the solid off the side with a cotton swab.

— Never use a brush on a cuvette! If this fails, one of the acidic cleaning solutions mentioned above can be used (but never HF)

— It is not recommended that base bath be used on cuvettes, because it tends to etch glass surfaces.

— **Protein Contamination:** Usually proteins can be removed scrubbing with detergent, but protein defies removal. In that event, you can proceed to the more aggressive acidic solutions, or you can prepare a peptidase solution (an enzyme that degrades proteins). The enzymatic approach is a bit slower than the forcing methods, but it is gentler and so can be used in situations that contaminated item is incompatible with acid.

✸ **Common Conversions in Molecular Biology**

1 gram (g) = 1×10^3 milligrams (mg)	1 liter = 1×10^3 milliliters (mL)
1 gram (g) = 1×10^6 micrograms (µg)	1 liter = 1×10^6 microliters (µL)
1 gram (g) = 1×10^9 nanograms (ng)	1 liter = 1×10^9 nanoliters (nL)
1 gram (g) = 1×10^{12} picograms (pg)	1 liter = 1×10^{12} picoliters (pL)

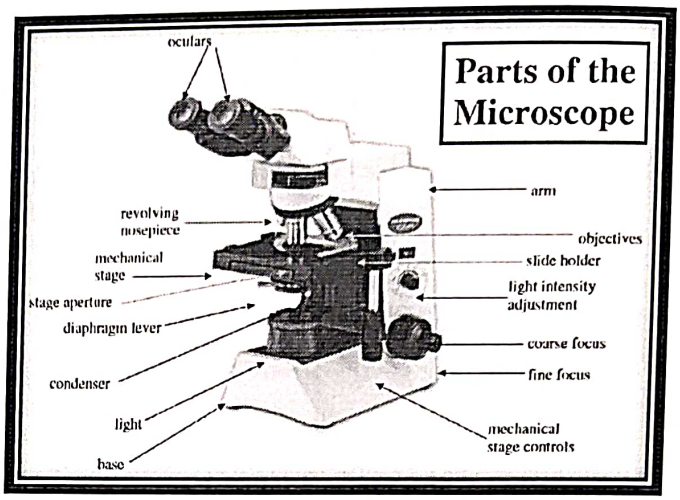
1 molar solution = 1 mole/liter

Conversion Exercises

1. How many grams are there in 0.22 nanograms?
2. 0.5 grams is equivalent to how many micrograms?
3. 0.56 liters equals how many milliliters?

USE AND HANDLING OF INSTRUMENTS/ EQUIPMENTS

1. Microscope



A microscope (from the Ancient Greek: *mikrós*, "small" and *skopeîn*, "to look" or "see") is an instrument used to see objects that are too small for the naked eye. The science of investigating small objects using such an instrument is called microscopy. Microscopic means invisible to the eye unless aided by a microscope.

There are many types of microscopes. The most common (and the first to be invented) is the optical microscope, which uses light to image the sample. Other major types of microscopes are the electron microscope (both the transmission electron microscope and the scanning electron microscope), the ultra microscope, and the various types of scanning probe microscope.

∇ Rules for Use of Microscope

— Carry the microscope properly.

— Always begin focusing with the 4X objective.

— Use the coarse focus only with the 4X objective in place.

— Use immersion oil only with the 100X objective (oil immersion lens) in place.

— Use only ONE drop of oil.

— Lower the stage and then remove the slide when you are done.

— ALWAYS clean the microscope when you are done. Use a lens paper and the alcohol in the labeled jars.

— Always place the 4X objective over the stage and be sure the stage is at its lowest position before putting the microscope away.

— Always turn off the light before putting the microscope away.

— Always wrap the cord correctly before putting the microscope away.

— Always return the microscope to the correct cabinet

— Always place the oculars toward the BACK of the cabinet.

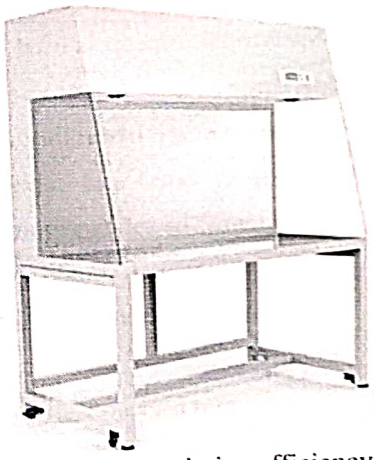
2. Analytical balance

An analytical balance (often called a "lab balance") is a class of balance designed to measure small mass in the sub-milligram range. The measuring pan of an analytical balance (0.1 mg or better) is inside a transparent enclosure with doors so that dust does not collect and so any air currents in the room do not affect the balance's operation. This enclosure is often called a draft shield.

