



Ethiopian TVET-System



MEDICAL LABORATORY Level -III

Based on Apr.2018G.C. Occupational Standard

Module Title: -	Preparing Laboratory Solutions
TTLM Code:	HLT MLT3 TTLM 1019v1

This module includes the following Learning Guides

LG39: Prepare a working solution

LG40: Standardize solution

LG41: Monitor the quality of laboratory solutions

LG42: Maintain safe work environment



This learning guide is developed to provide you the necessary information regarding the following **content coverage** and topics –

- Introduction to solution preparation
- Equipments and materials for solution preparation
- Measurement
- Estimate uncertainty of measurement
- Chemicals
- Making dilution
- Solution preparation
- Labeling and storage of reagents
- Record working solution details in laboratory register

This guide will also assist you to attain the learning outcome stated in the cover page.

Specifically, upon completion of this Learning Guide, **you will be able to –**

- Select the relevant/appropriate standard procedure for solution and/or working **solutions** preparation
- Select **equipment**, materials and solvent of specified purity
- calculated and recorded Data
- measure appropriate quantities of reagents for solution preparation and record data
- Select and assemble Specified laboratory equipment and appropriate grade of glassware
- mix or dilute the required working solution in accordance with procedures
- prepare Solutions to achieve homogeneous mix of the specified concentration
- label and store Solutions to maintain identity and stability
- record Working solution details in laboratory register

Learning Instructions:

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described below 3 to 6.
3. Read the information written in the information “Sheet 1, Sheet 2, Sheet 3 and Sheet 4,---”in **page ---, ---, --- and ---** respectively.
4. Accomplish the “Self-check 1, Self-check t 2, Self-check 3 and Self-check 4” ,---”in **page ---, ---, --- and ---** respectively



5. If you earned a satisfactory evaluation from the “Self-check” proceed to “Operation Sheet 1, Operation Sheet 2 and Operation Sheet 3 ”in **page ---**.
6. Do the “LAP test” in **page – ---**



1.1. Definition of terms

- **Solution** is a homogeneous mixture of two or more substances. OR a mixture of substance dissolved in another so the properties are the same throughout.
 - ✓ Solution: composed of a solute and the solvent
- **Solute** is the dissolved substance, OR the substance found in small amount
- **Solvent** is a substance in which solutes dissolve to make the mixture or the substance that is present in the greatest amount.
 - ✓ Water is the Universal Solvent but there are many things it cannot dissolve. For example water and oil do not mix. We say oil is immiscible in water. Water is a good solvent due to its polarity.
- **Mixtures**: combinations of different substances where each substance retains its chemical properties.
- **Concentration**- amount of a substance dissolved in a given amount of solvent
- **Compound**- composed of two or more substances (elements) but in a ratio that cannot vary.
 - ✓ Eg. water, there are 8 grams of oxygen for each gram of hydrogen. It won't be water if that ratio changes.

1.2. Ways of preparing a solution

- Dissolution
- Dilution

1.2.1. Dissolution

- IS the process by which a solute forms a solution in a solvent. weighed amount of solid dissolved in a required solvent. The solute, in the case of solids, has its crystalline structure disintegrated as separate ions, atoms, and molecules form.
- **Factors affecting dissolution**
 - ✓ **Surface area**: the larger the surface area, the faster it gets dissolved.

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- ✓ **Temperature:** as the temperature increases, it dissolves more quickly.
- ✓ **Volume of solvent:** The higher the amount of solvent, the quicker the dissolution
- ✓ **Solubility of the solid:** It depends how soluble the solute is to water.
- ✓ **particle size:** the smaller particle size, the faster to dissolved
- ✓ **pH of the dissolving medium:** neutral medium is best for dissolution
- ✓ **Agitation:** produced by stirring or mixing a solution increases the rate of dissolution
- E.g. if there are 10 grams of salt (the solute) dissolved in 1 liter of water (the solvent), this solution has a certain salt concentration

1.2.2. Dilution of solution

- Dilution is a process by which the concentration or activity of a given solution is decreased by the addition of solvent.
- A dilution represents the ratio of concentrated or stock material of the total final volume of a solution. Dilution is made to prepare:
 - ✓ A working solution from the stock
 - ✓ Measurable concentration of a sample (for reporting the actual concentrations of body-fluid constitutes)
 - ✓ If the specimen at hand is less than a procedure calls for
 - ✓ If the concentration of substances (analyte) is too high to be accurately measured.
- Whenever a solution is diluted, its volume is increased and its concentration decreased, but the total amount of solute remains unchanged
- They are of two types of dilution
 - A. Simple dilution
 - B. Serial dilution



Self-Check -1

Written Test

Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page:

1. Which of the following is false about **Solution**
 - A. is a homogeneous mixture
 - B. is a heterogeneous mixture
 - C. composed of a solute and the solvent
 - D. is a substance in which solutes dissolves
2. Concentration of solution is the
 - A. Quantity of solvent in solute
 - B. Quantity of solute in given solvent
 - C. Unite to measure concentration
 - D. Volume of solvent in solution
3. Which of the following is true about dissolution of solute in solvent?
 - A. The smaller the surface area, the faster it gets dissolved.
 - B. Temperature decrease, it dissolves more quickly.
 - C. The higher the amount of solute, the quicker the dissolution
 - D. the smaller particle size, the faster to dissolved
4. Dilution is a process by which the concentration or activity of a given solution is increased by the addition of solute
 - A. True
 - B. false

Note: Satisfactory rating - 2 points

Unsatisfactory - below 2 points

Answer Sheet

Score = _____
Rating: _____

Name: _____

Date: _____



2.1. Materials used to prepare solution

- ✓ balance
- ✓ flask
- ✓ measuring cylinder
- ✓ funnel
- ✓ desiccators
- ✓ labeling materials
- ✓ reagent bottles
- ✓ Burette stand
- ✓ Clamp
- ✓ Burette
- ✓ glass rod
- ✓ glass bead
- ✓ spatula
- ✓ scoop
- ✓ pipettes
- ✓ water bath/incubator
- ✓ Mortar and pestles.

2.1.1. Laboratory glass wares and plastic wares

- Laboratory glassware and plastic wares are materials used in clinical laboratory for: measuring pipetting transferring Preparation of reagents Storage etc.
- Most of the routine laboratory wares used to be of glass, but recent advantage made in the use of plastic resin to manufacture a wide range of plastic ware having led to a gradual replacement of glass wares with durable plastic ware. The plastic ware used in the laboratory should be of high quality. also cheaper and safer to use than glassware.
- The glass wares have the minor advantage of being re-usable and autoclavable. But heavier, more costly and easily broken. In fact, in this age of good awareness of the dangers posed by hepatitis and human immunodeficiency viruses (HIV), most of the plastic wares are disposable, thereby cutting down on the cost of cleaning.
- The plastic ware are fashioned and shaped exactly like the glass ware

2.2. Classification of Laboratory glass wares

A. can be divided in to five main types according to their composition

1. **Glass with high thermal resistance** – borosilicate glass can resist about 500^oc and low alkaline contact.
2. **High silica glass**- contains 96% silicon, It is thermal endurable, chemically stable and electric resistant



3. **Glass with high resistance to alkali-** Boron free, used in strong alkali low thermal resistance
4. **Low actinic glass** – amber color to protect light
5. **Standard flint glass-** soda lime glass, poor resistance to increased temp. Contains free soda in its walls

B. Based on their use

- a) volumetric wares
- b) Semi-volumetric Glass wares
- c) Non- volumetric glass wares.

a) Volumetric wares: Apparatus used for measurement of liquids Can be made either from glass or plastic. It includes:

- ✓ Volumetric flasks
- ✓ Graduated centrifuge tubes
- ✓ Graduated serological pipette
- ✓ Medicine dropper
- ✓ Burettes
- ✓ Micropipettes
- ✓ Diluting or thoma pipettes etc

b). Non- volumetric glass wares: are not calibrated to hold a particular or exact volume, but rather are available for various volumes, depending on the use desired .

- ✓ Erlenmeyer flask
- ✓ Round bottom flask
- ✓ Flat bottom flask
- ✓ Beaker
- ✓ Centrifuge tube
- ✓ Test tube
- ✓ Pasture pipette

C).Semi-volumetric Glass wares: are used for approximate measurement. It includes;

- ✓ Graduated cylinder
- ✓ Graduated specimen glass
- ✓ Beakers
- ✓ Conical flask
- ✓ Medicine droppers with or with out calibration mark

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- ✓ Graduated beaker with double beaks
- ✓ Graduated glass

2.2.1. Pipettes

- There are several types each having their own advantages and limitations. They are designated as class “A” or “B” according to their accuracy.
 1. **Class “A” pipettes** are the most accurate and the tolerance limits are well defined that is, ± 0.01 , ± 0.02 and ± 0.04 ml for 2, 25, and 50 ml pipettes respectively.
 2. **Class “B” pipettes:** are less accurate but quite satisfactory for most general laboratory.
- Read the volume at lower meniscus
- Significant errors will result if the temperature of the liquid pipetted is widely different from the temperature of calibration. The usual temperature of calibration is 20°C and this is marked on the pipette.

2.2.1.1. Micropipettes



Fig.2.1 automatic pipette

- Micropipettes are frequently used in
 - ✓ Medical chemistry
 - ✓ Virology
 - ✓ Immunology and serology laboratories.
- This is because in these laboratories often only small quantities of materials are available for measurement. They are found in different capacities such as 5, 10, 25, 50, 100 and 1000 micro liter.
- There are also other kinds of pipettes that are used in medical laboratories.
 - ✓ Example: Toma pipette, Pasteur pipette, automatic pipettes and others.



2.2.1.2. Volumetric pipettes

- Volumetric pipettes are calibrated to deliver a constant volume of liquid.
 - The most commonly used sizes are 1, 5, and 10ml capacities.
 - Less frequently used sizes are those which deliver 6, 8, 12, and so on ml.
 - They have a bulb mid – way between the mouthpiece and the tip
 - The main purpose of the bulb is to decrease the surface area per unit volume and to diminish the possible error resulting from water film.
 - The Volume (capacity) and calibration temperature of the pipettes are clearly written on the bulb.
 - They should be used when a high degree of accuracy is desired.
 - The pipette is first rinsed several times with a little of the solution to be used, and then filled to just above the mark.
 - Then the liquid is allowed to fall to the mark and the tip is carefully wiped with filter paper.
 - The contents are allowed to drain in to the appropriate vessel. A certain amount of liquid will remain at the tip and this must not be blown out
- **N.B:** *The reliability of the calibration of the volumetric pipette decreases with an increase in size and therefore, special micropipettes have been developing for chemical microanalysis.*

2.2.1.3. Graduated or measuring pipettes

- Graduated pipettes consist of a glass tube of uniform bore with marks evenly spaced along the length. The interval between the calibration marks depends up on the size of the pipette.
- Two types calibration for delivery are available:
 - A. One is calibrated between two marks on the stem (Mohr).
 - B. The other has graduation marks down to the tip (serological pipette)
- These pipettes are intended for the delivery of predetermined volumes. The serological pipette must be blown out to deliver the entire Volume of the liquid and it has an etched ring (pair of rings) near the mouth end of the pipette signifying that it is a blow out pipette.
- Measuring pipettes are common only in 0.1, 0.2, 0.5, 1.0 5.0, and 10.0 ml sizes.
- The liquid is delivered by allowing it to fall from one calibration mark to another.

N.B. *The classification of pipettes may not always be based on the presence or absence of a bulb and etched ring.*

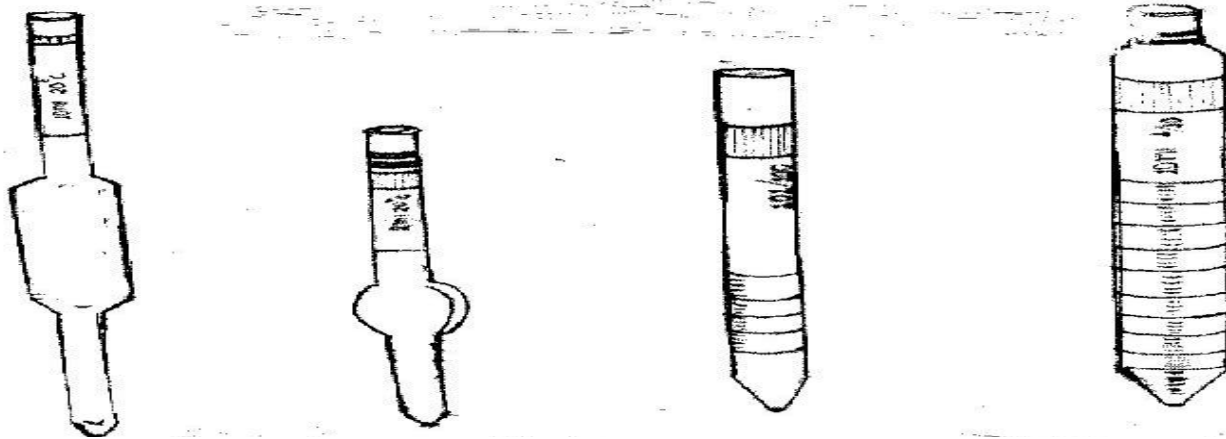


Fig 2.2 :- A. Volumetric (transfer) B. Ostwald volin (transfer). C. Measuring (Mohr) D. Serological (Graduated)

2.2.2. Burettes

- Burettes are used for measuring variable quantities of liquid that are used in volumetric titrations. They are made in capacities from 1 to 100 milliliters.
- They are long graduated tubes of uniform bore and are closed at the lower end by means of a glass stopper, which should be lightly greased for smooth rotation.



Fig 2.3:- burette

2.2.3. Flasks

- There are four types of flasks having 25 to 6,000 milliliter (ml) capacities.
 1. **Conical (Erlenmeyer) flasks:** Conical (Erlenmeyer) flasks are useful for titrations and also for boiling solutions when it is necessary to keep evaporation to a minimum. Some have a side arm suitable for attachment to a vacuum pump.

2. **Flat bottomed round flasks:** Flat-bottomed round flasks are convenient containers to heat liquids. These flasks are widely used in the preparation of bacteriological culture media.
 3. **Round bottomed flasks:** Round bottomed flasks can withstand higher temperatures than the flat-bottomed type. They may be heated in a necked flame or in an electro-thermal mantle. As a result used for boiling
 4. **Volumetric flasks:** Volumetric flasks are flat – bottomed, pear-shaped vessels with long narrow necks fitted with ground glass stoppers.
- Most flasks are graduated to contain a certain volume, and these are marked with the liters. A horizontal line etched round the neck denotes the stated volume of water at given temperature. They are used to prepare various kinds of solutions. The neck is narrow so that slight errors in reading the meniscus results in relatively small volumetric differences (minimizes volumetric differences or errors)

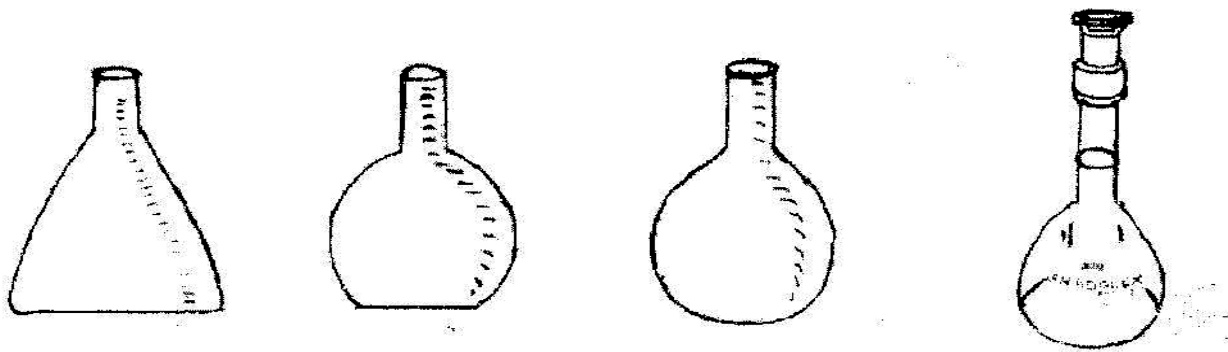


Fig2.4:- A. Conical B. Flat bottomed C. round bottomed D.Volumetric

2.2.4. Beakers

- Beakers have capacities from 5 to 5,000 ml. They are usually made up of heat resistant glass and are available in different shapes. The most commonly used is the squat form, which is cylindrical and has a spout. There is also a tall form, usually without a spout



Fig 2.5:- beakers

2.2.5. Cylinders

- Cylinders are supplied in 10 to 2,000 ml capacities. Some are of heat resistant glass or plastic. Measurement of liquids can be made quickly with these vessels, but a high degree of accuracy is impossible because of the wide bore of the cylinders



Fig 2.6:- cylinders

2.2.6. Test tube

- Test tubes are made of hardened glass or plastic materials that can withstand actions of chemicals, thermal shock and centrifugal strains. They are used to hold samples and solutions during medical laboratory procedures. These include simple round hollow tubes conical centrifuge tubes, vacutainer tubes. Test tubes can be with or without rims (lips)

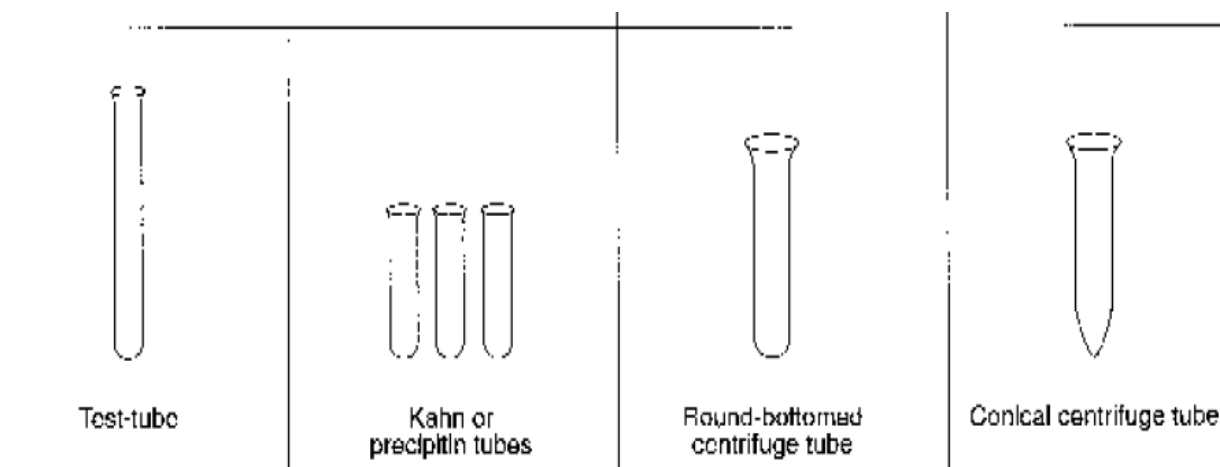


Fig 2.7:- test tubes

2.2.7. Reagent bottles

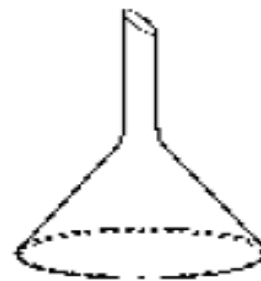
Reagent bottles are used to store different types of laboratory reagents. They are made from glass or plastics. Depending on their use, they are available in various sizes and type



Fig 2.8:- Reagent bottles

2.2.8. Funnels

- There are two types of funnels that are widely used in a medical laboratory. These are filter funnel and separating funnel.



Filter funnel

Fig. 2.9:- funnels

2.2.8.1. Filter Funnels: Filter funnels are used for pouring liquids into narrow mouthed containers, and for supporting filter papers during filtration. They can be made from glass or plastic materials

2.2.8.2. Separating funnels: They are used for separating immiscible liquids of different densities. Separating funnels are used for separating immiscible liquids of different densities. Example, ether and water

2.2.9. Pestle and mortar

- Pestle and mortar are used for grinding solids, for example, calculi and large crystals of chemicals. After each use always clean the pestle and mortar thoroughly. This is because chemicals may be driven into the unglazed surfaces during grinding, resulting in contamination when the apparatus is next used.



Fig. 2.10 Pestle and mortar

2.2.10. Pasture pipette

- They are non-volumetric glassware used in transferring liquid. It has a long –drown-out tip with a rubber bulb or teat to suction. Eye droppers or medicine droppers can use instead of pasture pipettes

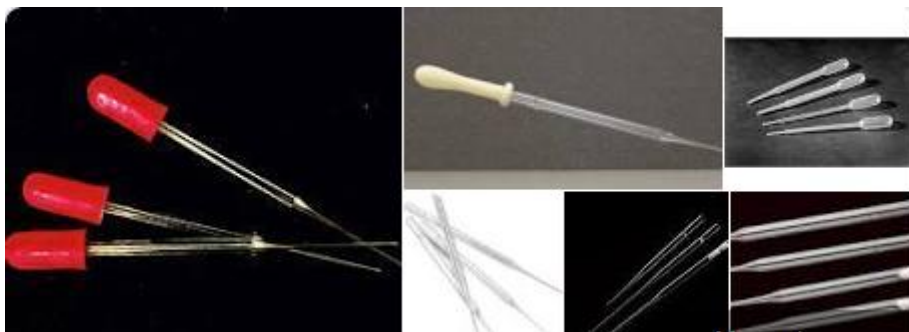


Fig 2.11:- pasture pipettes

2.3. Equipment for purifying water

2.3.1. DISTILLER

- A process by which impure water is boiled and the steam condensed on cold surface (condenser) to give pure distilled water is called distillation. Distilled water is free from dissolved salts and clear colorless, odorless and tasteless. It is sterile too. The apparatus is called distiller.



water distiller small industrial

Fig: - 2.12. Water distiller

- A considerable volume of cool running water is required to operate or to condense the steam

2.3.2. Deionizer

- A deionizer is an apparatus used to produce ion free water.
- A deionizer is an apparatus for demineralizing water by means of cartridges filled with ion-exchange resin.
- Deionization is a process in which chemically impure water is passed through anion and cation exchange resins to produce ion free water.
- Deionized water has low electrical conductivity, near neutral pH and is free from water-soluble salts but is not sterile.

2.4. Equipment for weighing/Balances

2.4.1. Balances:

- Essential laboratory instruments that are widely used for weighing of various substances (powders, crystals and others) in the laboratory. For instance, to prepare reagents, stains and culture media, balances are required to weigh accurately and precisely within the needed range. They should be kept carefully clean and located in an area away from heavy traffic, large pieces of electrical equipment, and open windows. To minimize any vibration, as interference that may happen, a slab of marble is placed under the balance
- Balances in medical laboratory may be:
 - ✓ Rough balances (mechanical balances)
 - ✓ Analytical balances/electrical/

2.4.1.1. Rough balances

- Rough balances are several types. Some of them use sliding scale, some have a single or double pan (s) and others utilize dial - operated fractions. They are used for weighing substances, which do not call for extreme accuracy. While operating, they do not require mains electricity or battery power and are currently less expensive than analytical balances of the similar sensitivity. Some rough balances weigh accurately to 0.1 gm of a substance. Two - pan balance is a rough balance, which has two copper pans supported by shafts.
- **It is used:**
 - ✓ To weigh large amounts (up to several kilo grams).
 - ✓ When a high degree of accuracy is not required.
 - ✓ The sensitivity of a two pan balance is 0.5 gm.
- The sensitivity of a balance is the smallest weigh that moves the pointer over one division of the scale. For routine laboratory purposes the sensitivity of a balance can be considered to be the smallest weigh that it will measure accurately. Usually the larger the amount of substance to go into a reagent, the least accuracy is required. For instance, if the sensitivity of balance is 1 mg, this means that a weight of at least 1.0 mg is needed to move the pointer over one scale.



Fig:- 2.13. Rough balance

2.4.1.2. Analytical balances

- Nowadays analytical and electronic balances (single pan balances that use an electron magnetic force instead of weights) are the most popularly used balances in medical laboratories to provide a precision and accuracy for reagent and standard preparation. Analytical balance is a highly sensitive instrument. It may have two pans suspended from a cross beam, inside a glass case. It requires mains electricity or battery (D.C) supplied power.

- **These balances are used:**

- ✓ To weigh small quantities usually in milli gram (mg) range.
- ✓ When great accuracy is required. E.g., 2.750mg, 0.330 mg, 5.860mg, etc.
- ✓ Its sensitivity is 0.5 mg to 1 mg depending on the model.
- ✓ **N.B:** The accuracy of a balance should be checked regularly as recommended by the manufacturer.



Fig:-2.14. Analytical balance

2.4.1.3. Use and care of balances

- A balance is a delicate instrument that requires practical instruction in its correct use. The following should be applied when using a balance:
 - ✓ Read carefully the manufacturer's instructions.
 - ✓ Always handle a balance with care.
 - ✓ Position the balance on a firm bench away from vibration, draughts and direct sunlight.
 - ✓ Before starting to weigh, zero the balance as directed by the manufacturer. If using a beam balance, check the position of the beam.
 - ✓ Weigh the chemicals at room temperature in a weighing scoop or small beaker. And Never put the chemicals directly on the balance pan.
 - ✓ When adding or removing a chemical, remove the container to avoid spilling any chemical on the balance.
 - ✓ When using an analytical double pan balance, bring the pans to rest before adding or removing a chemical.

- ✓ Always use forceps to add or remove weights. Protect the weights from dust, moisture and fungal growth.
- ✓ Use small brush to remove any chemical, which may have been spilt on the balance.
- ✓ A container of self - indicating silica gel should be kept inside the analytical balance case to remove any moisture present in the atmosphere.
- ✓ Keeps the balance clean, being particularly careful not to let dirt accumulate near the pivots and bearings?

2.5. Incubator

- Incubation at controlled temperature is required for bacteriological cultures, blood transfusion, Serology, Hematology and clinical Chemistry tests.
- For bacteriological cultures, an incubator is required whereas for other tests a dry heat block or a water bath may be used. For the incubator, the air inside is kept at a specific temperature (usually at 37⁰c). When tubes are kept inside the incubator, they take the temperature of the incubator.
- The appropriate temperature is obtained by means of temperature regulator and is maintained by a thermostat. This permits a more accurate temperature control.



Fig:- 2.15 incubator

2.5.1. Use and Care of Incubator

- ✓ Read carefully the manufacturer's instruction.
- ✓ Make sure the incubator is positioned on a level surface and that none of the ventilation openings are blocked.

- ✓ If the incubator does not have a temperature display, insert a thermometer in the vent hole through the roof of the incubator. Adjust the thermostat dial until the thermometer shows the correct reading, i.e., 35 - 37°C for the routine incubation of bacteriological cultures.
- ✓ Before incubating cultures and tests, check the temperature of the incubator.
- ✓ Clean the incubator regularly; making sure it is disconnected from its power supply.
- ✓ Every three to six months check the condition of the incubator
- ✓ At the time of purchase, it is advisable to buy a spare thermostat and thermometer if these are of special type and are not available locally.

2.6. Water bath

- The water bath, like the incubator, is required for controlled temperature incubation of culture and liquids, and many other laboratory tests. The temperature of the water bath is thermostatically controlled and can be set at any desired level ranging usually from 20°C to 100°C. The heating coil may be of immersion type or enclosed in a case, some models have propellers to help to circulate water so that identical temperature is maintained throughout the water bath.



Fig:- 2.16. Water bath

2.6.1. Use and care of water bath

- ✓ Maintain the minimum level in the water bath with chemically pure water. Avoid use tap water. Avoid use of water as salts from tap water may get deposited on coil and so affect its function
- ✓ Always use a thermometer to check that the temperature is stable at the desired level.
- ✓ Make sure that the substance being incubated is below the surface of water in the bath

- ✓ It is advisable to cover the tubes, flasks or plates during incubation to avoid contamination and dilution as a result of condensation of water from the lid of the water bath.
- ✓ Clean the water bath regularly

2.7. Mixers

- Are instruments used for preparation of reagents for mixing or dissolving purpose. Also used for homogenization.



fig 2.17 mixer

2.8. Desiccators

- Desiccators are instruments, which are used for drying of chemicals or to keep other chemicals from being hydrated. As chemicals stay for long period of time out of desiccators, they sometimes absorb water. The chemical is dried in an oven at 110°C for 1 hour, and then it is placed in a desiccator over night before weighing on the analytical balance.
- The purpose of the oven is to remove the water and that of the desiccator is to store the chemical at an ambient temperature where it cannot reabsorb water.
- A desiccator contains substances called drying agents. These absorb the water in the air of the desiccators. The most commonly used drying agents (desiccants) are calcium chloride and concentrated sulfuric acid. The chemical that is to be dried is placed in another bottle or test tube and is put on top of the desiccants present in a securely closed desiccator.

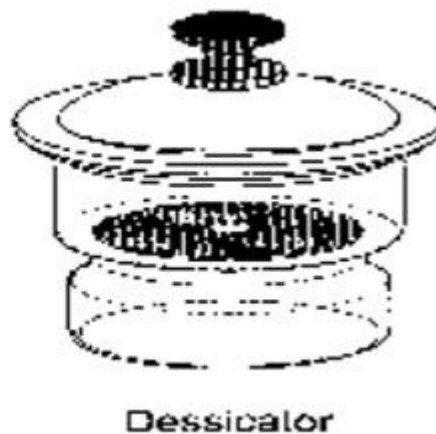


Fig :- 2.18. desiccator

2.9. PH meter

- Definition: is an instrument which is used to measure Potential of ion hydrogen (i.e. acidity or alkalinity of a substance) or Is an instrument used to measure the PH or H⁺ ion concentration.
- Potential of hydrogen pH scale is 0 – 14
 - ✓ Acid pH: 0-6.9
 - ✓ Neutral pH: 7.0
 - ✓ Alkaline pH: 7.1-14.0
- The pH meter is composed of
 1. Glass bulb electrode(PH- electrode)
 2. Reference(Calomel) electrode
 3. Potentiometer (Sensitive meter) which measures the electric volt.
- The glass bulb electrode contains a solution of a certain fixed PH or H⁺ conc. When the electrodes are placed in a solution of unknown PH, an electrical potential is produced between them(i.e the solution and the H⁺ ions in the PH-electrode) This potential which is proportional to the H⁺ ion concentration of the test solution, is measured with the aid of reference electrode which is compared to the potential of the PH-electrode. The mili volt (MV) potential difference is displayed as digital or galvanometric readings (PH0-14)
OMV=7.0



Fig 2.19 ph meter

2.10. Glass rod,

- Also called stirring rod, stir rod or solid glass rod, commonly uses borosilicate glass and quartz as material. Its diameter and length can be customized according to your requirements. Glass rod are corrosion resistant. It can resist most acid and alkali. It has strong hardness and can work in 1200 °C high temperature for long time. Thanks to these features, stirring rod is widely used in laboratory and industry. In laboratory, stirring glass can be used to speed up the mix of chemical and liquid



fig 2.20. Glass rod

2.11. Burette clamp

- Is a scientific equipment which used specifically to hold and secure a burette on a stand, so that a burette is fixed and more convenient for the experiment



fig 2.21 burette clamp

2.12. Spatula:

- can also refer to a tongue depressor are small stainless steel utensils or wooden utensils, used for scraping, transferring, or applying powders and paste like chemicals or treatments. Many spatula brands are also resistant to acids, bases, heat, and solvents, which make them ideal for use with a wide range of compound.

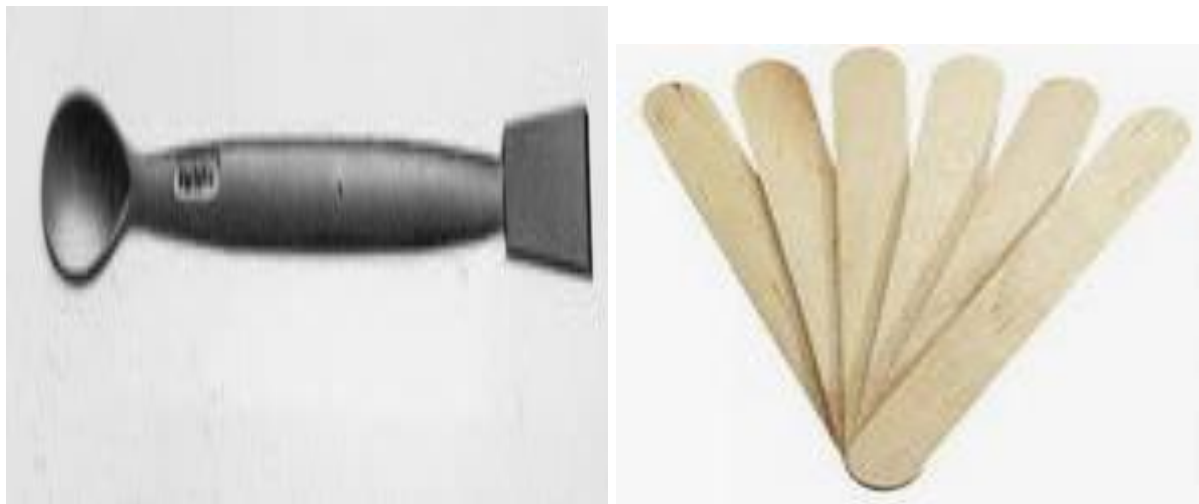


fig 2.22. Spatula

Self-Check -2

Written Test

Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page:

- When comparing glassware with plastic wares, plastic wares are?
 - Cheaper and safer to use.
 - Re-usable and autoclavable.
 - Heavier, more costly
 - easily broken
- _____ is/are calibrated to deliver a constant volume of liquid?
 - Micropipettes
 - Volumetric pipettes
 - Graduated pipettes
 - measuring pipettes
- Glass ware not calibrated to hold a particular or exact volume, but rather are available for various volumes, depending on the use desired
 - Volumetric glass wares
 - Non- volumetric glass wares
 - Semi-volumetric Glass wares
 - Volumetric flasks
- _____ is/are types of flasks useful for titrations and also for boiling solutions when it is necessary to keep evaporation to a minimum.



- A. Flat bottomed round flasks
 - B. Conical (Erlenmeyer) flasks
 - C. Round bottomed flask
 - D. Volumetric flasks
5. _____ is/are instruments, which are used for drying of chemicals or to keep other chemicals from being hydrated.
- A. Mixers
 - B. Desiccators
 - C. Water bath
 - D. Incubator
6. _____ is/are an apparatus for demineralizing water by means of cartridges filled with ion-exchange resin
- A. Deionizer
 - B. Distiller
 - C. Incubator
 - D. Water bath
7. Class "A" pipettes are the most accurate type of glass ware than Class "B" pipettes
- A. True
 - B. false

Part II matching: Match the following types of laboratory glass wares listed on column A from their correct composition listed on column B.

- | | |
|--|------------------------------------|
| <u> A </u> | <u> B </u> |
| 1. _____, high thermal resistance glass | A, Boron free |
| 2. _____, chemically stable and electric resistant | B, amber color to protect light |
| 3. _____, Glass with high resistance to alkali | C, borosilicate glass |
| 4. _____, Low actinic glass | D, Contains free soda in its walls |
| 5. _____, Standard flint glass | E, High silica glass |

Note: Satisfactory rating - 6 points

Unsatisfactory - below 6 points

Answer Sheet

Score = _____
Rating: _____

Name: _____

Date: _____



3.1. Introduction to Measurements

• **Measurement:**

- The action of measuring.
- An amount, size, or extent as established by measuring.

• **Measure/measuring**

- Ascertain the size, amount, or degree of (something) in comparison with a standard unit or with an object of known size.

3.1.1. Measurement units

- There are many measurement units used to describe measurements. for example, Traditional units, cgs units and Metric units (SI units)
- Majority of medical laboratory test methods and reagent preparations are required an appropriate measurement unit. Following World Health Organization recommendations an SI units (Système International d'Unités) are used in measurement.

3.1.1.1. International System of Units (SI)

- from French: *Le Système international d'unités*
 - ✓ is the modern form of the metric system
 - ✓ It is the world's most widely used system of measurement used in both everyday commerce and science. It comprises a coherent system of units of measurement built around seven base units, 22 named and an indeterminate number of unnamed coherent derived units
 - ✓ A set of prefixes that act as decimal-based multipliers.
- The International System of Units has been developed and agreed internationally to make uniform
 - ✓ In reporting of test results language (overcomes language barriers)
 - ✓ In enabling an exchange of health information within health institute, country or nation It is therefore important for health authorities and laboratories to adopt SI units
- The International System of Units is based on the *meter-kilogram-second* system and replaces both the foot-pound-second (Imperial) system and the centimetre-gram-second (cgs) system.
- The seven SI base units from which all the other units are derived are as follows:



SI base units	Symbol	Quantity measured
meter	M	Length
kilogram	KG	mass
second	S	time
mole	MOL	amount of substance
ampere	A	electric current
kelvin	K	temperature
candela	CD	luminous intensity

- SI derived units consist of combinations of base units. For ease of understanding and convenience Special names and symbols have been given to those derived units with complex base combination.