An electronic balance is a device used to find accurate measurements of weight. It is used very commonly in laborites for weighing chemicals to ensure a precise measurement of those chemicals for use in various experiments.

3. Laminar flow cabinet

A laminar flow cabinet or laminar flow closet or tissue culture hood is a carefully enclosed bench designed to prevent contamination of semiconductor wafers, biological samples, or any particle sensitive materials. Air is drawn through a HEPA (High-efficiency particulate arrestance or high-efficiency particulate arresting or high-efficiency particulate air, is a type of air filter) filter and blown in a very smooth, laminar flow towards the user. The cabinet is usually made of stainless steel with no gaps or joints where spores might collect.



Such hoods exist in both horizontal and vertical configurations, and there are many different types of cabinets with a variety of airflow patterns and acceptable uses.

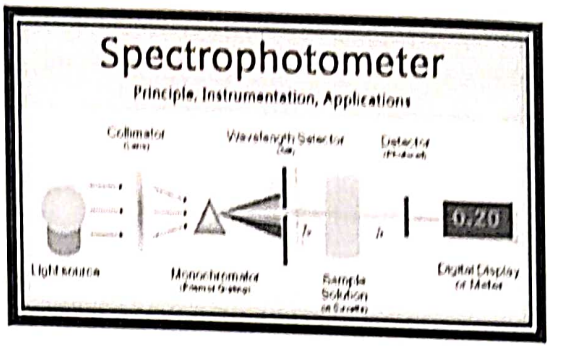
Laminar flow cabinets may have a **UV-Germicidal lamp to sterilize** the interior and contents before usage to prevent contamination of experiment. Germicidal lamps are usually kept on for 15 minutes to sterilize the interior and no contact is to be made with a laminar flow hood during this time. During this time, scientists normally prepare other materials to maximize efficiency. (It is important to switch this light off during use, to limit exposure to skin and eyes as stray ultraviolet light emissions can cause cancer and cataracts.)

4. Incubator

An incubator is a device used to grow and maintain microbiological cultures or cell cultures. The incubator maintains optimal temperature, humidity and other conditions such as the carbon dioxide (CO_2) and oxygen content of the atmosphere inside. Incubators are essential for a lot of experimental work in cell biology, microbiology and molecular

biology and are used to culture both bacterial as well as eukaryotic cells. Maintenance of uniform temperature within the incubator is essential and is achieved by fan, blower or a water jacket containing heated water.

5. Spectrophotometer



- Spectrophotometer is one of the most important instruments in Biochemistry and Chemistry laboratory. It is used for the colorimetric analysis for plant, soil, food etc. It is an instrument that measures the amount of light absorbed by a sample. Spectrophotometer techniques are mostly used to measure the concentration of solutes in solution by measuring the amount of the light that is absorbed by the solution in a cuvette (sample holder) placed in the spectrophotometer.
- Spectrophotometer have a light source (lamp), monochromator with gratting (which convert polychromatic light into monochromatic light i.e. it convert light of different wavelength into single (desired) wavelength), cuvette and detector.
- Spectrophotometer works on Beer- Lambert's Law.
- Spectrophotometer is used for the analysis of protein, carbohydrates, phenol, etc.

6. Centrifuge

- For an average laboratory a small table top centrifuge with maximum revolutions per minute of 6000 and capable of accommodating 10-12 tubes of 15 ml capacity is sufficient. The tubes should be placed exactly opposite to each other, should be of the same weight and should contain same amount of fluid. The speed is adjusted by a rheostat and should be allowed to rise slowly. A timer for fixed duration of centrifugation is preferred.

A few common uses are:

— Sediment examination

— Separation of serum from clotted blood.

— Concentration of the materials

7. Magnetic stirrer

- A magnetic stirrer or magnetic mixer is a laboratory device that employs a rotating magnetic field to cause a stir bar immersed in a liquid to spin very quickly, thus stirring it. The rotating field may be created either by a rotating magnet or a set of stationary electromagnets, placed beneath the vessel with the liquid.

8. Water bath

Water bath is a water container having an electrically operated heating device to provide a fixed and uniform temperature. A thermometer is inserted inside the water bath for recording temperature. A mixer immersed inside water is also desired to maintain uniform temperature throughout the water bath.

It is used to incubate samples in water at a constant temperature over a long period of time. All water baths have a digital or an analogue interface to allow users to set a desired temperature. Utilizations include warming of reagents, melting of substrates or incubation of cell cultures. It is also used to enable certain chemical reactions to occur at high temperature. For all water baths, it can be used up to 99.9 °C. When temperature is above 100 °C, alternative methods such as oil bath, silicone bath or sand bath may be used.

Precautions/handling of water bath

- It is not recommended to use water bath with moisture sensitive or reactions.
- Do not heat a bath fluid above its flash/boiling point.
- Water level should be regularly monitored, and filled with distilled water only. This is required to prevent salts from depositing on the heater.
- Disinfectants can be added to prevent growth of organisms.
- Raise the temperature to 90 °C or higher to once a week for half an hour for the purpose of decontamination.
- The cover is closed to prevent evaporation and to help reaching high temperatures

9. Oil bath

An oil bath is a type of heated bath used in a laboratory. These baths are commonly used to heat reaction mixtures. An oil bath is essentially a container of oil that is heated by a hot plate. Generally, silicone oil is used in modern oil baths, although mineral oil, cottonseed oil and even phosphoric acid have been used in the past.

10. Sand bath

A sand bath is a common piece of laboratory equipment made from a container filled with heated sand. It is used to provide even heating for another container, most often during a chemical reaction. A sand bath is most commonly used in conjunction with a hot plate or heating mantle. A beaker is filled with sand and is placed on the plate.

11. Fume Hood

This is an air lifting cabinet in which toxic gas releasing reactions during solution preparation and use as well as chemical processes are performed and which provides the safe discharge of environmentally harmful gases.

✸ Earthing

- If there is a fault in your electrical installation you could get an electric shock if you touch a live metal part. This is because the electricity may use your body as a path from the live part to the earth part. Earthing is used to protect you from an electric shock. It does this by providing a path (a protective conductor) for a fault current to flow to earth. It also causes the protective device (either a circuit-breaker or fuse) to switch off the electric current to the circuit that has the fault. A key function of equipment earthing is to provide a controlled method to prevent the buildup of static electricity, thus reducing the risk of electrical discharge in potentially hazardous environments.

    — Current - Flowing electricity

    — Earth - A connection to the ground

    — Earthing - A way of preventing electric shocks

    — Electrical installation - a fixed wiring system

RULES FOR HANDLING OF CHEMICALS AND IT'S SAFELY

Wide range of chemicals is used in research laboratories of the Institute, each with its own inherent hazards.

An understanding of the potential hazards and precautions required in handling of chemicals is of outmost importance in preventing exposure to chemicals and mishaps.

- **Routes of entry**

The main routes of entry of the chemicals into the human body are:

— By mouth (contaminated fingers!)

— By breathing in gases, aerosols or powder

— By skin contact or damage

— By absorption through intact skin

— By splashes into the eyes

- **Basically, there are four types of chemicals**

— Toxic agents

— These are chemicals that are poisonous to you, and can act upon the body very rapidly.

— Corrosives

— This type of chemical is usually an irritant. Corrosives can damage your body by burning, scalding or inflaming body tissues

— Flammables

Flammables are the chemicals that burn readily. They may explode or burn if sparks, flames or other ignition sources are present

— Reactive

Reactive chemicals are those that require stability and careful handling. Some of them can explode or react violently if the container is dropped or hit.

- **Good handling practice**

— Obtain the minimum amounts needed for your work

— Ensure that all containers are clearly labelled with their contents

— Toxic materials must be locked away

— Corrosive substances must be stored securely at a low level in trays

— Keep flammable materials in specially designed cupboards and only have out the minimum for immediate use

— Store acids, bases & solvents separately

— Never mouth-pipette

— Always dilute concentrated acids by adding the acid to water, never the reverse

— Never carry Winchesters by the neck – always use a carrier

— Always leave benches, balances etc clean & tidy after use

— Don't mix chemicals without specific authorization from the formulator.

— Always use personal protective equipment. Protect your eyes and hands from exposure to harsh chemicals; gloves, goggles or whatever is appropriate.

— When pouring chemicals, pour concentrates into the water and not vice-versa.

— Never pour chemicals into an unlabeled container.

— Don't store flammable chemicals near a source of heat.

— Pesticides, fungicides, etc. always must be stored in a safe and elevated position.

— Ventilate when engaging in cleaning or other applications using strong chemicals, especially dry solvents.

In case of fire:

— If your clothing catches fire, immediately drop to the floor and roll to smother the flames and call for help.

— If a compound or solvent catches on fire, *if you can*, quickly cover the flames with a piece of glassware

— If it is feasible, use a fire extinguisher to put the fire out.

— Do not put water on an organic chemical fire because it will only spread the fire.

— If the fire is large, do not take chances: evacuate the lab and the building immediately

If you inhale vapors:

- Leave the area immediately - at least into the hallway. Tell your Coordinator; they will take you outside into the fresh air, and if necessary provide first aid or take you to get medical attention.

If you spill a chemical on yourself:

— Immediately rinse the affected area with lots of water. Use soap if you wish, but never try to "treat" the spill with another solvent or chemical unless directed to do so by your Coordinator. If the affected area remains more than slightly red after the rinsing period, seek medical attention.

pH Meter

In practice, a pH value is defined by the equation: $pH = - \log_{10} [H^+]$. This equation means that the pH value is a common logarithm expressing the reciprocal of the hydrogen ion concentration.