Derived quantity	Name	Symbol
area	square meter	m^2
volume	cubic meter	m^3
speed, velocity	meter per second	m/s



Derived quantity	Name	Symbol	Expression in terms of other SI units	Expression in terms of SI base units
frequency	hertz	Hz	-	s^{-1}
force	newton	N	-	$m \cdot kg \cdot s^{-2}$
pressure, stress	pascal	Pa	N/m^2	$m^{-1} \cdot kg \cdot s^{-2}$
energy, work, quantity of heat	joule	J	$N \cdot m$	$m^2 \cdot kg \cdot s^{-2}$
power, radiant flux	watt	W	J/s	$m^2 \cdot kg \cdot s^{-3}$
electric charge, quantity of electricity	coulomb	C	-	$s \cdot A$
electric potential difference, electromotive force	volt	V	W/A	$m^2 \cdot kg \cdot s^{-3} \cdot A^{-1}$
capacitance	farad	F	C/V	$m^{-2} \cdot kg^{-1} \cdot s^4 \cdot A^2$
electric resistance	ohm	Ω	V/A	$m^2 \cdot kg \cdot s^{-3} \cdot A^{-2}$
electric conductance	siemens	S	A/V	$m^{-2} \cdot kg^{-1} \cdot s^3 \cdot A^2$
catalytic activity	katal	kat	-	$s^{-1} \cdot mol$
magnetic flux density	tesla	T	Wb/m^2	$kg \cdot s^{-2} \cdot A^{-1}$
Celsius temperature	degree Celsius	$^{\circ}C$	-	K

3.1.2. SI unit prefixes

- To enable the measurement of quantities larger or smaller than the base units or derived units, the SI Unit System also includes a set of prefixes.
- The use of a prefix makes a unit larger or smaller
- The range of SI unit prefixes commonly used in laboratory work are listed as follow

Prefixes	Symbol	Function	DIVIDE BY
deci	d	10^{-1}	10
centi	c	10^{-2}	100
milli	m	10^{-3}	1000
micro	μ	10^{-6}	1000000
nano	n	10^{-9}	1000000000
pico	p	10^{-12}	1000000000000
femto	f	10^{-15}	1000000000000000



• Prefix	Symbol	Function	multiply BY:
• deka	da	10^1	10^1
• hecto	h	10^2	10^2
• Kilo	k	10^3	10^3
• Mega	M	10^6	10^6
• giga	G	10^9	10^9
• tera	T	10^{12}	10^{12}
• peta	P	10^{15}	10^{15}
• exa	E	10^{18}	10^{18}



Self-Check -3	Written Test
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Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page:

1. Which of the following is/are SI base units

A. kilogram	C. newton
B. square meter	D. pascal

2. Majority of medical laboratory test methods and reagent preparations are required an appropriate measurement unit

A. true	B. false
---------	----------

3. Which of the following unite of measurement is recommended by World Health Organization
 - A. SI units (Système International d'Unités)
 - B. foot-pound-second (Imperial) system
 - C. Traditional units of mesurement
 - D. centimetre-gram-second (cgs) system

4. SI derived units consist a combinations of base units

A. true	B. false
---------	----------

Note: Satisfactory rating - 2 points

Unsatisfactory - below 2 points
Answer Sheet

Score = _____
Rating: _____

Name: _____

Date: _____

Information Sheet-4	Estimate uncertainty of measurement
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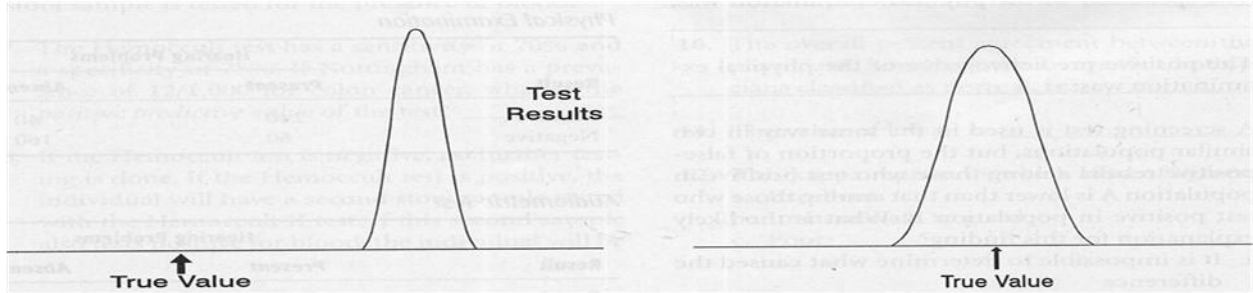
4.1. Accuracy and precision of measurement

4.1.1. Precision (Reliability)

- Is defined as the extent to which a questionnaire, test, observation or any measurement procedure produces the same results on repeated trials. In short, it is the stability or consistency of scores over time or across raters. Keep in mind that reliability pertains to *scores* not people

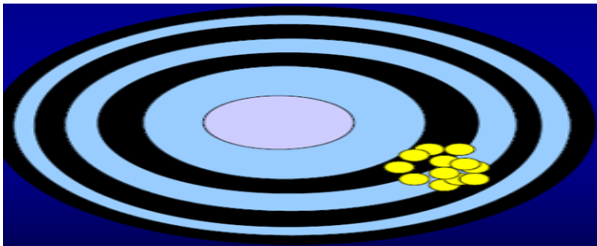
4.1.2. Accuracy (Validity)

- Is defined as the extent to which the instrument measures what it purports to measure. Or Is the ability of a test to indicate which individuals have the disease and which do not have the disease.
- For example, a test that is used to screen applicants for a job is valid if its scores are directly related to future job performance.

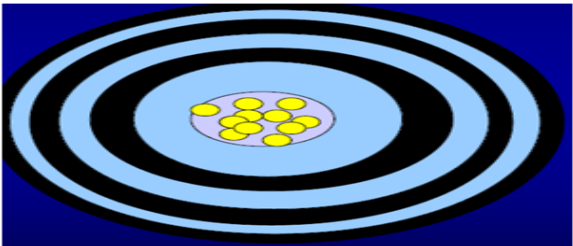


Results reliable but NOT valid

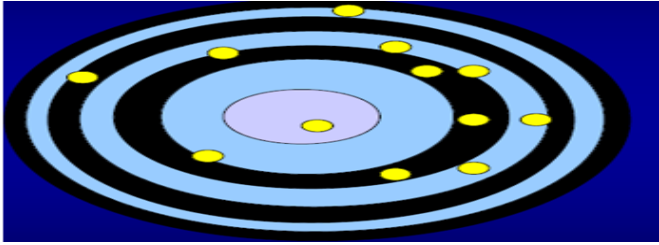
Results reliable and valid



Reliable, not valid



Both reliable and valid



Unreliable and invalid

4.2. Key measures of validity

1. Sensitivity



2. Specificity

3. Predictive value

- **Sensitivity** is the ability of the test to identify correctly those who have the disease from all individuals with the disease
- **Specificity** is the ability of the test to identify correctly those who do not have the disease from all individuals free from the disease.
- To determine the sensitivity and specificity of a new test **Gold standard test** is required.
- This helps to know the correct disease status of an individual. Use a 2 x 2 table to compare the performance of the new test to the gold standard test.
- **Predictive value:** Is the probability that the test result (positive or negative) will give the correct diagnosis (has a disease or does not have). In other words: the proportion of patients who test positive (negative) actually have (do not have) the disease in question.
 - Positive predictive value**
 - Negative predictive value**

		Gold standard test (disease)	
		+	-
New test	+	a (True positives)	b
	-	c	d (True negatives)

$$\begin{aligned}
 \text{Sensitivity} &= \frac{\text{True positives}}{\text{True positives} + \text{false negatives}} = \frac{\text{True positives}}{\text{All persons with the disease}} \times 100 \\
 &= \frac{\text{TP}}{\text{TP} + \text{FN}}
 \end{aligned}$$

$$\begin{aligned}
 \text{Specificity} &= \frac{\text{True negatives}}{\text{True negatives} + \text{false positives}} = \frac{\text{True negatives}}{\text{All persons without the disease}} \times 100 \\
 &= \frac{\text{TN}}{\text{TN} + \text{FP}}
 \end{aligned}$$

- **Positive Predictive Values:-** The positive predictive value (PPV) of a test is defined as the proportion (probability) of people with a positive test result who actually have the disease

$$\text{Predictive Value of a Positive Result (\%)} = \frac{\text{TP}}{\text{TP} + \text{FP}} \times 100$$

- Negative predictive value (NPV) is the probability that a person with a negative (normal) test result is truly free of disease.

$$\text{Predictive Value Negative Result (\%)} = \frac{\text{TN}}{\text{FN} + \text{TN}} \times 100$$



- **Example:-** Assume a population of 1,000 people 100 have a disease 900 do not have the disease A diagnostic test says 80 have a disease and 920 have no disease. Calculate sensitivity and specificity of a diagnostic test and Calculate positive and negative Predictive values of individuals



Self-Check -4	Written Test
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Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page:

1. _____ Is/are the extent to which a questionnaire, test, observation or any measurement procedure produces the same results on repeated trials.

A. Accuracy	C. Precision
B. Validity	D. uncertainty

2. _____ is/are the ability of the test to identify correctly those who do not have the disease from all individuals free from the disease.

A. Sensitivity	C. Positive predictive value
B. Specificity	D. Negative predictive value

3. Suppose a new HIV rapid test kit is evaluated by the gold standard (ELISA) as shown in the table below.

		gold standard test (ELISA)	
		Diseased	Not Diseased
New test	Positive	8	10
	Negative	2	90

What is the sensitivity and specificity of the new test in percentage respectively?

- | | |
|-------------|---------------|
| A. 20%, 80% | C. 90%, 80% |
| B. 80%, 90% | D. 100%, 100% |
4. From the above table what is the Positive and Negative predictive value of new test in percentage respectively?

A. 20%, 92%	B. 44%, 98%
C. 68%, 92%	D. 80%, 100%

Note: Satisfactory rating - 2 points

Unsatisfactory - below 2 points

Answer Sheet

Score = _____
Rating: _____

Name: _____

Date: _____

5.1. Introduction

- **Chemicals-** is a form of matter that has constant chemical composition and characteristic properties can be elements, compounds, ions and alloys


5.2. Characteristics of chemical

- It cannot be separated into components
- chemical can be pure or any mixture
- has the same properties and ratio
- exist as solids, liquids, gases
- Phases of matter may change with changes in temperature or pressure.
- May be combined or converted to others by means of chemical reactions.

5.3. Grade of chemicals

- **Lab Grade** - A line of solvents suitable for histology methods and general laboratory applications
- **AR** :- The standard grade of analytical reagents; suitable for laboratory and general use.
- **Guaranteed Reagent (GR)** - Suitable for use in analytical chemistry, products meet or exceed American Chemical Society (ACS) requirements where applicable.
- **Reagent A.C.S.** - This designates a high quality chemical for laboratory use

**Self-Check –5****Written Test**

Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page:

1. Which of the following is **NOT** chemical?
 - A. Pure water
 - B. Sugar
 - C. Vitamin C
 - D. Electricity
2. Which of the following is correct about Characteristics of chemicals?
 - A. can be separated into components
 - B. cannot be pure substance
 - C. exist as solids, liquids, gases state
 - D. Cannot combine or converted by chemical reactions.
3. Which grade of chemical have low quality
 - A. Lab Grade
 - B. AR
 - C. Guaranteed Reagent (GR)
 - D. Reagent A.C.S

Note: Satisfactory rating - 2 points

Unsatisfactory - below 2 points

Answer Sheet

Score = _____

Rating: _____

Name: _____

Date: _____



Information Sheet-6

Making dilution

6.1. Introduction

- Dilution is a process by which the concentration or activity of a given solution is decreased by the addition of solvent.
- A dilution represents the ratio of concentrated or stock material of the total final volume of a solution. Dilution is made to prepare:
 - ✓ A working solution from the stock
 - ✓ Measurable concentration of a sample (for reporting the actual concentrations of body- fluid constitutes)
 - If the specimen at hand is less than a procedure calls for
 - If the concentration of substances (analyte) is too high to be accurately measured.
- Whenever a solution is diluted, its volume is increased and its concentration decreased, but the total amount of solute remains unchanged

6.2. types of dilution

- Simple dilution
- Serial dilution

6.2.1. Simple dilution:

- A general process of preparing less concentrated solutions from a solution of greater concentration, a **unit volume of a liquid** material of interest is combined with an appropriate **volume of a solvent** liquid to achieve the desired concentration. To dilute a solution means to add more solvent without the addition of more solute to bring a solution into the desired concentration. The resulting solution is thoroughly mixed so as to ensure that all parts of the solution are identical
- the ratio of concentrated or stock solution to the total volume equals the dilution factor

$$\text{Dilution factor (df)} = \frac{\text{volume of stock}}{\text{Total volume of solution}}$$
- The df is inversely related to the concentration thus, the dilution factor increases as the concentration decreases.
 - ✓ a 1:5 dilution (verbalize as "1 to 5" dilution) entails combining 1 unit volume of **solute** (the material to be diluted) + 4 unit volumes of the **solvent** medium
 - ✓ hence, dilution factor could be: $1 + 4 = 5$



- Mathematically this relationship can be shown in the equation:

$$D = V_s/T_v$$

- Where:
 - D = dilution
 - V_s = volume of solute(sample)
 - T_v = Final volume
- In the performance of dilution, the following equation is used to determine the volume (V_2) needed to dilute a given volume (V_1) of solution of a known concentration (C_1) to the desired lesser concentration (C_2).

$$C_1 \times V_1 = C_2 V_2$$

- Likewise, this equation also is used to calculate the concentration of the diluted solution when a given solution is added to the starting solution.
- In making a simple dilution, the laboratory technician must decide on the total volume desired & amount of stock solution to use

6.2.1.1. Using Proportion

- It is used when reagents are prepared by adding a specific amount of one solution to a specific amount of another solution.

$$V = C/A+B$$

Where: C – total volume of final reagent

A – total parts of solution A

B – total parts of solution B

V – volume of each part

- ✓ Example 1: a buffer made by adding two parts of 'solution A' to five parts of solution B would be required to make 70 mL of the buffer.

➤ Formula: $C/A+B$ $V = \underline{70\text{mL required}}$

2 parts of A + 5 part of B

= 70 mL

7 part

= 10 volume of one part

A = 2 x 10 = 20 mL

B = 5 x 10 = 50 mL

- ✓ Example 2: a 100mg/mL N_2 standard is diluted 1:10. then the concentration of the resulting solution is

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$$100\text{mg/mL} \times 1/10 = 10 \text{ mg/mL}$$

6.2.1.2. Using $C_1V_1 = C_2V_2$

- This formula is useful only if the units for concentration & volume are the same & if three of the four variables are known.
- ✓ Example1. What volume is needed to make 500ml of 0.1M solution of tris-buffer from a solution of 2 M tris-buffer?

✓ **Example:2** To make 45 ml of 30% Solution from 70% solution $C_2 = 30\%$ $V_1 = C_2V_2$

$$V_2 = 45\text{ml}, C_1 = 70\% \quad V_1 = \frac{30 \times 45}{70} = 19.3 \text{ ml}$$

70

- Therefore, 19.3 ml of 70% solution must be diluted with 25.7 ml of distilled water to obtain 45ml of a 30% solution
- Diluting body fluids/standards
- ✓ Example: To make 8ml of a 1 in 20 dilution of blood.

$$C_1xV_1=C_2V_2 \quad \Rightarrow 20xV_1=1x8V_1 \text{ (blood volume)} =0.4$$

- Therefore, to prepare 8 ml of a 1 in 20 dilution, add 0.4 ml of blood to 7.6 ml of the diluting fluid.
- To make 4ml of a 1 in 2 dilution of serum in physiological saline.
- ✓ $C_1xV_1=C_2V_2 \Rightarrow 2xV_1=1x4 \Rightarrow V_1 \text{ (serum volume)} =0.4$

- To prepare 4ml of a 1 in 2 dilution, add 2ml of serum to 2 ml of physiological saline.

- Calculating the dilution of a body fluid

✓ Examples: Calculate the dilution of blood when using 50 micro liters (μ l) of blood and a 50 μ l of diluting fluid.

$$\text{Total volume of blood and diluting fluid } 50 + 50 = 100 \mu \text{ l}$$

$$\text{Sample: total } 50:100 \quad 1 \text{ in } 2 \text{ dilutions}$$

- Calculate the dilution of urine using 0.5 ml of urine and 8.5 ml of diluting fluid (physiological saline)

✓ Total volume of urine and diluting fluid, $0.5 + 8.5 = 9.0 \mu \text{ l} \Rightarrow 0.5: 9 = 1 \text{ in } 18 \text{ dilutions}$

6.2.2. Serial dilutions

- A serial dilution may be defined as multiple progressive dilutions ranging from more concentrated solutions to less concentrated solutions.
- Simply a series of simple dilutions which amplifies the dilution factor quickly beginning with a small initial quantity of material (bacterial culture, a chemical, orange juice, etc.). The source



of dilution material (solute) for each step comes from the diluted material of the previous dilution step.

- Final dilution factor (DF) = DF1 * DF2 * DF3 etc.
- It is the stepwise dilution of a substance in solution. Usually the dilution factor at each step is constant, resulting in a geometric progression of the concentration in a logarithmic fashion.
 - ✓ Used to accurately create highly diluted solutions as well as solutions for experiments resulting in concentration curves with a logarithmic scale.
 - ✓ used to reduce the concentration of microscopic organisms or cells in a sample
 - ✓ It is required for certain quantitative tests
 - ✓ Serial dilution is extremely useful when the volume of the concentrate &/or diluents is in short supply
 - ✓ Large dilutions may be difficult to make because of the amount of diluent that needs to be added.
 - For example, a 1/1000 dilution may be difficult to create accurately even with 0.1 mL of serum & 99.9 mL of diluent. A series of dilutions, also called serial dilutions, may be a better way to make the dilutions.
- The dilution fold of a system can be determined by the formula:

$$\frac{1}{\text{Dilution fold}} = \frac{\text{volume transferred}}{\text{total volume}}$$

- Volume transferred = is equal to the constant volume transferred to each successive tubes in the serial dilution system.
- Total volume = is equal to the volume being transferred plus the volume of diluents already in the tube.
- ✓ g. 1. What is the dilution fold of the following serial dilution system consisting of five tubes? The following amount of diluents have been added to the tubes; 0.5 mL to tube 1 & 0.5 mL to tube 2 to 5. Next, 0.5 mL of patient serum is added to tube 1 and 0.5 mL is serially transferred through tube 5. finally, 0.5 mL is discarded from tube 5.
 - $1/Y = 0.5/1.0$
 - $Y \times 0.5 = 1$
 - $Y = 1/0.5 = 2$

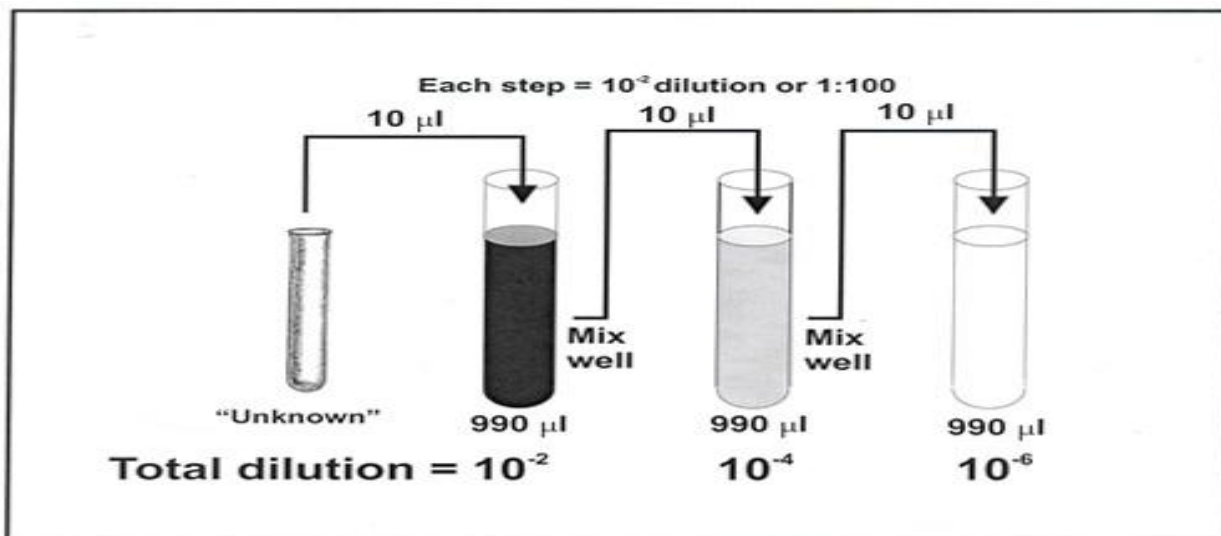
- ✓ E.g. 2. it is often desirable to determine the dilution of a given tube (Y) in a serial dilution system. This dilution can be calculated by Solution of tube 1 = dilution of Y x [1/dilution fold]

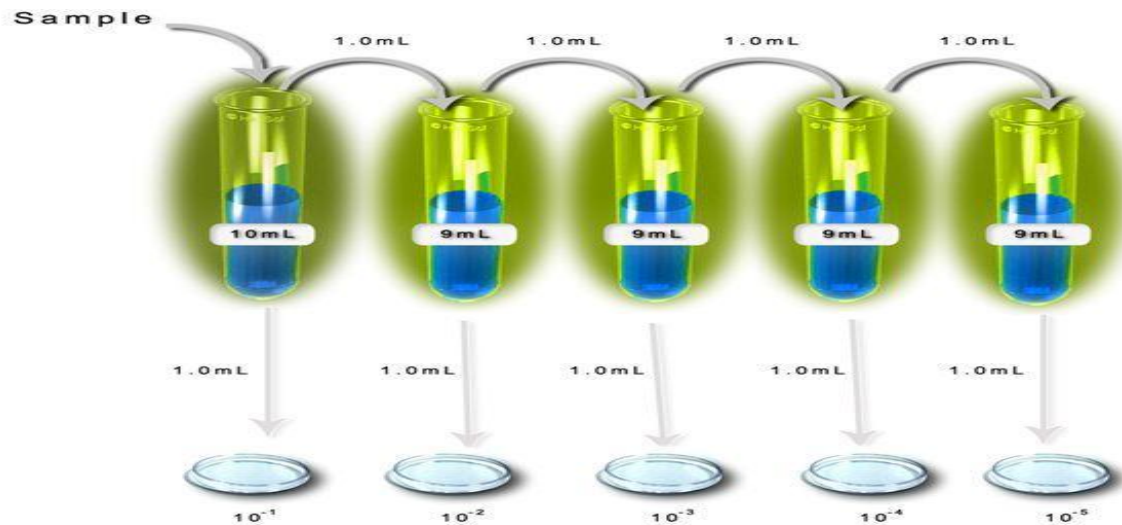
- What is the dilution of tube 3 in the preceding example?

$$\begin{aligned}
 Y &= \frac{1}{2} \times (\frac{1}{2})^{(Y-1)} \\
 &= \frac{1}{2} \times (\frac{1}{2})^2 \\
 &= \frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} \\
 &= \frac{1}{8} ;
 \end{aligned}$$

The dilution of serum in the tube 3 is 1/8.