The pH meter consists of a glass electrode and a reference electrode. Reference electrodes are the calomel and silver-silver chloride electrodes. It allows the pH value of the sample to be obtained by measuring the potential difference between the two electrodes with a potential difference meter.

How pH meter works?

- When one metal is brought in contact with another, a voltage difference occurs due to their differences in electron mobility.
- When a metal is brought in contact with a solution of salts or acids, a similar electric potential is caused, which has led to the invention of batteries.
- Similarly, an electric potential develops when one liquid is brought in contact with another one, but a membrane is needed to keep such liquids apart.
- A pH meter measures essentially the electro-chemical potential between a known liquid inside the glass electrode (membrane) and an unknown liquid outside.
- The glass electrode measures the electro-chemical potential of hydrogen ions or the potential of hydrogen.
- To complete the electrical circuit, also a reference electrode is needed.
- The pH meter measures the electrical potential. Only the potential between the unknown liquid and the solution inside the glass electrode change from sample to sample.

  - The potassium chloride inside the glass electrode is a neutral solution with a pH of 7, so it contains a certain amount of hydrogen ions (H+). Suppose the unknown solution you're testing is much more acidic, so it contains a lot more hydrogen ions. What the glass electrode does is to measure the difference in pH between the two solutions by measuring the difference in the voltages their hydrogen ions produce. Since we know the pH of the potassium chloride solution (7), we can figure out the pH of the unknown solution.

  - To calibrate the pH meter, a standard solution with a known pH value is used. As standard solutions, phthalic acid (pH4.01), neutral phosphate (pH6.86), and borate (pH9.18) are mainly used.

Preparation of media and methods of sterilization

- **Medium/Media:** a nutrient blend used to support microbial growth. Culture medium is a liquid or gel designed to support the growth of microorganisms or cells, or small plants.
  - **Culture:** Is part of specimen grown in culture media.
  - **Culture Media:** is a medium (liquid or solid) that contains nutrients to grow bacteria *in vitro*.

There are three physical forms of media: broth, solid, and semisolid.

1. ***Liquid (Broth):*** Mostly used for biochemical tests (blood culture, Broth culture). Growth of bacteria is shown by turbidity in medium. e.g. Nutrient broth, Selenite F broth, alkaline peptone water.
2. ***Semisolid agar*** (soft agar): Contains small amounts of agar (0.5-0.7%).
3. ***Solid (agar):*** Is Broth plus agar (seaweed). Are prepared by adding a solidifying agent (agar 1.5 -3%).

   The most common growth media for microorganisms are nutrient broths (liquid nutrient medium). Liquid media are often mixed with agar and poured into Petri dishes to solidify. These agar plates provide a solid medium on which microbes may be cultured.

♣ **Properties of Media:**

— Support the growth of the bacteria.

— Should be nutritive (contains the required amount of nutrients).

— Suitable pH (neutral to slightly alkaline 7.3-7.4).

— Suitable temperature, and suitable atmosphere. (Bacteria grow at 37^0C)

♣ **Types of Culture Media**

— **Simple (basal, ordinary):** media that contain the basic nutrients. E.g. Nutrient broth, nutrient agar, peptone water.

— **Enriched Culture Media:** are media that are enriched with: Whole blood e.g. blood agar.

— **Selective Media:** it is a media, which contains substances that prevent or slow the growth of microorganisms other than the bacteria for which the media is prepared for. e.g**TSI** (triple sugar iron agar).

— **Differential Media (indicators):** Contains indicators, dyes, etc, to differentiate microorganisms.e.g. MacConkey agar, which contains neutral red (pH indicator) and is used to differentiate lactose fermenter and non-lactose fermenter. (e.g. *E. coli* and *Salmonella*).

ORGANIC FARMING

- As per the definition of the United States Department of Agriculture (USDA), "organic farming is a system which avoids or largely excludes the use of synthetic inputs (such as fertilizers, pesticides, hormones, feed additives etc) and to the maximum extent feasible rely upon crop rotations, crop residues, animal manures, off-farm organic waste, mineral grade rock additives and biological system of nutrient mobilization and plant protection".

∇ Why farm organically

Organic farming provides long-term benefits to people and the environment.

Organic farming aims to:

— Increase long-term soil fertility
— Control pests and diseases without harming the environment
— Ensure that water stays clean and safe
— Use resources which the farmer already has, so the farmer needs less money to buy farm inputs
— Produce nutritious food, feed for animals and high quality crops to sell at a good price.