- ✓ E.g. In a typical microbiology exercise the students perform a *three step* 1:100 serial dilution of a bacterial culture in the process of quantifying the number of viable bacteria in a culture. Each step uses a 1 ml total volume. The initial step combines 1 unit volume of bacterial culture (10 μ l) with 99 unit volumes of broth (990 μ l) = 1:100 dilution. In the second step, one unit volume of *the 1:100 dilution* is combined with 99 unit volumes of broth now yielding a total dilution of 1:100x100 = 1:10,000 dilution. Repeated again (the third step) the total dilution would be 1:100x10,000 = 1:1,000,000 total dilution. The concentration of bacteria is now one million times *less* than in the original sample.





Self-Check -6

Written Test

Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page:

1. Buffer solution is made by adding two parts of ‘solution A’ to three parts of solution B. which volume of solution A and solution B would be required to make 100 mL of the buffer respectively.

A. 10, 90	C. 30, 70
B. 20, 80	D. 40, 60

2. Calculate the dilution of urine using 0.5 ml of urine and 8.5 ml of diluting fluid (physiological saline)

A. 1: 3 dilutions	C. 1:12 dilutions
B. 1:9 dilutions	D. 1:18 dilutions

3. What is the dilution fold of tube 3 in serial dilution system consisting of five tubes? 0.5 mL diluents have been added to tube 1 to 5. Next, 0.5 mL of patient serum is added to tube 1 and 0.5 mL is serially transferred through tube 5. Finally, 0.5 mL is discarded from tube 5.

A. 2	C. 6
B. 4	D. 8

Note: Satisfactory rating - 2 points

Unsatisfactory - below 2 points

Answer Sheet

Score = _____

Rating: _____



Information Sheet-7

Solution preparation

7.1. Definition

- Solution is a homogenous mixture of two or more components that can be varied in composition within certain limit. Every solution consists of two parts, the solvent and solute

7.2. Characteristics of solutions

- homogeneous
- The particles of solute in a solution cannot be seen by naked eye.
- Does not allow beams of light to scatter.
- Stable.
- The solute from a solution cannot be separated by filtration (or mechanically).
- Composed only one phase.

7.3. Functions of solutions

- involved in a chemical reaction, especially one used to detect, measure, or produce another substance
- Preserve/fix substances to protect from deterioration
- Maintain Ph of a solution
- Decrease concentration of a substance
- Maintain osmotic pressure of a substance
- cleaning, disinfecting and sterilizing materials
- Increase refractive index of light
- Gives artificial color to be visualized

7.4. Types of clinical laboratory solution

- There are different types of solutions used in medical laboratory procedures. These include reagent solution, staining solution, standard solution and buffer solution.

7.4.1. Reagents solutions

- Any solution that is used in conjunction with a given sample and expected to produce a measurable or noticeable change is called a reagent solution.
- Necessary care, including the followings should be taken in preparing a given reagent solution:
 - ✓ Chemical selection;
 - ✓ Following instruction of preparation;



- ✓ Using of accurate measurements of substances (ingredients)
- ✓ Using of appropriate type of glass or plastic wares.

- There are two Types of reagent solutions

7.4.1.1. Stock reagent solution

- Is a concentrated reagent solution which is diluted to prepare a working solution.
- Has a longer shelf life and occupies less space in storage than the working solution.

7.4.1.2. Working –reagent solution

- Can be diluted from the stock solution or prepared directly from the reagent chemical following the recommended procedures

7.4.2. Staining solution

- Staining solutions are solutions that contain colored dyes. These solutions can contain basic, acidic or neutral dyes.
- Different strains are used in medical laboratories to give an artificial color for the substances to be identified from a given biological specimen (whole blood, body fluids, urine, etc.). The substances may be identified by their characteristic reaction with the staining solutions.
- Different types of blood cells, bacteria, parasites, and tissues together with their cellular elements can be stained by using appropriate types of stains (differential stains) such as Giemsa stain, Wright stain, Gram stain, Leishman stain, Acid Fast Stain, etc. Simple stains are used to reveal the morphology (shape, size and content) of an organism(s) and single dye is utilized for the procedure.
- Based on their reaction, there are three kinds of stains:
 1. Basic stains
 2. Acidic stains
 3. Neutral stains

7.4.2.1. Basic Stains

- Are stains in which the colouring substance is contained in the base part of the stain and the acidic part is colourless.

✓ **Example :** Methylene blue stain, Safranin, Genetian violet, Carbolfuchsin etc

7.4.2.2. Acidic Stains

- Are stains in which the colouring substance is contained in the acidic part of the stain and the base part is colourless. E.g. eosin.

7.4.2.3. Neutral Stains

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- Are stains in which the acidic and basic components of stains are coloured.
- Neutral dyes stain both nucleic acid and cytoplasm. e.g Giemsa's stain, Wright's stain

7.4.3. Standard solutions

- These are solutions in which the concentration of a given chemical is precisely known
- They are used to determine the value of an identical chemical with unknown concentration of a given solution.
- Chemicals that are used to prepare these solutions should be of analytical grade.
- Since poor standard solutions cause errors in the estimation of the intended substances, their accurate preparation is of utmost importance in order to obtain accurate and precise laboratory findings in medical laboratories
- There are two types of standard solution

7.4.3.1. Primary standard solution

- Primary standard solution is a chemical solution that has the highest purity and can be used directly for the exact measurement of substances of unknown concentration in a given solution. These solutions include sodium chloride, sodium bicarbonate, potassium iodide, etc.
- Primary standard solution should be made of substances that are:
 - ✓ Free of impurities,
 - ✓ Stable on keeping in solid state and in solution,
 - ✓ Able to be accurately weighed or measured to give a solution of exactly known concentration,
 - ✓ Not hygroscopic (does not absorb moisture) and vaporize at 20^oc.

7.4.3.2. Secondary standard solutions

- Secondary standard solutions are solutions of lower purity and their concentrations are determined by comparison to primary standard solutions. Secondary standard solutions are used for analytical procedures after their concentration is already determined. Some examples of these solutions are nitric acid, hydrochloric acid, sulfuric acid, etc.
- In the preparation of secondary standard solutions, the following points should be taken into consideration:
 - ✓ Using analytical balance for weighing
 - ✓ Dissolving the weighted substance in the prescribed volume of solvent
 - ✓ Determining the exact concentration by comparison against a primary standard solution

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- ✓ Diluting stock secondary standard solutions using exact measurements.

7.4.4. Buffer solutions

- A buffer is a solution of a weak acid or base and one of its respective salts. Buffers are able to resist changes in the PH.
- Buffers are used when the pH needs to be carefully controlled for the diagnostic procedures, such as in measuring enzyme activities.

7.5. Classification of solutions

7.5.1. Based on the **states(phase)** of the solution

- solutions can be gaseous, liquid and solid, solute can be **gas, liquid or solid**, Solvents can also be **gas, liquid or solid**

	Gas	Liquid	Solid
Gas	Oxygen and other gases in nitrogen (air), Br ₂ gas (solute) dissolved in Ar gas (solvent).	Water vapor in air (humidity), Ar gas (solute) dissolved in liquid H ₂ O (solvent).	The odor of a solid - molecules of that solid being dissolved in the air
Liquid	Carbon dioxide in water (carbonated water)	Ethanol in water, various hydrocarbons in each other (petroleum) *, Br ₂ liquid (solute) dissolved in liquid H ₂ O (solvent).	Sucrose (table sugar) in water; sodium chloride (table salt) in water, NaCl (solute) dissolved in liquid H ₂ O (solvent).
Solid	Hydrogen dissolved to palladium	Water in activated charcoal	Steel, Brass, other metal alloys

7.5.2. Based on the **strength** of the solution:

- strength of a solution is measured by its solubility



- **Solubility** is the ability of one compound to dissolve in another compound at any one temperature, When a liquid can completely dissolve in another liquid the two liquids are ***miscible***. But two substances that can never mix to form a solution are called ***immiscible***.
- Here **law of dissolution** is applied “ like dissolve like”
 - ✓ Dilute or Weak Solution
 - ✓ Concentrated Solution
 - ✓ Unsaturated
 - ✓ Saturated Solution
 - ✓ Supersaturated Solution

7.5.3. Based on the types of solvent of the solution

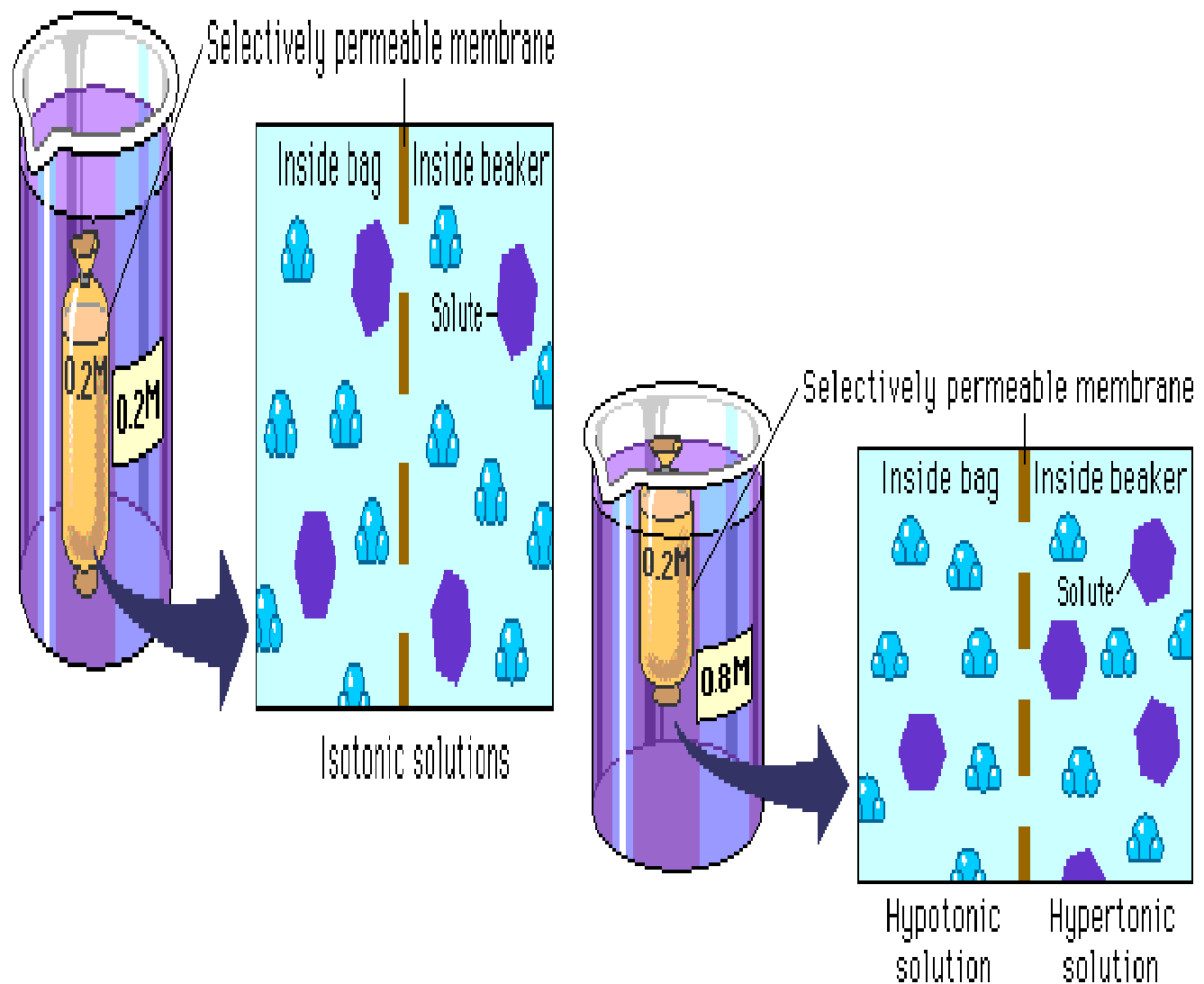
- **Aqueous solutions:** contain water as the solvent. example, sugar in water, carbon dioxide in water, etc.
- **Non-aqueous solution:** contain a solvent other than water. Ether, benzene, petrol, carbon tetrachloride etc., are some common solvents. example, sulfur in carbon disulphide, naphthalene in benzene, etc.

7.5.4. Based on the **concentration of H_3O^+** of the solution, solutions can be

- Acids eg. HCl
- Bases eg. KOH
- Neutral (Salts) eg. NaCl solution

7.5.5. Based on the **osmotic pressure (solute concentration)** of the solution

- Hypertonic
- Isotonic
- hypotonic



7.5.6. Based on the **dye content**

- Based on the content of dye it can be classified as **stains and non-stains**
- **Stains**-reagent or dye used in treating a specimen for microscopic examination
- Stains are used to bind and make visible specific structures within the cell so that they are more easily visible in the microscope.
- Stains (Dyes) are coloured chemical compounds that are used to selectively give (impart) colour to the colourless structures of bacteria or other cells

- Staining reactions are made possible because of the Physical phenomena of capillary osmosis, solubility, adsorption, and absorption of stains or dyes by cells of micro-organisms.
- **Basic principle:** The cellular components of mammalian as well as microbial cell are different. For example the nuclei of cell is negatively charged because of the presence of acidic component (DNA) hence it combines with positively charged compounds, (basic dyes). and the cytoplasm parts of a cell is generally positively charged therefore combines with negatively charged compounds (acidic dyes).

7.6. Expressing concentration of solutions

- **Concentration-** amount of a substance dissolved in a given amount of solvent. Concentration of solutions should be accurately expressed for the appropriate use in the desired procedures. Concentration of a solution can be expressed in three ways
 - 1) Qualitative Expressions of Concentration
 - 2) Semi-Quantitative Expressions of Concentration
 - 3) Quantitative Expressions of Concentration

7.6.1. Qualitative Expressions of Concentration

- A solution can be qualitatively described as
 - ✓ **Dilute:** a solution that contains a small proportion of solute relative to solvent
 - ✓ **Concentrated:** a solution that contains a large proportion of solute relative to solvent



Diluted ←————→ **Concentrated**

7.6.2. Semi-Quantitative Expressions of Concentration

- A solution can be semi-quantitatively described as:
 - ✓ **Unsaturated:** a solution in which more solute will dissolve
 - ✓ **Saturated:** a solution in which no more solute will dissolve



- ✓ **Supersaturated Solution**-a solution that contains more dissolved substance than does a saturated solution; the solution is not in equilibrium with the pure substance.

7.6.3. Quantitative Expressions of Concentration

- Quantitative notation of concentration is far more informative and useful from a scientific point of view.
- Many units of concentration require measurement of a substance's volume, which is variable depending on ambient temperature and pressure.
- Unless otherwise stated, all the following measurements are assumed to be at standard state temperature and pressure (that is, 25 degrees Celsius at 1 atmosphere). There are a number of different ways to quantitatively express concentration

7.6.3.1. Physical Units

7.6.3.1.1. Percentage expression

- Percent (%W/V):** Weight of solute per unit volume of solution. Or mass of a substance in a mixture as a percentage of the volume of the entire mixture
 - ✓ Example, 40 % w / v glucose solution means, 40 gm of glucose is dissolved in 100 ml of a given solvent.
- Percent (%W/W):** Weight of solute per weight of solvent. Or Denotes the mass of a substance in a mixture as a percentage of the mass of the entire mixture.
 - ✓ Example, 30 % w / w HCl means, each 100 gm of hydrochloric acid solution contains 30 gm of HCl and the rest 70 gm is the solvent
- Percent (%V/V):** Volume of solute per volume of solvent. Or Describes the volume of the solute in mL per 100 mL of the resulting solution. This is most useful when a liquid - liquid solution is being prepared.
 - ✓ E.g. Beer is about 5% ethanol by volume. This means every 100 mL beer contains 5 mL ethanol (ethyl alcohol).

7.6.3.1.2. Parts by part

- Sometimes when solutions are too dilute, their percentage concentrations are too low.
- So, instead of using really low percentage concentrations such as 0.00001% or 0.000000001% choose another way to express the concentrations. Used to denote the relative abundance of trace elements in the Earth's crust, trace elements in forensics or other analyses, or levels of pollutants in the environment.

A. Parts per hundred (denoted by '%' and very rarely 'pph' 1 part in 10²):

- Denotes one particle of a given substance for every 99 other particles.

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B. **Parts per thousand** (denoted by '‰' [the per mil symbol], and occasionally 'ppt' 1 part in 10^3):

- denotes one particle of a given substance for every 999 other particles.

C. **Parts per million ('ppm')**

D. **Parts per billion ('ppb')**

E. **Parts per trillion ('ppt')**

F. **Parts per quadrillion ('ppq')**

7.6.3.2. Chemical units

- Most common acids and some basic solutions like ammonium hydroxide are usually found with their concentrations expressed in specific gravity and percentage by weight of the specific solution.
- These two information's (specific gravity and percentage by weight) should be changed to the commonly known expressions of concentration, like molarity and normality.

7.6.3.2.1. Molarity (M)

- Molarity : defined as the number of moles of solute in each liter of solution
- A **molar solution** is a solution that contains one mole of the solute in one liter of solution For example, the molar weight of sulfuric acid (H_2SO_4) is 98.

$$M = \frac{\text{Number of mole of solute}}{\text{Volume of solution in liter}}$$

$$M = \frac{\text{Amount of substance (weight)}}{\text{Molar weight} \times \text{volume of solution in liter}}$$

- E.G. A solution contains 5.7 grams of potassium nitrate dissolved in enough water to make 233 mL of solution. What is its molarity? (Formula weight of KNO_3 is 101.103 g/mol.)

$$\text{Mole of } KNO_3 = 5.7g/101.103g/mol = 0.056mol$$

$$M = 0.056mol/0.233L = 0.24 \text{ mol/L}$$

$$= 0.24M$$

- liters of liquid, containing 2.0 moles of dissolved particles, constitutes a solution of 0.5 M Such a solution may be described as "0.5 molar." Working with moles can be highly advantageous, as they enable measurement of the absolute number of particles in a solution, irrespective of their weight and volume.

7.6.3.2.2. Normality (N)

- Normality: is defined as the number of equivalent weight of a solute in a liter of solution.

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- A normal solution is a solution that contains one-gram equivalent weight of the solute in one liter of solution.

$$N = \frac{\text{Number of gram equivalents of solute}}{\text{Volume of solution in liter}}$$

$$N = \frac{\text{Amount of substance}}{\text{Equivalent weight} \times \text{volume of solution in liter}}$$

$$\text{Equivalent weight} = \frac{\text{Molecular weight}}{\text{Valancy}}$$

- In practice, this simply means one multiplies the molarity of a solution by the valence of the ionic solute. It has advantages when carrying out titration calculations
- The equivalent weight of H_2SO_4 is 98 divided for 2 (valancy of H_2SO_4), which is 49. Therefore, one normal solution of H_2SO_4 contains 49 gram of H_2SO_4 per liter of solution.

7.6.3.2.3. Molality (m)

- Molality (m): is defined as the number of moles of solute in 1 kilogram of solvent.
- A molal solution is a solution that contains one mole of the solute in one kilogram of solution.

$$m = \frac{\text{Number of mole of solute}}{\text{Volume of solvent in Kg}}$$

$$m = \frac{\text{Amount of substance (weight)}}{\text{Molar weight} \times \text{volume of solvent in Kg}}$$

- ✓ E.g. 80 grams of a simple sugar is added to 750 g of water. The sugar is glucose, with the composition $\text{C}_6\text{H}_{12}\text{O}_6$. What is the molality of glucose in the solution?

7.7. Basic steps in solution preparation

- ✓ Assembling necessary materials and equipment for preparation of solutions
- ✓ Calculating and recording data
- ✓ Measuring chemicals and solvents
- ✓ Dissolving/ diluting the solution in accordance SOPs
- ✓ Labeling and storage of reagents
- When preparing a solution decide whether the solution requires
 - ✓ An accurate volume preparation, E.g. a calibrate (standard)
 - ✓ Less accurate volume preparation, E.g. Stain
- **analytical reagents (AR)**:- chemicals that are labeled Analar, Univar, AR (Analytical Reagent), also known as GR (Guaranteed Reagent) which is prepared purely and used for the preparation of calibrates or any reagent which requires a pure chemical.

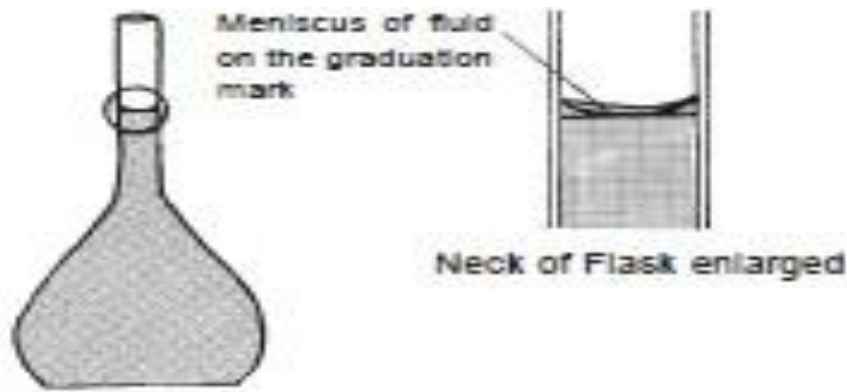
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- **Laboratory Grade (LG):** - has lower purity but may be Industrial grade chemicals. It's less expensive, useful for some less pure solution preparation.
- **Deliquescent:**- chemical dissolved in the moisture and go on taking in moisture until the vapor pressure of the solution equals the pressure of the water in the atmosphere
- **Hygroscopic:** a chemical absorbs water from the air but does not dissolve in the water it absorbs

7.7.1. Preparing accurate solutions:

- Use a balance of sufficient sensitivity
- Weigh the chemical as accurately as possible.
- The chemical should be of an analytical reagent grade.
- Hygroscopic and deliquescent chemicals need to be weighed rapidly
- Use calibrated, chemically clean glassware
- Read carefully the graduation marks and other information on flasks and pipettes
 - ✓ Example: check whether a pipette is of the containing (rinsing- out) type or of the delivery (non- rinsing-out) type. It is best to use a delivery type
- Use a funnel to transfer the chemical from the weighing container to a volumetric flask.
- Wash any chemical remaining in the container into the flask with a little of the solvent.
- Make the solution up to its final volume. If warm, make up to volume only when the solution has cooled to the temperature used to graduate the flask (written on the flask).
- To avoid over-shooting the graduation mark, use a Pasteur pipette or wash-bottle to add the final volume of solvent to the flask.
- Make sure the bottom of the meniscus of the fluid is on the graduation mark when viewed at eye level (see Fig. below).
- Mix the solution well by inverting the flask at least twenty times



7.7.1.1. Preparation of calibrating solutions (standards):

- When preparing calibrates the following are important:
 - ✓ Always use pure chemicals. The use of impure low grade chemicals can lead to serious errors in test results.
 - ✓ Avoid weighing a very small quantity of a calibrate substance. Instead prepare a concentrated stock solution which can be diluted to make working solutions.
 - ✓ Use good quality distilled water. Electrolyte calibrates require deionized water.
 - ✓ Use calibrated glassware and a volumetric technique.

N.B. calibrates should be prepared and standardized in a regional or control laboratory and distributed with instructions for use to district laboratories.

7.7.2. Preparing stains:

- There is no need to use expensive volumetric glassware when preparing stains. Weigh the dye in a small container and transfer the weighed dye direct to a leak-proof storage container, preferably a brown bottle. Add any other ingredients and the volume of solvent as stated in the method of preparation, and mix well.
- Adding a few glass beads will help the dye to dissolve more quickly. For some stains heat can be used to dissolve the dye (this will be stated in the method of preparation).
- **Note:** Instead of measuring the volume of solvent each time the stain is prepared, it is more practical to mark the side of the container with the volume which needs to be added.
- Transfer part of the stain to a stain dispensing container, filtering it if required. Always use dispensing containers with tops that can be closed when not in use.
- Label the container in a similar way to that described previously. Store as instructed in the method of preparation. Always protect stock containers of stain from direct sunlight

7.7.2.1. Giemsa stain preparation



- Giemsa stock solution of about 500 ml is prepared in the bottle of stain by mixing Giemsa powder (3.8g), Glycerol (250 ml) and Methanol (250ml) in a water bath at 50–60 °C

- **Necessary Materials:**

- ✓ To make about 500 ml:
- ✓ Giemsa powder 3.8 g
- ✓ Glycerol (glycerin)..... 250 ml
- ✓ Methanol (methyl alcohol)..... 250 ml
- ✓ Balance Funnel
- ✓ Weighing paper Labeling Marker
- ✓ Measuring Cylinder Plastic Adhesive Tape Reagent bottle (Brown bottle)
- Spatula (spoon)
- ✓ Glass Beads Filter Paper

- *Preparation of Giemsa working Solution (10% Giemsa solution):*

- Necessary Materials

- ✓ Giemsa stock solution
- ✓ Buffered Water (pH 7.2)
- ✓ Measuring Cylinder
- ✓ Empty container

7.7.2.2. Preparation of EDTA anticoagulant:

- Necessary Materials

- ✓ To Make about 250ml
- ✓ Di-potassium ethylene- diamine-tetra-acetic acid (K₂ –EDTA)... 2.5 g
- ✓ Distilled water..... 25 ml

- **Necessary Tools & Equipment:**

- ✓ Balance
- ✓ Funnel
- ✓ Weighing paper
- ✓ Micropipette
- ✓ Measuring Cylinder
- ✓ Test tubes
- ✓ Reagent bottle (Brown bottle)
- ✓ Spatula (spoon)
- ✓ Labeling Marker



- ✓ Plastic Adhesive Tape

7.7.2.3. Preparation of Diluted Sodium hypochlorite (Bleach) Disinfectants

To prepare 10ml 0.5% bleach

Necessary materials:

- Stock Sodium Hypochlorite Distilled water
- Measuring Cylinder Empty container
- Gloves

1. Calculate the total parts of water to be added using the formula Total parts of water added= (%concentrate/%dilute)-1

$$= (5\%/0.5\%)-1$$

$$=9 \text{ parts}$$

So 1 part of 5%bleach is mixed with 9 parts of water

Volume of water to be added = total volume × (parts of water/total parts)

$$=10\text{ml} \times (9/10)$$

$$=9\text{ml}$$

Volume of 5% bleach=total volume – volume of water added

$$=10\text{ml}-9\text{ml}=1\text{ml}$$

So 1ml of 5% bleach is mixed with 9ml of water

2. Measure 1ml of 5% bleach in to an empty container
3. Measure 9ml of water and mix well
4. Label as 0.5% bleach on the container

7.7.2.4. Preparation of 70% alcohol disinfectant:

- Creating dilutions reduces the concentration of one liquid with the addition of another. In order to create 70 percent alcohol, a solution of ethanol alcohol with a concentration greater than 70 percent must be diluted by a calculated amount of water.
- The formula for this calculation is $C_1V_1=C_2V_2$, where C1 and V1 is the starting concentration and volume of the solution and C2 and V2 is the final concentration and volume of the dilution. For the purpose of this example, the initial solution is 100 percent ethanol alcohol, creating a final volume of 500 mL of 70 percent alcohol

7.7.2.5. Gram’s stain reagents preparation

Crystal violet stain

- Crystal violet 20g
- Ammonium oxalate 9g

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- Ethanol or methanol absolute 95ml
- Distilled water to 1 litre

Acetone -alcohol

To make 1 litre

- Acetone 500ml
- Ethanol or methanol absolute 475 ml
- Distilled water 25ml
- Mix the acetone, ethanol and distilled water and transfer to a clean glass-stoppered bottle. Label the bottle “ACETONE–ETHANOL DECOLORIZER” and write the date.

Gram’s iodine

- Potassium iodide 20 g
- Iodine 10 g
- Distilled water to 1 litre
- Should be stored in a brown bottle

Safranin

1. Prepare a stock solution

- Safranin O ----- 2.5g
- Ethanol (95%) -----100ml

Mix until all the safranin is dissolved. Transfer the solution to a glass – stoppered bottle.

Label the bottle (Safranin stock solution) and write the date.

2. Prepare a working solution in a glass stoppered bottle

- Stock solution -----10ml
- Distilled water -----90ml
- Label the bottle (Safranin working solution) and write the date

Neutral red; 1g/l (w/v)

To make 1 litre

- Neutral red 1g
- Distilled water 1 litre

7.7.2.6. Ziehl Neelson stain reagents preparation

A. Carbofuschin

Solution A (saturated solution of basic fuchsin)

- Basic fuchsin 3gm
- Ehanol100ml



Solution B (phenol aqueous solution, 50g/L (5%)

- Phenol 10gm
- Distilled water 200ml
- Mix **10 ml** of solution A with **90ml** of solution B. Transfer resulting mixture to a glass stoppered amber bottle and label the bottle “CARBOL FUCHSIN SOLUTION” and write the date.

Warning: Phenol is highly corrosive and poisonous

Preparation of Acid alcohol, 3% v/v:

To make 1 liter:

Ethanol or methanol, absolute* 970 ml

Hydrochloric acid, concentrated 30 ml

1. Measure the ethanol or methanol and transfer to a 1 liter capacity leak-proof container.

Caution: Ethanol and methanol are highly flammable, therefore use well away from an open flame.

2. Measure 30 ml of concentrated hydrochloric acid, add to the solution, and mix well.

Caution: Concentrated hydrochloric acid is a corrosive chemical with an injurious vapor; therefore handle it with great care in a well-ventilated room.

3. Label the bottle, and mark it Flammable.
4. Store at room temperature in a safe place. The reagent is stable indefinitely.

For use: Transfer a small amount of the reagent to a dispensing container that can be closed when not in use.

Methylene Blue Stain

- Methylene blue chloride 0.5g
- Distilled water..... 100ml

Malachite green

Malachite green 0.5gm

Distilled water 100ml

Using a pestle and mortar, grind the malachite green crystal to a powder. Dissolve the ground powder in 100ml distilled water and store in a dark brown bottle.

7.7.2.7. Fluorescent stain Reagents preparation

Auramine O

Auramine-----0.1gm

95% Ethanol-----10 ml



Dissolve Auramin with Ethanol -----Solution1

Phenol

Phenol crystal-----3.0 gm

Distilled water-----87ml

Dissolve phenol with Distilled water-----Solution 2

Mix solution 1 and 2 and store in amber bottle away from light and heat

Potassium permanganate

Potassium permanganate (KMnO₄) -----0.5g

Dis. Water-----100ml

Dissolve and store in amber bottle

Acridine orange

Acridine orange-----0.01g

Anhydrous dibasic sodium phosphate (NA₂HPO₄) -0.01g

Distilled water-----100ml

7.7.2.8. Lens cleaning solution (Ethyl ether – alcohol)

- Absolute ethanol20ml
- Ethyl ether80ml
- Mix and stopper

7.7.2.9. Boric acid, saturated solution

- Boric acid4.8 g
- Distilled waterq.s. 1000ml
- Store in a glass-stoppered bottle.
- Label the bottle “BORIC ACID SATURATED SOLUTION” and write the date

7.7.2.10. Buffered water, pH 7.2

- Disodium hydrogen phosphate (Na₂HPO₄·2H₂O)..... 3.8 g
- Potassium dihydrogen phosphate ,anhydrous2.1 g
- Distilled waterq.s. 1000ml
- Dissolve the salts in the distilled water, stirring well. Check the pH using narrow range pH papers; it should be 7.0–7.2.
- Transfer the solution to a glass-stoppered bottle and Label the bottle “BUFFERED WATER” and write the date.

7.7.2.11. Eosin, 10g/l (1%) solution

- Eosin1 g



- Distilled waterq.s. 100ml
- Label the bottle “EOSIN 1% SOLUTION” and write the date

7.7.2.12. Formaldehyde, 10% solution

- formaldehyde (CH₂O) solution, 40%.....100ml
- Distilled water300ml
- Transfer the solution to a glass-stoppered bottle.
- Label the bottle “FORMALDEHYDE 10% SOLUTION” and write the date.
- *Warning: Formaldehyde is corrosive and poisonous.*

7.7.2.13. Potassium hydroxide, 200 g/l (20%) solution

- Potassium hydroxide (KOH) pellets20g
- Distilled waterq.s. 100ml
- Label the volumetric flask “POTASSIUM HYDROXIDE 20% SOLUTION” and write the date.
- *Warning: Potassium hydroxide is corrosive.*

7.7.2.14. Physiological saline, 8.5 g/l (0.85% w/v) solution (isotonic saline)

- Sodium chloride (NaCl)8.5 g
- Distilled waterq.s. 1000ml
- Label the volumetric flask “SODIUM CHLORIDE 0.85% SOLUTION” and write the date.

7.7.2.15. Trisodium citrate, anticoagulant

To make about 100 ml:

- Trisodium citrate, anhydrous (Na₃C₆H₅O₇)..... 3.8 g
- Distilled waterq.s. 100ml
- Dissolve and Keep in the refrigerator.

Use 1 ml of the solution per 4 ml of blood. Label the volumetric flask “TRISODIUM CITRATE 3.8% SOLUTION” and write the date.

**Self-Check -7****Written Test**

Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page:

- Which of the following is solid solution?
A. alloys
B. air
C. normal saline
D. 70% alcohol
- Which are the possible physical states of solutes and solvents in gaseous solutions respectively
A. Solid- gas
B. liquid – gas
C. gas- liquid
D. gas - gas
- _____ Is/are stains in which the colouring substance is contained in the acidic part of the stain and the base part is colorless
A. Acidic Stains
B. Basic Stains
C. Neutral Stains
D. Simple stains
- _____ is a solution in which the concentration of a given chemical is precisely known
A. reagent solution
B. staining solution
C. standard solution
D. buffer solution
- when comparing Stock solution with Working solution, Stock solutions are:
A. concentrated reagent solution
B. Diluted to prepare a working solution.
C. Has a shorter shelf life
D. occupies less space in storage
- _____, There is no need to use expensive volumetric glassware when preparing stains
A. True
B. false
- Which of the following is true during preparing accurate solutions?
A. Use a balance of low sensitivity
B. Weigh the chemical as accurately as possible.
C. The chemical should be laboratory reagent grade.
D. Hygroscopic and deliquescent chemicals need to be weighed slowly
- A chemical which absorbs water from the air but does not dissolve in the water it absorbs is called _____



- A. Guaranteed
- B. Analytical
- C. Deliquescent
- D. Hygroscopic

Note: Satisfactory rating - 3 points

Unsatisfactory - below 3 points

Answer Sheet

Score = _____

Rating: _____



Information Sheet-8

Labeling and storage of reagents

8.1. Definition

- **Label** is An item used to identify something or someone with a piece of paper, card, or other material attached to an object to identify it or give instructions or details concerning its ownership, use, nature, destination, etc.; **tag**
- Proper labeling is fundamental to a safe and effective laboratory operation. Reagents created in the laboratory also require labeling.
- All purchased reagent chemicals should be labeled with
 - ✓ Chemical name
 - ✓ date received
 - ✓ date of initial opening
 - ✓ shelf-life
 - ✓ hazard warnings
 - ✓ Storage classification location
 - ✓ Name and address of manufacturer
 - ✓ Reagent _____
 - ✓ Lot # _____
 - ✓ Concentration _____
 - ✓ Storage Temp _____
 - ✓ Open/Prep Date _____
 - ✓ Expiration Date _____
 - ✓ Prepared by _____
 - ✓ Location _____
 - ✓ Precautions _____
- All reagents created in the laboratory should be labeled with -
 - ✓ chemical name and formula
 - ✓ concentration
 - ✓ date prepared
 - ✓ name of person who prepared the reagent
 - ✓ storage condition
 - ✓ hazard warning label (available from a safety supplier)
 - ✓ Reference to original source of chemical (e.g., manufacturer, which jar, etc.)



8.2. Storage of Solution

- storing reagents incorrectly are important causes of unreliable test results
- Reagents should be stored in a clean, cool, dry location to maximize shelf life.
- Refrigerate reagents only if indicated on the label.
- Reagents that are stored at elevated temperatures or in humid locations may experience accelerated degradation and reduced shelf life
- Storage requirements are to be indicated on container labels:

A. Temperatures

- Store reagents at required temperatures indicated by manufacturer
- Reagents with temperature storage requirements shall have these listed on the labels.
- Store reagents that must be refrigerated or frozen in freezers or Refrigerators with the required temperature ranges.

B. Light sensitivity

- If indicated store in cabinets or in a dark container.
- Secure storage areas against unauthorized removal of chemicals.
- Where possible, storage areas should have two separate exits.
- Maintain clear access to and from the storage areas.
- Do not store chemicals in aisles or stairwells, on desks or laboratory benches, on floors or in hallways, or in fume hoods.
- Use an appropriate "Acid Cabinet" for any acid solutions of 6 M concentration or higher. Nitric acid needs to be isolated.
- Label storage areas with a general hazard symbol to identify hazardous chemicals and indicate correct fire fighting procedures.
- File a Material Safety Data Sheet (MSDS) for every chemical stored in the laboratory.
- Store all reagent chemicals in compatible family groups. Do not alphabetize.
- Store all chemicals at eye level and below. The preferred shelving material is wood treated with polyurethane or a similar impervious material.
- All shelving should have a two-inch lip. If you use shelving with metal brackets, inspect the clips and brackets annually for corrosion and replace as needed.
- Store chemical reagents prepared in the laboratory in plastic bottles (if possible and appropriate to the chemical) to minimize the risk of breakage.
- Date containers upon receipt and again when opened.
- Attach chemical labels with all necessary information to all containers.

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- When opening newly received reagent chemicals, immediately read the warning labels to be aware of any special storage precautions such as refrigeration or inert atmosphere storage.
- Test peroxide-forming substances periodically for peroxide levels; dispose of these substances after three months unless the MSDS for the substance indicates a longer shelf life.
- Check chemical containers periodically for rust, corrosion, and leakage.
- Store bottles of especially hazardous and moisture-absorbing chemicals in chemical-safe bags.
- Maintain a complete inventory in the room where the chemicals are stored, and make a copy available to fire fighters.
- Keep storage areas clean and orderly at all times.
- Have spill cleanup supplies (absorbents, neutralizers) in any room where chemicals are stored or used.
- Limit the amount of flammable and combustible materials stored to that required for one year of laboratory work.
- Use only metal flammables cabinets to store flammable and combustible liquids. Label the cabinets *FLAMMABLE - KEEP AWAY FROM FIRE*.