♣ Components of Organic Farming

(1) Manure (2) Green manuring (3) Vermicompost
(4) Bio-fertilizers (5) Crop rotation (6) Animal husbandry
(7) Biological managements

Principals of organic farming: Care..... Health..... Ecology..... Fairness

✓ What is seed viability?

- The viability of the seed accession is a measure of how many seeds are alive and could develop into plants which will reproduce themselves, given the appropriate conditions.

✓ Why do we test seed viability?

- It is important to know that the seeds that are stored in a gene bank will grow to produce plants. Therefore they must have a high viability at the start and during storage. The viability of seeds at the start of storage will also determine, within the environmental conditions, the storage life of the accession.

✓ **When should viability be determined?**

- Viability will need to be determined at the start of storage and at regular intervals during storage to predict the correct time for regeneration of the accession. The viability test takes from a few days to weeks or even months to give an accurate result. If possible the results should be available before the seeds are packaged and placed in the gene bank so that poor quality seeds can be identified and regenerated before storage. Where the viability cannot be determined before storage, the seeds should be placed into long-term storage to ensure their safety whilst awaiting the results of the test.

TISSUE CULTURE OF CROP PLANTS

- **Tissue culture:** The aseptic culture of plant protoplasts, cells, tissues or organs under conditions which lead to cell multiplication or regeneration of organs or whole plants.
- Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. Plant tissue culture is widely used to produce clones of a plant in a method known as **micro propagation**. Different techniques in plant tissue culture may offer certain advantages of propagation, including:
- The production of exact copies of plants that produce particularly good flowers, fruits, or have other desirable traits.
- To quickly produce mature plants.
- The production of multiples of plants in the absence of seeds or necessary pollinators to produce seeds.
- The regeneration of whole plants from plant cells that have been genetically modified.
- The production of plants in sterile containers that allows them to be moved with greatly reduced chances of transmitting diseases, pests, and pathogens.
- To clear particular plants of viral and other infections and to quickly multiply these plants as 'cleaned stock' for horticulture and agriculture.

❖ **Techniques**

- Preparation of plant tissue for tissue culture is performed under aseptic conditions under HEPA filtered air provided by a laminar flow cabinet. Thereafter, the tissue is grown in sterile containers, such as Petri dishes or flasks in a growth room with controlled temperature and light intensity.

Factors Affecting Plant Tissue Culture

— Growth Media: Minerals, Growth factors, Carbon source , Hormones

— Environmental Factors: Light, Temperature , Photoperiod

— Explants Source: Usually, the younger, less differentiated the explants, the better for tissue culture

— Genetics: Different species show differences in amenability to tissue culture. In many cases, different genotypes within a species will have variable responses to tissue culture; response to somatic embryogenesis has been transferred between melon cultivars through sexual hybridization

Preparation and standardization of some common reagent solution

- The volumetric (titrimetric) analysis, involves essentially in determining the volume of a solution of accurately known concentration which is required to react quantitatively with the solution of the substance being determined. **The solution of accurately known strength is called the standard solution.** It contains a definite number of gram equivalents per litre. The weight of the substance to be determined is then calculated from the volume of the standard solution and the known laws of chemical equivalence.
- In chemical analysis, many normal solutions are used. Usually the normality varies. A **normal solution of a reagent is one that contains 1 gram equivalent weight per litre of solution.** Standardization is must for accurate analytical results, many process control decisions, legal requirements and analyst confidence.
- If a reagent is available in the pure state, a solution of definite normality is prepared simply by weighing out an eq. weight or a definite fraction or multiple thereof, dissolving it in the solvent, usually water, and making up the solution to a known volume. The following is a list of some of the substances which can be obtained in a state of high purity and are therefore suitable for the preparation of standard solutions.

A. Acid-Base Titration

Sodium carbonate	Na_2CO_3
Benzoic acid	$H(C_7H_5O_2)$

B. Redox Titration

Potassium dichromate	$K_2Cr_2O_7$
Sodium oxalate	$Na_2C_2O_4$

C. Complex Formation Titration

Silver nitrate	$AgNO_3$
Sodium chloride	NaCl
disodium Ehtylenediamine Tetra Acetate dihydrate	$Na_2H_2C_{10}H_{12}O_8N_2 \bullet 2H_2O$

D. Precipitation Titration

Silver nitrate	$AgNO_3$
Sodium chloride	NaCl

- When a reagent is not available in the pure form. *e.g.* most alkali hydroxides, some inorganic acids and various deliquescent substances, solutions of the appropriate normality are first prepared. These are then standardized against a solution of a pure substance (as above list) of known normality.