Self-Check -8	Written Test
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Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page:

1. Giemsa is light sensitive reagent and it needs to store dark or brown bottle.

A. True	B. false
---------	----------
2. Which of the following is true about Storage of prepared laboratory solution?
 - A. Store reagents in completely clean containers
 - B. use brown bottles for storing light sensitive reagents
 - C. Don't Label the container If a reagent is Harmful
 - D. Protect all reagents from sunlight and heat
3. Which information is should be labeled on laboratory prepared reagents

A. chemical name	C. date prepared
B. concentration	D. all of the above

Note: Satisfactory rating - 3 points

Unsatisfactory - below 3 points

Answer Sheet

Score = _____
Rating: _____

Name: _____

Date: _____



9.1. Laboratory Solutions Register

- All solutions prepared at Lab are recorded, by solution type, into the Laboratory Solutions Register. This register contains all the solution identification details that are recorded on the label, as well as the details of the preparation of the solution.
- Each solution is identified as either a primary or a secondary standard in the Laboratory Solutions Register.
- If the solution is a primary standard, then all the raw data used to calculate the concentration of the solution is recorded in the Laboratory Solutions Register. This information includes:
 - ✓ details, including batch numbers, of the reagents used to make up the solution
 - ✓ the amount of reagent (volume or mass) used to make up the solution
 - ✓ the final volume of the solution.
- In the case of a primary standard, the label and the Laboratory Solutions Register provide a comprehensive record of the solution.
- However, when a secondary solution is prepared, a Standardized Solution Sheet is used to record the titration results and the calculations of the concentration of the solution. The solution identification details are then recorded on the label and in the Laboratory Solutions Register. The solution identification number is included in the Standardised Solution Sheet for tracking purposes.



Self-Check -9

Written Test

Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page:

- 1. All solutions prepared at Lab are recorded, by solution type, into the Laboratory Solutions Register.
 - A. True
 - B. False
- 2. This register contains all the solution identification details that are recorded on the label, as well as the details of the preparation of the solution.
 - A. True
 - B. False

Note: Satisfactory rating - 3 points

Unsatisfactory - below 3 points

Answer Sheet

Score = _____
Rating: _____

Name: _____

Date: _____



Operation Sheet 1	Prepare Giemsa stock solution
--------------------------	--------------------------------------

Necessary Materials:

To make about 500 ml:

- Giemsa powder 3.8 g
- Glycerol (glycerin)..... 250 ml
- Methanol (methyl alcohol)..... 250 ml
- Materials & Equipment
- Balance Funnel
- Weighing paper Labeling Marker
- Measuring Cylinder Plastic Adhesive Tape
- Reagent bottle (Brown bottle)
- Spatula (spoon)
- Glass Beads
- Filter Paper

Procedure:

Step1. Weigh 3.8 gms of Giemsa on a piece of clean paper (pre weighed), and transfer to a dry brown bottle of 500 ml capacity which contains a few glass beads.

✓ Note: Giemsa stain will be spoiled if water enters the stock solution during its preparation or storage.

Step2. Using a dry cylinder, measure the methanol, and add to the stain. Mix well.

✓ **Caution:** Methanol is toxic and highly flammable; therefore handle it with care and use well away from an open flame.

Step3. Using the same cylinder, measure the glycerol, and add to the stain. Mix well.

Step4. Place the bottle of stain in a water bath at 50–60 °C, or if not available at 37 °C, for up to 2 hours to help the stain to dissolve. Mix well at intervals.

Step5. Label the bottle, and mark it Flammable and Toxic. Store at room temperature in the dark. If kept well-stoppered, the stain is stable for several months.

✓ For use: Filter a small amount of the stain into a dry stain dispensing container.



Operation Sheet 2

Prepare *Giemsa working Solution (10% Giemsa solution)*

Necessary Materials

- Giemsa stock solution
- Buffered Water(PH7.2)
- Measuring Cylinder
- Empty container

Procedure

To prepare about 100ml

Step1. Pour 10 ml of filtered Giemsa stock in the measuring cylinder

Step2. Add 90 ml of buffered water (PH 7.2) in the measuring cylinder.

Step3. Mix the stain well.

Step4. Filter before using.

- ✓ NOTE: Reagent Stability and Storage: Giemsa working solution is stable for 12 hours but it depends on the climatic condition of an area so there should be time adjustment according to that specific area to maintain the stability of working solution of Giemsa. It is light sensitive reagent and it needs to store dark or brown bottle



Operation Sheet 3	Prepare <i>EDTA anticoagulant</i>
--------------------------	--

- Necessary Materials
 - ✓ To Make about 250ml
 - ✓ Di-potassium ethylene- diamine-tetra-acetic acid (K₂ –EDTA)... 2.5 g
 - ✓ Distilled water..... 25 ml
- **Necessary Tools &Equipment:**

<ul style="list-style-type: none"> ✓ Balance ✓ Funnel ✓ Weighing paper ✓ Micropipette ✓ Measuring Cylinder 	<ul style="list-style-type: none"> ✓ Test tubes ✓ Reagent bottle (Brown bottle) ✓ Spatula (spoon) ✓ Labeling Marker ✓ Plastic Adhesive Tape
---	--

Procedure:

Step1. Weigh 2.5gms Di-potassium ethylene- diamine-tetra-acetic acid, and transfer it to a small glass bottle.

Step2. Measure 25 ml of water; add to the chemical, and mix to dissolve. Label the bottle.

Step3. For use, pipette 0.04 ml of the reagent into small bottles marked to hold 2.5 ml of blood.

Step4. Place the small bottles without tops, on a warm bench for the anticoagulant to dry. Protect from dust and flies.

Step5. When dry, replace the bottle tops, and store ready for use



Operation Sheet 4

Prepare 70% alcohol

Materials

- Stock ethyl alcohol
- Distilled water
- Funnel
- Measuring Cylinder
- Empty container
- PPE (glove, gown, etc.)
- Safety box

Precautions:

Ethanol and methanol are highly flammable, therefore use well away from an open flame which can endanger the life of trainees and any other health work force who are working in the skills laboratory/laboratory where the trainees are practice this task.Hence, use universal precautions when handling ethanol or other material. In the event of an exposure, administer first aid immediately, notify your manager or supervisor and seek prompt medical attention. First aid includes washing cuts and needle sticks with soap and water; flushing splashes to the nose, mouth, or skin with copious amounts of water; and irrigating eyes with clean water, saline, or sterile irrigates.

Step 1- Wearing gown

Step 2- Washing your hand with soap and water

Step 3- Wearing glove

Step 4 Cleaning the working area

Step 5 Confirming the working area fit for purpose(i.e. safe to work)

Step 6 Assembling necessary materials

Step 7 Calculate the volume by $V_1 = C_2V_2/C_1$ to get initial volume

Step 8 Decide the added volume by $V_2 - V_1$

Step 9 Measure the proper volume of the ethyl alcohol

Step 10 Dispense the solution, mix

Step 11 Label the prepared solution as 70%

Step 12 Safely dispose used materials (safety precautions practiced during the procedure)



Operation Sheet 5

Prepare 0.5% sodium hypochlorite (bleach)

Materials

- Stock sodium hypochlorite
- Distilled water / buffered water
- Funnel
- Measuring Cylinder
- Empty container Safety materials and
- Safety materials and
- Disinfectant PPE (glove, gown, goggle etc.), Safety box, Dust pin

Precautions:

Sodium hypochlorite is hazardous; therefore handle it with care and use well away from an open flame in skills/laboratory where the trainees are practice this task.

Hence, use universal precautions when handling of the chemicals. In the event of an exposure, administer first aid immediately, notify your manager or supervisor and seek prompt medical attention. First aid includes washing cuts and needle sticks with soap and water; flushing splashes to the nose, mouth, or skin with copious amounts of water; and irrigating eyes with clean water, saline, or sterile irrigates.

Procedures

Step 1- Wearing gown

Step 2- Washing your hand with soap and water

Step 3- Wearing glove

Step 4 Cleaning the working area

Step 5 Confirming the working area fit for purpose(i.e. safe to work)

Step 6 Assembling necessary materials

Step 7- Compute the calculation to get proper volume

Step 8 - Measure the proper volume of the stock (5%

Step 9- sodium hypochlorite) and distilled water

Step 10 - Transfer each to the container, mix

Step 11- Label the prepared solution as 0.5%sodium hypochlorite and date of preparation

Step 12- Safely dispose used materials (safety precautions practiced during the procedure)



LAP Test	Practical Demonstration
-----------------	--------------------------------

Name: _____ Date: _____

Time started: _____ Time finished: _____

Instructions: Given necessary templates, tools and materials you are required to perform the following tasks within --- hour.

Task1. Prepare 1000ml Giemsa stock solution

Task2. Prepare 50ml Giemsa working Solution (10% Giemsa solution)

Task3. Prepare 10ml EDTA anticoagulant

Task4. Prepare 1L 70% alcohol

Task5. Prepare 1L 0.5% sodium hypochlorite (bleach)



This learning guide is developed to provide you the necessary information regarding the following **content coverage** and topics –

- Assembling laboratory equipments for solution preparation
- Making serial dilution
- Standardization of solutions
- determining concentration of standard solution
- Labeling and storage of standard solution

This guide will also assist you to attain the learning outcome stated in the cover page.

Specifically, upon completion of this Learning Guide, **you will be able to –**

- Assemble appropriate laboratory equipment
- perform Serial dilutions as required
- standardize the solution to the required specified range and precision
- determine the concentration of standardize solutions
- label and store Solutions to maintain identity and stability and re-standardized if require

Learning Instructions:

7. Read the specific objectives of this Learning Guide.
8. Follow the instructions described below 3 to 6.
9. Read the information written in the information “Sheet 1, Sheet 2, Sheet 3 and Sheet 4, ---” **in page ---, ---, --- and --- respectively.**
10. Accomplish the “Self-check 1, Self-check t 2, Self-check 3 and Self-check 4” ,---” **in page - --, ---, --- and --- respectively**
11. If you earned a satisfactory evaluation from the “Self-check” proceed to “Operation Sheet 1, Operation Sheet 2 and Operation Sheet 3 ” **in page ---.**
12. Do the “LAP test” **in page – ---**

Information Sheet-1	Assembling laboratory equipments for solution preparation
----------------------------	--

1.1. Introduction

- To prepare the solutions correctly the appropriate grade of glassware needs to be used.

1.2. Grades of Glassware

- There are many kinds and grades of glassware available in the Analytical (diagnostic) Laboratory. The following lists glassware that may be used.

- ✓ Hard Glass - as in combustion tubes
- ✓ Borosilicate Glass - most laboratory grade glassware is borosilicate glass
- ✓ Low Alkali Borosilicate Glass
- ✓ Heat Resistant Borosilicate Glass (eg Pyrex brand) - widely used in laboratories

A. Class A Glassware: for accuracy

burettes > volumetric flasks > pipettes > graduated cylinders > graduated beakers

(most accurate) (least accurate)

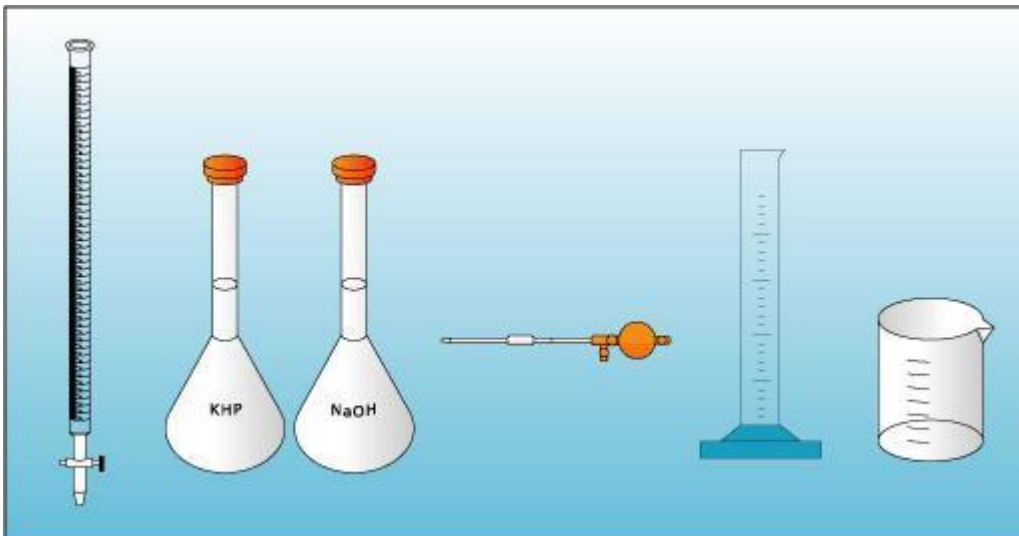


Fig:- 2.1 Class A Glassware

B. Class B Glassware: used when accuracy not critical

C. Specially Washed Glassware: for cleanliness

acid washed > Milli-Q water > distilled/deionised water > cold water > hot water

(most clean) (least clean)



Fig:- 1.2 **Specially Washed Glassware:** for cleanliness

D. Specially Treated Glassware: eg coated with silicone, Teflon, other coatings on inside; plastic film covering on outside to reduce risk of cracking. Sterile Glassware – autoclaved.

- In laboratories there are two main grades of reagents, used according to their purity.
 - ✓ **Analytical reagent grade (designated AR)** which is used for high precision work where accuracy is the most important outcome. For example, AR would be used when you wish to calculate the precise amount of a substance.
 - ✓ **Technical reagent grade** used for work where accuracy of results is secondary to the outcome. An example of use would be in the preparation of tissue stains, where the exact quantity of stain in solution is not critical for the result.
- The above information is usually found on the reagent label; however it is not a guarantee of the purity because:
 - ✓ tests for some impurities may not have been done by the manufacturer
 - ✓ the reagent may have been contaminated in the laboratory after being opened
 - ✓ The reagent may not be sufficiently dry, owing to absorption of moisture.



Self-Check -1

Written Test

Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page:

- 1. Which of the following correct sequence of Glassware from most accurate to least accurate
 - A. Burettes > volumetric flasks > pipettes > graduated cylinders > graduated beakers
 - B. Graduated cylinders > graduated beakers> pipettes > Burettes > volumetric flasks
 - C. Graduated beakers > graduated cylinders > pipettes > volumetric flasks > Burettes
 - D. Pipettes > Burettes > volumetric flasks > graduated beakers > graduated cylinders
- 2. Analytical reagent grade is used for high precision work where accuracy is the most important outcome
 - A. True
 - B. False
- 3. information found on the reagent label is not a guarantee of the purity
 - A. True
 - B. False

Note: Satisfactory rating – 1.5 points

Unsatisfactory - below 1.5 points

Answer Sheet

Score = _____
Rating: _____

Name: _____

Date: _____



Information Sheet-2

Making serial dilution

2.1. Materials used for serial dilution

- Buffer used to dissolve the sample
- The sample
- Multiple tubes
- Pipette or graduated cylinder
- Stirring rod
- Test tube rack
- Funnel
- Stationery
- Labeling materials

2.2. Procedure

- Shake the solution by hand or use the stirring rod to swirl the solution.
- Make sure the solution is uniformly mixed.
- Determine initial dilution
- Take half of the solution out to a new tube and add equal amount of buffer into it.
- Take half of the newly made solution to another new tube and add equal amount of buffer into it.
- Calculate the dilution at each point

2.3. Considerations for making a serial dilution

- Depending on circumstances you do not necessarily have to set up the first tube containing the undiluted material
- The last tube will contain 10 mL. Usually this is not a problem as more reagent is made up than required.
- If it is a problem, simply remove exactly 1 mL of the final dilution and discard it according to the appropriate laboratory procedures.



Self-Check -2	Written Test
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Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page:

1. the volume of solution is increase in a series of test tubes through Serial dilution
 - A. true
 - B. false

2. it is necessarily have to set up the first tube containing the undiluted material in serial dilution
 - A. true
 - B. false

Note:Satisfactory rating - 1 points

Unsatisfactory - below 3 points

Answer Sheet

Score = _____
Rating: _____

Name: _____

Date: _____



Information Sheet-3

Standardization of solutions

3.1 Introduction

- Standardization is the process of determining the accurate concentration of a standard solution by titrating it against a solution of accurate concentration with high degree of purity.

3.2. **Standard solutions**

- These are solutions in which the concentration of a given chemical is precisely known
- They are used to determine the value of an identical chemical with unknown concentration of a given solution.
- Chemicals that are used to prepare these solutions should be of analytical grade.
- Since poor standard solutions cause errors in the estimation of the intended substances, their accurate preparation is of utmost importance in order to obtain accurate and precise laboratory findings in medical laboratories

3.3. **Classification of standard solutions**

3.3.1. **Primary standard solution**

- Primary standard solution is a chemical solution that has the highest purity and can be used directly for the exact measurement of substances of unknown concentration in a given solution. These solutions include sodium chloride, sodium bicarbonate, potassium iodide, etc.
- Primary standard solution should be made of substances that are:
 - ✓ Free of impurities,
 - ✓ Stable on keeping in solid state and in solution,
 - ✓ Able to be accurately weighed or measured to give a solution of exactly known concentration,
 - ✓ Not hygroscopic (does not absorb moisture) and vaporize at 20^oc.

3.3.2. **Secondary standard solutions**

- Secondary standard solutions are solutions of lower purity and their concentrations are determined by comparison to primary standard solutions. Secondary standard solutions are used for analytical procedures after their concentration is already determined. Some examples of these solutions are nitric acid, hydrochloric acid, sulfuric acid, etc.
- In the preparation of secondary standard solutions, the following points should be taken into consideration:



- ✓ Using analytical balance for weighing
- ✓ Dissolving the weighted substance in the prescribed volume of solvent
- ✓ Determining the exact concentration by comparison against a primary standard solution
- ✓ Diluting stock secondary standard solutions using exact measurements.

3.4. Prepare standard solution

- Your ability to prepare reagents (solutions) containing the correct constituents in the correct concentrations is a competency that is critical to laboratory performance. Many other activities of the laboratory rely on the correct preparation of laboratory reagents.
- There are two methods of preparing **standard solutions**.
 1. By direct weighing of a pure reagent and making up a known volume of solution.
 2. By preparing a solution of approximately the required concentration and standardizing against a reference material.
- While the first method is simple and can be used in many cases, many standard solutions cannot be prepared in this way. If, for example, we attempt to prepare a standard sodium hydroxide (NaOH) solution by weighing out pure sodium hydroxide, dissolving it in water and making up the volume, we would find that solid NaOH reacts rapidly with moisture and carbon dioxide in the atmosphere, making it difficult to handle and keep pure. The result is an uncertainty in the concentration of the solution. In such cases it is necessary to prepare a solution of approximate concentration as best we can, then standardize the solution against a reference material. A pure reference material that can be readily titrated is required for the standardization.
- Examples of activities that rely on appropriately prepared reagents include:
 - ✓ sample preparation
 - ✓ sample storage
 - ✓ mobile phases in liquid chromatography
 - ✓ dilution of cells such as bacteria or red blood cells
 - ✓ titration of unknown samples
 - ✓ Staining of specimens.

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- In the modern laboratory many reagents can be purchased 'off-the-shelf' in a ready-to-use format. However, you may still be asked to make up solutions or even to test that the ready-to-use reagents are indeed at the correct concentration.