✸ BUFFER SOLUTIONS

- A buffer solution (more precisely, pH buffer or hydrogen ion buffer) is an aqueous solution consisting of a mixture of a weak acid and its conjugate base, or vice versa. Its pH changes very little when a small or moderate amount of strong acid or base is added to it and thus it is used to prevent changes in the pH of a solution. A buffer solution is one that resists a change in pH on the addition of acid (H+) or base (OH−). Buffer solutions are used as a means of keeping pH at a nearly constant value in a wide variety of chemical applications. Many life forms thrive only in a relatively small pH range so they utilize a buffer solution to maintain a constant pH.
- Most commonly, the buffer solution consists of a mixture of a weak acid and its conjugate base; for example, mixtures of acetic acid and sodium acetate or of ammonium hydroxide and ammonium chloride are buffer solutions. A buffer system consists of a weak acid (the proton donor) and its conjugate base (the proton acceptor).

♣ Biological Buffers

Biological buffers should meet the following general criteria:

— Their pKa should reside between 6.0 to 8.0.
— They should exhibit high water solubility and minimal solubility in organic solvents.
— They should not permeate cell membranes.
— They should not exhibit any toxicity towards cells.
— The salt effect should be minimum.
— Ionic composition of the medium and temperature should have minimal effect of buffering capacity.
— Buffers should be stable and resistant to enzymatic degradation.
— Buffer should not absorb either in the visible or in the UV region.

Experiment: **Preparation of acetate buffer known strength and pH**		
Aim	:	To prepare 100 ml of 0.1 M acetate buffer of pH 5.2
Reagents	:	Sodium acetate, acetic acid and distilled water
Apparatus	:	i. Volumetric flask (100 ml) ii. Graduated pipettes iii. Beakers iv. pH meter
Principle	:	Buffer solutions resist the change in the pH. They contain a weak acid and its salt (acidic buffer) or weak alkali and its salt (alkaline buffer) According to Henderson-Hasselbalch equation $\text{pH} = pK_a + \log \frac{[\text{Salt}]}{[\text{Acid}]}$ From the above equation the amount of sodium acetate and acetic acid required to make 100 ml of 0.1 M acetate buffer of pH 5.2 will be calculated. pKa of acetic acid is 4.76. $CH_3COOH \overset{pKa}{\rightleftharpoons} CH3COO^- + H^+$ $CH_3COONa \longrightarrow CH3COO^- + Na^+$
Calculation	:	$\text{pH} = pK_a + \log \frac{[\text{Salt}]}{[\text{Acid}]}$ 5.2 = 4.76 + log [salt]/[acid] log [salt]/[acid] = 5.2-4.76 log [salt]/[acid]= 0.44 [salt]/[acid]= antilog of 0.44 = 2.754 Let 'A' be the concentration of salt and 'B' that of acid A/B= 2.754 Therefore, A = 2.754B --------(1) In a buffer solution, the sum of proportions of acetic acid and sodium acetate concentration will be 100%. So, A + B = 100 ---------(2) Substituting the value of 'A' of (1) in (2), we get 2.754B + B = 100 3.754B = 100 B = 100/3.754

		B = 26.64% and A = 73.36% Hence, proportion of sodium acetate and acetic acid in the buffer will be 73.36% and 26.64% respectively. For 1 M acetate buffer, we need 0.7336 moles of sodium acetate and 0.2664 moles of acetic acid. Therefore, 100 ml of 0.1 M acetate buffer, we require 0.007336 moles of sodium acetate and 0.002664 moles of acetic acid. Now, number of moles is given by the formula = weight in grams/molecular weight Therefore, Weight in grams of acetic acid (mol. wt. -60.05) = 0.002664 x 60.05 = 0.16 Weight in grams of sodium acetate trihydrate (mol. wt. -136.09) = 0.007336 x 136.09 = 0.998 i.e ~ 1.00
Procedure	:	Weigh 1.00 g of sodium acetate and 0.16 g of acetic acid in separate beaker. The contents are transferred into a 100 ml volumetric flask and the volume is made up to 100 ml with distilled water. Check the pH with the help of pH meter
Result	:	The pH of the prepared buffer is 5.4

STERILIZATION

— **Sterilization** is a term referring to any process that eliminates (removes) or kills all forms of life. Sterilization can be achieved by applying the proper combinations of heat, chemicals, irradiation, high pressure, and filtration.

— A widely-used method for heat sterilization is the autoclave. Autoclaves commonly use steam heated to 121–134°C. To achieve sterility, a holding time of at least 15 minutes at 121°C or 3 minutes at 134°C is required.

— Sterilization methods include; autoclaving, dry-heat, filtration, UV exposure and ethylene oxide.