Self-Check -3	Written Test
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Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page:

Secondary standard solutions are used for analytical procedures before their concentration is determined true false

- Which of the following is true about Primary standard solution?
 - Free of impurities
 - Stable in solid state and in solution
 - Able to be accurately weighed
 - Should be hygroscopic
- Standardization is the process of determining the accurate concentration of a standard solution
 - True
 - false
- Primary standard solution is a chemical solution that has the highest purity
 - True
 - false
- Secondary standard solutions are solutions of lower purity and their concentrations are determined by comparison to primary standard solutions
 - True
 - false

Note: Satisfactory rating - 5 points

Unsatisfactory - below 5points

Answer Sheet

Score = _____
Rating: _____

Name: _____

Date: _____

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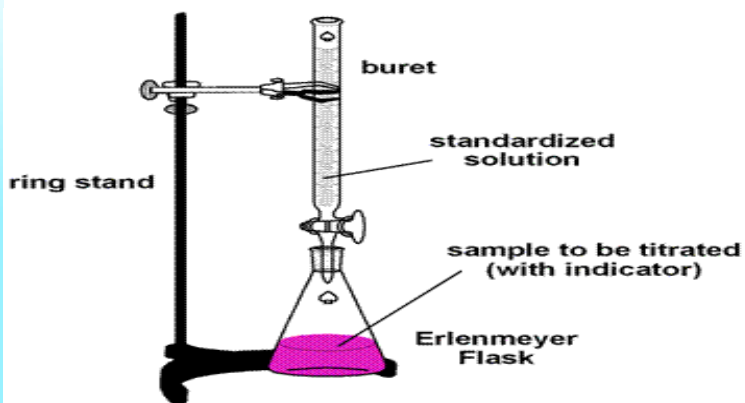
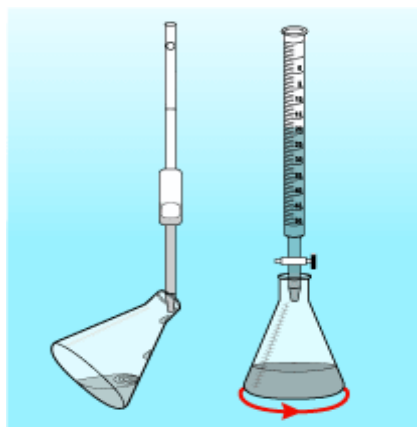


4.1. Standardizing solutions

- The process of determining the unknown's concentration
- Why standardizing a solution?
 - ✓ To find the precise concentration of the solution.
 - ✓ To quantify the purity of chemicals or double check it.
- A volumetric analysis (standardization) often can be done through **titrations** between two different chemicals, usually an acid or a base

4.2. Titration

- Titrations are very valuable laboratory procedures, the main purpose of which is to determine the concentration of some type of unknown solution. The most common type of titrants is acids and bases. Concentrations of other types of solutions may also be calculated by means of titration, but this method is more advanced and beyond the scope of this unit. One can determine the concentration of a known acid or base in a solution and, from this, determine the pH of the unknown solution. This is very useful, especially in the analytical laboratory.
- In a titration, a solution of known concentration (titrant) is added to the solution of unknown concentration. This is done in such a way that the volume of solution that is added can be measured very accurately. The known solution should react with the unknown solution. For example, if the unknown solution is a base, the titrant should be an acid. The reaction is carried out to completion (until all of the unknown solution is reacted). The technician knows the reaction is complete when the solution changes colour, because of the addition of an indicator. By using stoichiometry, the technician can calculate how much of the unknown was present, and therefore calculate the concentration of the unknown solution.
- The simplest titrations are monoprotic acids and bases, such as:
$$\text{NaOH(aq)} + \text{HCl(aq)} \longrightarrow \text{NaCl(aq)} + \text{H}_2\text{O(aq)}$$
- Indicators are special chemicals that change colour, depending on the pH of the solution they are in. Indicators are a vital part of the titration process, since they signal the completion of the titration. Different indicators are used, depending on the pH range of the titration.
- The titrant should be (if possible) a strong acid or strong base to ensure a large pH change at the neutral point so that the indicator will change colour for a sharp end point.



pH Range	Indicator
0 - 1.6	Methyl Violet
2.9 - 4.0	Methyl Yellow
3.0 - 4.6	Bromophenol Blue
3.2 - 4.4	Methyl Orange
4.8 - 6.0	Methyl Red
5.5 - 8.0	Litmus
6.0 - 7.6	Bromothymol Blue
6.6 - 8.0	Phenol Red
8.2 - 10.6	Phenolphthalien
9.4 - 10.6	Thymolphthalien
10.0 - 12.0	Alizarin Yellow

- The following diagrams show the different colours of a solution containing phenolphthalien, at stages doing a titration.



Acidic Solution

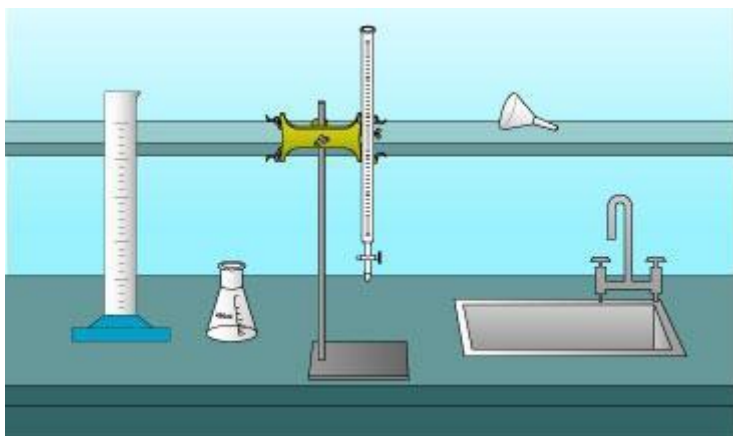


Endpoint



Basic Solution

4.3. Equipment used

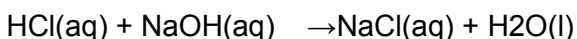


- In a titration, a burette is used to deliver a measurable amount of a solution with a known concentration to a known volume of the solution with an unknown concentration contained in an Erlenmeyer flask. The Erlenmeyer flask is used because it can be swirl without spilling any of the contents.
- An indicator with a suitable pH range must be selected and added to the flask before the titration begins, so that the reaction completion may be detected.
- A graduated cylinder or bulb pipette is used to measure a known volume of the solution with the unknown concentration to the flask. The unknown solution is then reacted with just enough titrant to react completely. The volume of titrant used is noted, and the technician uses the stoichiometry of the reaction and the SOP to do the calculations and determine the concentration of the unknown solution.

4.4. Titration- Reaction and Calculation

- Performing a titration is completely pointless if you do not understand the stoichiometry of the reaction. Understanding the reaction is the starting point for correct calculations.
- Look at the following reaction:

Strong Acid + Strong Base → Neutral solution (pH = 7.0)



- It is evident from the equation that one mole of HCl reacts with one mole of NaOH producing one mole of common salt and one mole of water as products. Thus a simple calculation:
 ✓ E.g. If 20 ml of 0.1M HCL reacts with exactly 20 ml of NaOH what is the molarity of the NaOH?
- A useful equation that can be used when calculations are not as simple as this example is:



$$C = \frac{n}{V} = \frac{m}{MrV}$$

- This equation can be modified to suit a number of different purposes.

A more complex calculation

Weak Acid + Strong Base → Weak Base + Water

(pH > 7.0; in these situations equilibrium will be achieved slightly above neutrality)

- ✓ For example, in a titration: 10.25 mL of 0.25M NaOH neutralises 25 mL of a solution of CH₃COOH. What is the molarity of the CH₃COOH? This example is worked through in the following five steps.

Step1.

What is the reaction? Choose the correct reaction from those listed below.

- 2NaOH(aq) + CH₃COOH(aq) → CH₃COONa(aq) + H₂O(l)
- NaOH(aq) + CH₃COOH(aq) → CH₃COONa(aq) + H₂O(l)
- NaOH(aq) + 2CH₃COOH(aq) → CH₃COONa(aq) + H₂O(l)
- NaOH(aq) + CH₃COOH(aq) → CH₃COOH₂O(aq) + Na
- NaOH(aq) + CH₃COONa(aq) → CH₃COOH(aq) + H₂O(l)

Step2.

In the previous reaction, how many moles of NaOH react with how many moles of CH₃COOH? Choose the correct ratio from those listed below.

- 2:1
- 1:2
- 1:1.5
- 1.5:1
- 1:1

Step3.

Calculate the number of moles that were present in the titrant NaOH.

$$C = \frac{n}{V}$$

$$\text{Therefore } n = C \times V$$

$$n(\text{NaOH}) = C(\text{NaOH}) \times V(\text{NaOH}) \quad C(\text{NaOH}) = 0.25\text{M}$$

$$V(\text{NaOH}) = 10.25 \text{ mL} = 0.01025 \text{ L} \quad n(\text{NaOH}) = 0.25 \times 0.01025$$

$$= 0.00256 \text{ Moles}$$

Step4.

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Each mole of NaOH reacted with one mole of CH₃COOH. Therefore there were 0.00256 moles of CH₃COOH in the 25 mL volume.

Step5.

Calculate the number of moles of CH₃COOH in one litre of the CH₃COOH solution and this gives the molarity of the CH₃COOH.

$$n(\text{CH}_3\text{COOH}) = 0.00256 \text{ mol}$$

$$V(\text{CH}_3\text{COOH}) = 25 \text{ mL} = 0.025 \text{ L}$$

$$C(\text{CH}_3\text{COOH}) = \frac{n(\text{CH}_3\text{COOH})}{V(\text{CH}_3\text{COOH})} = \frac{0.00256}{0.025 \text{ L}} = 0.10 \text{ M}$$

- ✓ NOTE: when there is a 1:1 molar ratio between the reactants the following equation can be used to simplify the calculation. $C_1V_1 = C_2V_2$
- ✓ Thus as long as you have three of the four values, the fourth can be calculated rapidly.
- ✓ One final example:

Strong acid + Weak Base Weak Acid + Water

- (pH < 7.0; in these situations equilibrium will be achieved slightly below neutrality)
- In a titration, 72 mL of 2.2M NH₄OH neutralises 15.74 mL of HCl (the strong acid titrant). Calculate the molarity of the HCl solution. Can you calculate the molarity of the HCl? The answer is **10.1M**, a concentrated acid indeed!

Step1. Assemble appropriate laboratory equipment

- Volumetric analysis involves the use of standard solutions and standardised solutions. A standard solution has an accurately determined concentration, whereas a standardised solution is one that has its concentration determined by titration against a standard solution.
- The chemical used to make up the standard solution must be cheap, readily available and stable, or it will not be suitable as a standard solution.
- The standardisation and use of volumetric solutions involves weighing, dilution and titration, using an appropriate end-point indicator.

Step2. Perform serial dilutions as required

- Serial dilutions are made in a sequential manner with the dilution factor between each sample in the series equal. The dilution factor may be any magnitude but the most often used are $\frac{1}{2}$ and $\frac{1}{10}$.

Step3. Standardize the solution to the required specified range and precision

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5.1. Labeling of Reagents

- Purpose of labeling. Workplace reagent labeling primarily serves two purposes, to:
 - ✓ identify the contents of the container
 - ✓ Warn of hazards.
- Reagent labeling is a complement to other sources of information such as the MSDS and other labeling requirements. It aims to assist with the safer use of a substance by identifying hazards likely to be associated with the use of the substance.
- **Responsibility:-** Chemical suppliers and employers have the primary responsibility to ensure that in the workplace, hazardous substances are correctly labeled.
- Employers must ensure that:
 - ✓ chemicals are appropriately and correctly labeled
 - ✓ labeling is not removed or modified
 - ✓ Decanted substances are labeled
 - ✓ There are prescribed measures for lost labels and unknown substances.
- **Workplace labels:** Hazardous substances must be labeled to show
 - ✓ contents
 - ✓ significant hazards
 - ✓ complementing other information (including MSDS information such as directions for use, first aid and emergency procedures)
 - ✓ Date opened.
- **Scope:** Workplace labels are required for containers containing:
 - ✓ hazardous substances
 - ✓ drugs and poisons
- And labeling is also required for:
 - ✓ decanted hazardous substances, not for immediate use
 - ✓ items (and substances) that can produce hazardous substances in use
 - ✓ Containers not cleaned.
- **Lost Labels:-** If the label is lost and the contents are unknown, the container should be:
 - ✓ marked **CAUTION DO NOT USE: UNKNOWN SUBSTANCE**
 - ✓ stored in isolation until the contents can be identified
 - ✓ If contents cannot be identified, the contents should be suitably disposed of (with advice from relevant authorities).
- **Replacement of labels:** A new label must be issued and placed on the container when:



- ✓ the substance changes (including new ingredients)
- ✓ new information becomes available that affects the information provided on the label (often instigated through a change of MSDS)
- ✓ A new expiry date (if used) is required

Each solution is identified as either a primary or a secondary standard in the Laboratory Solutions Register.



Operation Sheet 1	Make serial dilution
--------------------------	-----------------------------

To carry out successive 1/10 dilutions, 5 times

Step1. Set up seven clean tubes that hold about 20 mL as you will need to leave some room for mixing.

Step2. Carefully label each tube with the concentration and name of the reagent.

Step3. Leaving the first tube empty carefully pipette 9 mL of the diluent into each of the remaining five tubes

Step4. Carefully pipette 10 mL of the solution to be diluted into the first tube.

Step5. Using a 1 mL pipette carefully transfer 1 mL of the starting solution into the second tube.

Step6. Discard the pipette and using a fresh pipette, mix the contents in the second tube by pipetting up and down ten times. You now have a solution that is 1/10 the strength of the starting solution in the second tube.

Step7. Using the same pipette carefully transfer 1 mL from the second tube to the third tube and repeat step 6. You now have a 1/100 dilution of the starting solution in the third tube.

Repeat steps 6 and 7 until you have finished the dilution series.



Procedures

Preparation of a 0.1 Molar Sodium Hydroxide Solution

- This procedure describes the make-up of a sodium hydroxide (NaOH) solution to approximately molar concentration (0.1M).
- All volumetric glassware used in this procedure is Class A. Standard PPE should be worn when performing this work.

Step1- Locate the 6M sodium hydroxide stock solution in the laboratory store (**CAUTION: sodium hydroxide is very corrosive**)

Step2. Prepare an approximate 0.1M solution by diluting 20 mL of the 6M stock solution to 1 liter with distilled water. This can be safely done using a 50 mL measuring cylinder and a 1 liter graduated beaker.

Step3. Transfer the contents to a plastic bottle for storage and mix the contents well.

Preparation of Potassium Hydrogen Phthalate Standard Solution:

Step1. Place 50.1 g of KHP (Analytical Reagent Grade) in a weighing bottle and dry in an oven at 110 C for 2 hours. Store the KHP in a desiccator after this time.

Step2. Weigh the weighing bottle to 4 decimal places on the analytical balance.

Step3. Quantitatively transfer the KHP to a 250 mL volumetric flask.

Step4. Reweigh the weighing bottle and find the weight of the KHP to 4 decimal places, m (KHP).

Step5. To the volumetric flask, add 100 to 150 mL of distilled water (you may use a wash bottle) and swirl until the KHP dissolves.

Step6. Dilute to the mark with distilled water, replace the stopper and mix the contents by inverting and swirling the flask a number of times.

Step7. Calculate the molarity of the standard KHP solution, M (KHP), to 4 decimal places: $M(\text{KHP}) = m(\text{KHP}) \times 0.0196$

Standardization of 0.1M Sodium Hydroxide Solution

Step1. Using a bulb pipette, quantitatively transfer a 25 mL aliquot of the standard KHP solution to a 250 mL Erlenmeyer flask. Wash down the inside of the flask with about 50 mL of distilled water delivered from a wash bottle.

Step2. Add 2 drops of phenolphthalein indicator and mix well.

Step3. Carefully fill a 50 mL burette with the prepared sodium hydroxide solution

Step4. Use a magnetic stirrer to stir the solution during the titration process.



Step5. Titrate the KHP solution to the first sign of a permanent pink end point (use a white tile beneath the Erlenmeyer flask during the titration). Record the titre to the nearest 0.01 mL.)

Step6. Repeat the titration (steps 1 to 3) until three titers are obtained that agree within 0.10 mL. Average these readings, T (NaOH).

Step7. Calculate the molarity of the sodium hydroxide solution M (NaOH) to 4 decimal places:

$$\frac{M(\text{KHP}) \times 25}{M(\text{NaOH})} = T(\text{NaOH})$$

$$M(\text{NaOH}) = \frac{M(\text{KHP}) \times 25}{T(\text{NaOH})}$$

Where T (NaOH) is the average of the 3 titres in mL.



LAP Test	Practical Demonstration
-----------------	--------------------------------

Name: _____ Date: _____

Time started: _____ Time finished: _____

Instructions: Given necessary templates, tools and materials you are required to perform the following tasks within --- hour.

Task 1. Perform successive 1/2 dilutions, 5 times

Task 2. Perform Standardization of 0.1M Sodium Hydroxide Solution



Instruction Sheet

LG41: Monitor the quality of laboratory solutions

This learning guide is developed to provide you the necessary information regarding the following **content coverage** and topics –

- Methods of checking the quality of solution
- Monitoring the quality of stored solution
- Recording quality monitoring

This guide will also assist you to attain the learning outcome stated in the cover page.

Specifically, upon completion of this Learning Guide, **you will be able to –**

- check the quality of prepared solution before use
- monitor the quality of stored solution
- record quality monitoring details

Learning Instructions:

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described below 3 to 6.
3. Read the information written in the information “Sheet 1, Sheet 2, Sheet 3 and Sheet 4, ---” in page ---, ---, --- and --- respectively.
4. Accomplish the “Self-check 1, Self-check t 2, Self-check 3 and Self-check 4” , ---” in page --, ---, --- and --- respectively
5. If you earned a satisfactory evaluation from the “Self-check” proceed to “Operation Sheet 1, Operation Sheet 2 and Operation Sheet 3 ” in page ---.
6. Do the “LAP test” in page – ---

Information Sheet-1	Methods of checking the quality of solution
----------------------------	--

1.2. Introduction

- Quality check up can be performed in two ways
 - ✓ Physically
 - ✓ Chemically

1.2.1. Checking quality of solution Physically

- This is done by Checking reagents for visual deterioration and observing expiry date

Reagents must be visually inspected for

- | | |
|---|---|
| <ul style="list-style-type: none"> ✓ loudiness/ turbidity ✓ olor change ✓ particulate matter ✓ olume ✓ ontainer ✓ xpiration | <ul style="list-style-type: none"> ✓ C
abel ✓ C
vaporation ✓
eakage ✓ V
topper ✓ C
torage condition ✓ E
dulteration/Contamination |
|---|---|

1.2.2. Checking quality of solution chemically

- In each day of use, one must confirm that the reagents react as expected when used as described in the laboratory's procedure manual.
- If a reagent does not give the expected result, it is a sign of deterioration.
 - ✓ Change in staining xics
 - ✓ alteration of PH
 - ✓ poor preservation
 - ✓ Solute concentration (isotonicity)
 - ✓ Stated parameters of performance (absorbance, controls...)

**Self-Check -1****Written Test**

Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page:

1. No matter how the solution is prepared and stored, it will deteriorate over time
 - A. True
 - B. False
2. The Quality of solutions can be check up by
 - A. Physically
 - B. Chemically
 - C. all

Note: Satisfactory rating - 3 points

Unsatisfactory - below 3 points

Answer Sheet

Score = _____

Rating: _____

Name: _____

Date: _____



Information Sheet-2

Monitoring the quality of stored solution

2.1. Check solutions for visual deterioration and expiry date.

- A carefully prepared solution will only be viable for a certain period of time. No matter how the solution is prepared and stored, it will deteriorate over time. Deterioration can be caused by many factors.
- The following factors can reduce the quality of laboratory solutions.
 - ✓ **Incorrect Storage** - temperature, light and cleanliness are all factors here
 - ✓ **Chemical Contamination** - caused by sloppy procedures
 - ✓ **Microbial Contamination** - reagents may be autoclaved to avoid this
 - ✓ **Chemical Instability** - unstable reagents may break down or react to form other chemicals
 - ✓ **Calculation Error** - not a cause of deterioration necessarily, but a significant quality concern
 - ✓ **Precipitation** - reagent components may precipitate out of solution and sometimes adhere strongly to the interior of the container (eg protein solutions) thus reducing the molarity of the solution.
- Many of the factors mentioned previously can be controlled by 'shelf life'. If it takes six months for a reagent to deteriorate to a point where it is no longer usable, then putting a shelf life of three months on the container should solve the problem.
- This, of course, relies on the user checking solutions for visual deterioration and expiry dates. In microbiology laboratories it is second nature to check expiry dates and then to hold reagents, growth media etc up to the light and look for evidence of microbial contamination.
- Always check expiry dates and check the solution visually for signs of deterioration.