The various methods of sterilization are:

1. Physical Method
   a. Thermal (Heat) methods (autoclaving, dry-heat)
   b. Radiation method (UV exposure)
   c. Filtration method (Membrane filtration)
2. Chemical Method
   a. Gaseous method (ethylene oxide)

— The most tedious parts of in vitro techniques are sterilizing plant materials and media and maintaining aseptic conditions once they have been achieved. Bacteria and fungi are the two most common contaminants observed in cell cultures. Fungal spores are light weight and present throughout our environment. When a fungal spore comes into contact with the culture media used in tissue culture, conditions are optimal for germination of the spore and subsequent contamination of the culture.

♣ Sterilizing Culture Rooms and Transfer Hoods

— Large transfer rooms are best sterilized by exposure to ultraviolet (UV) light. Sterilization time varies according to the size of the room and should only be done when there are no experiments in progress. Ultraviolet radiation is harmful to the eyes. Transfer rooms can also be sterilized by washing them 1-2 times a month with a commercial brand of antifungal spirocyte. Smaller transfer rooms and hoods also can be sterilized with UV lights or by treatment with bactericides and/or fungicides. Laminar flow hoods are easily sterilized by turning on the hood and wiping down all surfaces with 95% ethyl alcohol 15 min before initiating any operation under the hood.

— Culture rooms should be initially cleaned with detergent-brand soap and then carefully wiped down with a 2% sodium hypochlorite solution or 95% ethyl alcohol.

All floors and walls should be washed gently on a weekly basis with a similar solution; extreme care must be used to avoid stirring up any contamination that has settled. Commercial disinfectants such as Lysol, Zephiram, and Roccal diluted at manufacturer's recommended rates can be used to disinfect work surfaces and culture rooms.

♣ **Sterilizing glassware and Instruments**

— Metal Instruments are best sterilized using a glass bead sterilizer, Product Number S636 or S637. These sterilizers heat to approximately 275-350° C and will destroy bacterial and fungal spores that may be found on your instruments. The instruments simply need to be inserted into the heated glass beads for a period of 10 to 60 sec. The instruments should then be placed on a rack under the hood to cool until needed.

— Metal instruments, glassware, aluminum foil, etc., can also be sterilized by exposure to hot dry air (130°-170°C) for 2-4 hr in a hot-air oven. All items should be sealed before sterilization but not in paper, as it decomposes at 170°C. Autoclaving is not advisable for metal instruments because they may rust and become blunt under these conditions.

— Instruments that have been sterilized in hot dry air should be removed from their wrapping, dipped in 95% ethyl alcohol, and exposed to the heat of a flame. After an instrument has been used, it can again be dipped in ethyl alcohol, reflamed, and then reused. This technique is called flame sterilization. Safety is a major concern when using ethyl alcohol. Alcohol is flammable and if spilled near a flame will cause an instant flash fire. This problem is compounded in laminar flow hoods due to the strong air currents blown towards the worker. Fires commonly start when a flamed instrument is thrown back into the alcohol beaker. In case of fire do not panic. Limiting the supply of oxygen can easily put out fires.

— Autoclaving is a method of sterilizing with water vapor under pressure. Cotton plugs, gauze, labware, plastic caps, glassware, filters, pipettes, water, and nutrient media can all be sterilized by autoclaving. Nearly all microbes are killed by exposure to the super-heated steam of an autoclave for 10-15 minutes. All objects should be sterilized at 121°C and 15 psi for 15-20 min.

♣ **Sterilizing Nutrient Media**

— Two methods (autoclaving and membrane filtration under positive pressure) are commonly used to sterilize culture media. Culture media, distilled water, and other stable mixtures can be autoclaved in glass containers that are sealed with cotton plugs,

aluminum foil, or plastic closures. However, solutions that contain heat-labile components must be filter-sterilized.

— Generally, nutrient media are autoclaved at 15 psi and 121°C. For small volumes of liquids (100 ml or less), the time required for autoclaving is 15-20 min, but for larger quantities (2-4 liter), 30-40 min is required. The pressure should not exceed 20 psi, as higher pressures may lead to the decomposition of carbohydrates and other thermo-labile components of a medium.

♣ **Sterilizing Plant Material**

— Obtaining sterile plant material is difficult, and despite any precautions taken, 95% of cultures will end up contaminated if the explant is not disinfected in some manner. Because living materials cannot be exposed to extreme heat and retain their biological capabilities, plant organs and tissues are sterilized by treatment with a disinfecting solution. Solutions used to sterilize explants must preserve the plant tissue but at the same time destroy any fungal or bacterial contaminants.

— Once explants have been obtained, they should be washed in a mild soapy detergent before treatment with a sterilizing solution. Some herbaceous plant materials (e.g., African violet leaves) may not require this step, but woody material, tubers, etc., must be washed thoroughly. After the tissue is washed, it should be rinsed under running tap water for 10-30 min and then be submerged into the disinfectant under sterile conditions. All surfaces of the explant must be in contact with the sterilant. After the allotted time for sterilization, the sterilant should be decanted and the explants washed at least three times in sterile distilled water. For materials that are difficult to disinfect, it may be necessary to repeat the treatment 24-48 hr before making the final explants. This allows previously unkilled microbes time to develop to a stage at which they are vulnerable to the sterilant.

♣ **Sterile Culture Techniques**

— Successful control of contamination depends largely upon the operator's techniques in aseptic culture. You should always be aware of potential sources of contamination such as dust, hair, hands, and clothes. Obviously, your hands should be washed, sleeves rolled up, long hair tied back, etc. Washing your hands with 95% ethyl alcohol is an easy precautionary measure that can be taken. Talking or sneezing while culture material is exposed also can lead to contamination. When transferring plant parts from one container to another, do not touch the inside edges of either vessel. By observing where contamination arises in a culture vessel, you may be able to determine the source of contamination.

— In plant tissue culture, small pieces of plant tissue are placed on or in a medium rich in nutrients and sugar. If bacteria or fungi come in contact with the plant tissue or the medium, the culture becomes contaminated. The contaminants (bacteria and fungi) will use nutrients from the medium and the plant, which quickly destroys the plant tissue. Our aim is to surface sterilize the plant tissue and put it on a sterile growth medium without any bacteria or fungi getting on the plant or medium. This is not easy because bacteria and fungal spores are in the air, on us, in us, and under us!

— When you see sunlight shining in a window you can, from certain angles, see dust particles in the air. There are hundreds of bacteria attached to each dust particle. A horizontal laminar flow unit is designed to remove the particles from the air. Room air is pulled into the top of the unit and pushed through a HEPA (High Energy Particle Air) filter with a uniform velocity of 90 ft/min across the work surface. The air is filtered by the HEPA filter so nothing larger than 0.3 um (micrometer) can pass through. This renders the air sterile. The flow of air from the unit discourages any fungal spores of bacteria from entering. All items going inside the unit should be sterile or sprayed with ethanol or isopropyl alcohol. They will remain sterile unless you contaminate them.

— A transfer cabinet provides an enclosed environment that is not sterile but can be sterilized. A cardboard box lined with aluminum foil or plastic, or a well-cleaned aquarium, provides a shield to reduce contamination. A box that is 20-24 inches wide, 20-24 inches high, and 12-16 inches deep provides a good work area. Working inside any of these does not guarantee success.

The following precautions are necessary for all work areas.

- The room should be swept and if possible, mopped.
- Each work surface should be washed with a 10% Clorox^{R}, Lysol^{R}, or other disinfectant solution.
- Doors and windows should be closed.
- Air conditioners and fans should be turned off.
- If possible, each student must have a work space that can be properly treated against contamination. For example, the box or aquarium mentioned earlier, or a piece of poster paper lying on the table to indicate the student's sterile workspace.
- Have spray bottles filled with 70% ethanol or isopropanol (never methanol) placed so each student has access to one bottle. Spray everything going into the sterile area.

- Have a central supply area so all necessary items can be picked up and taken to the workspace as needed. Items can be returned to the central supply area after being used.
- Sterile instruments will be needed for each person. One way to accomplish this is to have a ½-pint jar of 70 % ethanol for scalpels and short forceps. When tissue has to be positioned in a vessel, long 10-inch forceps are needed. The long forceps need to be placed deep enough in alcohol so that any part of the forceps that might come into contact with the vessel is sterilized. A 100-ml graduated cylinder can be used to hold the alcohol and long forceps. A ½-pint jar of sterile water is needed for dipping the instruments to remove the residual alcohol that might dry out plant tissues.
- A sterile work surface is needed on which to place the sterile tissue to trim it. The easiest thing to use is a sterile petri dish. If you have glass ones, you can autoclave and reuse them. Presterilized plastic dishes are used and discarded. Spray the bag of dishes with 70 % alcohol before you open it and place the desired number of unopened dishes at each station. Each dish has two sterile surfaces-the inside top and inside bottom.
- Long hair should be tied back or covered.
- Hands should be washed, not scrubbed (scrubbing dries hands and creates flakes of skin that have bacteria) and sprayed with 70 % ethyl or isopropyl alcohol or coated with isopropyl alcohol gel.
- Gloves and masks provide extra protection.
- Do not talk while performing sterile operations.
- Do not lean over your work. Keep your back against the backrest of your chair.
- Try to work with your arms straight: this position may feel awkward, but it will reduce contamination.
- Do not pass non sterile items over sterile areas or items.
- Reach around rather than over. Make your movements smooth and graceful so that you do not disturb the air more than is necessary.
- Work quickly though, the longer it takes to manipulate the tissues the greater the chance of contamination.
- Have only the necessary items in sterile work area. Remove items that are no longer needed as quickly as possible. Act out each step before beginning so that you understand what you are about to do.