2.2. Re standardize or dispose of dated or deteriorated solutions

- Some solutions may not need to be discarded. For example, at expiry date, a 0.1M solution of sodium hydroxide may appear as clear and as fresh as the day it was made. An alternative to disposal is to re standardize the reagent. How do you find out it is safe to use?
- Sometimes reagents are beyond help. In the previous activity, the reagent might now have a very low molarity. This may indicate that the reagent container was not airtight or that there has been contamination of the reagent, eg carbon dioxide in the atmosphere would react with the sodium hydroxide. In these situations and in analyses that are very reagent sensitive or significant, the best approach is to discard the reagent.



- Max, the Senior Technician, asks you to discard those stock solutions you determined were deteriorated. He suggests you follow the correct procedure for waste disposal contained in the OHS Manual under **SOP**

Self-Check -2	Written Test
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Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page:

- many of the factors which causes deterioration of solutions can be controlled by 'shelf life'
 - true
 - false
- The following factors can reduce the quality of laboratory solutions.
 - correct Storage
 - careful procedures
 - Microbial Contamination
 - Stable reagents
- _____ is/are not necessarily a cause of deterioration of solution, but a significant quality concern
 - Precipitation
 - Calculation Error
 - Chemical Instability
 - Incorrect Storage

Note: Satisfactory rating - 3 points

Unsatisfactory - below 3 points

Answer Sheet

Score = _____
Rating: _____

Name: _____

Date: _____

**Information Sheet-3****Recording quality monitoring****3.2 Recording quality monitoring**

- Each reagent has been given a number.
- The reagents required and their numbers are indicated in the description of each technique.
- An alphabetical list of all the reagents used, with the numbers assigned to them, their composition, methods of preparation and storage requirements appears in the Annex at the end of the manual.

Self-Check -3**Written Test**



Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page:

1. The description of quality monitoring techniques of each reagents should be recorded.
 - A. True
 - B. False

Note: Satisfactory rating - 5 points

Unsatisfactory - below 5points

Answer Sheet

Score = _____
Rating: _____

Name: _____

Date: _____

Instruction Sheet	LG42: Maintain safe work environment
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This learning guide is developed to provide you the necessary information regarding the following **content coverage** and topics –



- Safety precautions for use of laboratory equipment and hazardous chemicals/reagents
- Using Appropriate laboratory glassware and measuring equipment
- Safe work practices and using personal protective equipment
- Cleaning of splashes of chemicals
- Waste generation and safe environment
- Waste management and disposal
- Cleaning and storage of glass ware
- Reagent storage according to OHS standard

This guide will also assist you to attain the learning outcome stated in the cover page.

Specifically, upon completion of this Learning Guide, **you will be able to –**

- Apply Appropriate safety precautions for use of laboratory equipment and hazardous chemical materials
- Use appropriate laboratory glassware and measuring equipment
- use established safe work practices and PPE to ensure personal safety and that of other laboratory personnel
- cleaned up Spills by using appropriate techniques to protect personnel, work area and environment
- minimize Generation of waste and environmental impacts
- ensure The safe collection of laboratory hazardous waste for subsequent disposal
- clean and store Glassware and equipment in accordance with enterprise procedures
- store equipment and reagents as required

Learning Instructions:

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described below 3 to 6.
3. Read the information written in the information “Sheet 1, Sheet 2, Sheet 3 and Sheet 4, ---” **in page ---, ---, --- and ---** respectively.
4. Accomplish the “Self-check 1, Self-check t 2, Self-check 3 and Self-check 4” ,---” **in page - --, ---, --- and ---** respectively
5. If you earned a satisfactory evaluation from the “Self-check” proceed to “Operation Sheet 1, Operation Sheet 2 and Operation Sheet 3 ” **in page ---.**
6. Do the “LAP test” **in page – ---**



Information Sheet-1	Appropriate safety precautions are applied for use of laboratory equipment and hazardous chemical materials
----------------------------	--

1.3. Introduction

- First of all, pay attention to what you are doing and what you are working with. Always wear safety equipment when working with dangerous chemicals. Recognize any accidents immediately. Keep food and drinks out of the laboratory work area. Always read labels of chemicals carefully.
- Never do unauthorized experiments. Never work alone in laboratory. Keep your lab space clean and organized. Do not leave an on-going experiment unattended. Always inform your instructor if you break a thermometer. Do not clean mercury yourself!! Never taste anything. Never pipette by mouth; use a bulb. Never use open flames in laboratory unless instructed by TA. Check your glassware for cracks and chips each time you use it. Cracks could cause the glassware to fail during use and cause serious injury to you or lab mates. Maintain unobstructed access to all exits, fire extinguishers, electrical panels, emergency showers, and eye washes. Do not use corridors for storage or work areas. Do not store heavy items above table height. Any overhead storage of supplies on top of cabinets should be limited to lightweight items only. Also, remember that a 36" diameter area around all fire sprinkler heads must be kept clear at all times. Areas containing lasers, biohazards, radioisotopes, and carcinogens should be posted accordingly. However, do not post areas unnecessarily and be sure that the labels are removed when the hazards are no longer present. Be careful when lifting heavy objects. Only shop staff may operate forklifts or cranes. Clean your lab bench and equipment, and lock the door before you leave the laboratory.
- Never mix two unknown chemicals. Wipe spill of chemicals immediately. In case if contact with chemical, rinse it off immediately with lots of water. Never taste, smell or touch anything chemical. Coats, backpacks, etc., should not be left on the lab benches and stools. Always wash your hands before leaving lab. Learn where the safety and first-aid equipment is located (fire extinguishers, fire blankets, and eye-wash stations)
- Consider **all** chemicals as if hazardous. Only liquid be put in the lab sinks. Always pour acids into water. Never leave burners unattended. Label all materials clearly. Never pipette anything by mouth. Clean up your work area before leaving. Avoid working alone. Do not use flammable liquids near open flames.



- ✓ Treat every chemical as if it were hazardous. Make sure all chemicals are clearly and currently labeled with the substance name, concentration, date, and name of the individual responsible.
- ✓ Never return chemicals to reagent bottles. (Try for the correct amount and share any excess.) Comply with fire regulations concerning storage quantities, types of approved containers and cabinets, proper labeling, etc. If uncertain about regulations, contact the building coordinator. Use volatile and flammable compounds only in a fume hood. Procedures that produce aerosols should be performed in a hood to prevent inhalation of hazardous material. Never allow a solvent to come in contact with your skin. Always use gloves. Never "smell" a solvent!! Read the label on the solvent bottle to identify its contents. Dispose of waste and broken glassware in proper containers. Clean up spills immediately. Do not store food in laboratories.
- ✓ Do not use any equipment unless you are trained and approved as a user by your supervisor. Never eat, drink, or smoke while working in the laboratory. Shorts and sandals should not be worn in the lab at any time. If you have long hair or loose clothes, make sure it is tied back or confined
- ✓ Turn off all ignition sources and lock the doors when leaving. Properly dispose wastes and used material in appropriate containers. Never return chemicals to reagent bottles.
- ***Personal and General laboratory safety***
- ✓ Never eat, drink, or smoke while working in the laboratory. Read labels carefully. Do not use any equipment unless you are trained and approved as a user by your supervisor. Wear safety glasses or face shields when working with hazardous materials and/or equipment. Wear gloves when using any hazardous or toxic agent. Clothing: When handling dangerous substances, wear gloves, laboratory coats, and safety shield or glasses. Shorts and sandals should not be worn in the lab at any time. Shoes are required when working in the machine shops. If you have long hair or loose clothes, make sure it is tied back or confined. Keep the work area clear of all materials except those needed for your work. Coats should be hung in the hall or placed in a locker. Extra books, purses, etc. should be kept away from equipment, that requires air flow or ventilation to prevent overheating. Disposal - Students are responsible for the proper disposal of used material if any in appropriate containers. Equipment Failure - If a piece of equipment fails while being used, report it immediately to your lab assistant or tutor. Never try to fix the problem yourself because you could harm yourself and others. If leaving a lab

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unattended, turn off all ignition sources and lock the doors. Never pipette anything by mouth. Clean up your work area before leaving. Wash hands before leaving the lab and before eating.



Information Sheet-2	Using Appropriate laboratory glassware and measuring equipment
----------------------------	---

- 4.1. Introduction/definition of terms/concepts/overview/principles

- 4.2. Components/Classification/Types/parts

- 4.3. purpose/use/Importance/advantages and disadvantages

- 4.4. OHS hazards and suitable PPE

- 4.5. Necessary tools and equipments

- 4.6. Rules to follow



Information Sheet-3

Safe work practices and using personal protective equipment

3.1. Introduction

- Safe laboratory practice is based on understanding and respect, not fear; the regulations intended to help you work safely with chemical reagents. Before beginning an experiment, be sure you have this information at hand and that you understand it. Do not hesitate to consult or questions about any experiment or about the regulations.

3.2. General safety rules in the lab

- Safety goggles must be worn at all times in the laboratory.
- No eating or drinking in the laboratory.
- Never taste or touch the laboratory chemicals.
- Always wash your hands before leaving the laboratory.
- Wear proper clothing: safety glasses, closed-toed shoes, and an apron; tie long hair back and remove all jewelry.
- Always follow the written directions, and never perform an unauthorized experiment.
- Always add acid to water. This prevents the acid from spatter.
- Point heating test tubes away from others and yourself, and heat them slowly.
- Never return unused chemicals to their original containers. This prevents contamination.
- Always use a pipette bulb or a pipetter to transfer when using a pipette. Never use your mouth.
- Always use a fume hood when working with toxic substances. Never inhale fumes directly.
- Never use an open flame near flammable liquids.
- Dispose of chemicals in the designated disposal site—not in the sink or trash can.
- Use laboratory equipment for its designed purpose only
- Use warning signs to designate particular hazards
- Never put solids in the sink

3.3. Personal protective equipment (PPE)

- PPE is comprised of clothing or equipment that is used to isolate a worker from direct exposure to workplace hazards.
- It is used to provide worker health and safety.
- PPE provide adequate protection if it is properly worn and appropriately used.
- PPE examples include:
 - ✓ Partial and full body protective garments (aprons, lab coats and coveralls)

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- ✓ Headwear
- ✓ Face and eyewear (goggles and mask)
- ✓ .Gloves
- ✓ Footwear (shoe covers/boots)
- ✓ Respirators (disposable, air purifying and air supplied)
- ✓ Hearing protectors (earplugs and earmuffs)

3.4. **Guidance for the Selection and Use of Personal Protective Equipment (PPE) in Healthcare Settings**

- Types of PPE Used in Healthcare Settings
 - ✓ Gloves – protect hands
 - ✓ Gowns/aprons – protect skin and/or clothing
 - ✓ Masks and respirators– protect mouth/nose
 - ✓ Respirators – protect respiratory tract from airborne infectious agents
 - ✓ Goggles – protect eyes
 - ✓ Face shields – protect face, mouth, nose, and eyes

3.5. **Factors Influencing PPE Selection**

- Type of exposure anticipated
 - ✓ Splash/spray versus touch
 - ✓ Durability and appropriateness for the task
 - ✓ Fit

• **Gloves**

Purpose:

- ✓ To reduce the risk of staff acquiring infections from patients,
- ✓ To prevent staff from transmitting their skin flora to patients,
- ✓ To reduce contamination of the hands of staff by microorganisms that can be transmitted from one patient to another (cross-contamination)

Glove material – vinyl, latex, nitrile, other

- ✓ Sterile or non-sterile
- ✓ One or two pair
- ✓ Single use or reusable

Gloves should be worn when:

- There is a reasonable chance of hand contact with blood or other body fluids, mucous membranes, or non-intact skin,
- Performing an invasive medical procedures,



- Before handling soiled instruments, contaminated waste items or touch contaminated surfaces.
- When disposing contaminated waste items
- Handling chemicals or disinfectants



Information Sheet-4

Cleaning of splashes of chemicals

3.3 Introduction

- A chemical spill is considered to be minor only if:
 - The person who spilled it is familiar with the chemical
 - Knows the associated hazards
 - Knows how to clean up the spill safely
- The recommended steps for dealing with a minor spill include:
 - Alert coworkers, then clean up spill
 - Follow procedures for disposal of materials used to clean up spill
 - Absorb free liquids with an appropriate absorbent, as follows
 - Caustic liquids—use polypropylene pads or diatomaceous earth
 - Oxidizing acids—use diatomaceous earth
 - Mineral acids—use baking soda or polypropylene pads
 - Flammable liquids—use polypropylene pads
 - Neutralize residues and decontaminate the area.
- Anything beyond a **minor spill** and that requires help from outside of the laboratory group constitutes a **major spill**.
- Steps to deal with **major spills** include:
 - Alerting coworkers
 - Moving to a safe location and
 - Calling authorities to report the situation



3.4 Introduction

- **Waste-** is any substance or object the holder discards, intends to discard or is required to discard
- **Hazardous waste-**is a waste that is dangerous or potentially harmful to health or env't

Key facts

- Of the total amount of waste generated by health-care activities, about 85% is general, non-hazardous waste. The remaining 15% is considered hazardous material that may be infectious, toxic or radioactive. Every year an estimated 16 billion injections are administered worldwide, but not all of the needles and syringes are properly disposed of afterwards.
- Open burning and incineration of health care wastes can, under some circumstances, result in the emission of dioxins, furans, and particulate matter.
- Measures to ensure the safe and environmentally sound management of health care wastes can prevent adverse health and environmental impacts from such waste including the unintended release of chemical or biological hazards, including drug-resistant microorganisms, into the environment thus protecting the health of patients, health workers, and the general public.

Classification of waste

1. Non-hazardous waste

- Include papers, packaging boxes, plastic bags and hand paper towels etc that have no contact with hazardous materials

2. Hazardous waste

- This includes different types

Waste category	Description and examples
Infectious waste	Waste suspected to contain pathogens e.g. laboratory cultures; waste from isolation wards; tissues (swabs), materials, or equipment that have been in contact with infected patients; excreta
Pathological waste	Human tissues or fluids e.g. body parts; blood and other body fluids; fetuses
Sharps	Sharp waste e.g. needles; infusion sets; scalpels; knives; blades; broken glass
Pharmaceutical waste	Waste containing pharmaceuticals e.g. pharmaceuticals that are expired or no longer needed; items contaminated by or containing pharmaceuticals (bottles, boxes)
Genotoxic waste	Waste containing substances with genotoxic properties e.g. waste containing cytostatic drugs (often used in cancer therapy); genotoxic chemicals
Chemical waste	Waste containing chemical substances e.g. laboratory reagents; film developer; disinfectants that are expired or no longer needed; solvents
Wastes with high content of heavy metals	Batteries; broken thermometers; blood-pressure gauges; etc.
Pressurized containers	Gas cylinders; gas cartridges; aerosol cans
Radioactive waste	Waste containing radioactive substances e.g. unused liquids from radiotherapy or laboratory research; contaminated glassware, packages, or absorbent paper; urine and excreta from patients treated or tested with unsealed radionuclides; sealed sources



The major sources of health-care waste are:

- hospitals and other health facilities
- laboratories and research centers
- mortuary and autopsy centers
- animal research and testing laboratories
- blood banks and collection services
- nursing homes for the elderly

High-income countries generate on average up to 0.5 kg of hazardous waste per hospital bed per day; while low-income countries generate on average 0.2 kg. However, health-care waste is often not separated into hazardous or non-hazardous wastes in low-income countries making the real quantity of hazardous waste much higher.

Health-care activities generating waste

- Diagnosis
- Treatment
- Prevention of diseases
- Alleviation of disablement
- Associated research

Who is at Risk of laboratory waste?

- Laboratory workers
- Doctors and nurses
- Patients
- Hospital support staff
- Waste collection and disposal staff
- General public

Health risks

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Health-care waste contains potentially harmful microorganisms that can infect hospital patients, health workers and the general public. Other potential hazards may include drug-resistant microorganisms which spread from health facilities into the environment.

Adverse health outcomes associated with health care waste and by-products also include:

- sharps-inflicted injuries;
- toxic exposure to pharmaceutical products, in particular, antibiotics and cytotoxic drugs released into the surrounding environment, and to substances such as mercury or dioxins, during the handling or incineration of health care wastes;
- chemical burns arising in the context of disinfection, sterilization or waste treatment activities;
- air pollution arising as a result of the release of particulate matter during medical waste incineration;
- thermal injuries occurring in conjunction with open burning and the operation of medical waste incinerators; and
- radiation burns.

Sharps-related

Worldwide, an estimated 16 billion injections are administered every year. Not all needles and syringes are disposed of safely, creating a risk of injury and infection and opportunities for reuse.

Injections with contaminated needles and syringes in low- and middle-income countries have reduced substantially in recent years, partly due to efforts to reduce reuse of injection devices. Despite this progress, in 2010, unsafe injections were still responsible for as many as 33 800 new HIV infections, 1.7 million hepatitis B infections and 315 000 hepatitis C infections (1).

A person who experiences one needle stick injury from a needle used on an infected source patient has risks of 30%, 1.8%, and 0.3% respectively of becoming infected with HBV, HCV and HIV.

Additional hazards occur from scavenging at waste disposal sites and during the handling and manual sorting of hazardous waste from health-care facilities. These practices are

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common in many regions of the world, especially in low- and middle-income countries. The waste handlers are at immediate risk of needle-stick injuries and exposure to toxic or infectious materials.

Environmental Impact

Treatment and disposal of healthcare waste may pose health risks indirectly through the release of pathogens and toxic pollutants into the environment.

- The disposal of untreated health care wastes in landfills can lead to the contamination of drinking, surface, and ground waters if those landfills are not properly constructed.
- The treatment of health care wastes with chemical disinfectants can result in the release of chemical substances into the environment if those substances are not handled, stored and disposed in an environmentally sound manner.
- Incineration of waste has been widely practised, but inadequate incineration or the incineration of unsuitable materials results in the release of pollutants into the air and in the generation of ash residue. Incinerated materials containing or treated with chlorine can generate dioxins and furans, which are human carcinogens and have been associated with a range of adverse health effects. Incineration of heavy metals or materials with high metal content (in particular lead, mercury and cadmium) can lead to the spread of toxic metals in the environment.
- Only modern incinerators operating at 850-1100 °C and fitted with special gas-cleaning equipment are able to comply with the international emission standards for dioxins and furans.
- Alternatives to incineration such as autoclaving, microwaving, steam treatment integrated with internal mixing, which minimize the formation and release of chemicals or hazardous emissions should be given consideration in settings where there are sufficient resources to operate and maintain such systems and dispose of the treated waste.

Safe working environment

- Rules concerning access to the laboratory and displaying of safety signs and notices for staff, patients, and visitors to the laboratory
- Procedures to follow to maintain local laboratory security

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- How to keep the laboratory clean
- How to separate and dispose of general waste, broken glass and other 'sharps', contaminated materials, and different specimens
- Decontamination procedures
- Washing of reusable specimen containers, needles, syringes, lancets, slides, cover glasses, pipettes
- Disinfectants and their use in the laboratory
- Sterilization procedures
- Ventilation of the laboratory
- How to check the laboratory for structural damage and wear that may lead to accidents or make the premise less secure
- Maintenance schedules and routine cleaning of equipment
- Inspecting electrical equipment for damage to insulation and loose connections in plugs
- Rules for the storage and labeling of chemicals and reagents and how to keep an inventory of chemicals
- Regulations covering the safe packing and transport of specimens
- Procedure for the reporting of faults

Safe working practices

- Personal hygiene measures and wearing of safe footwear
- Regulations concerning the wearing, storing, decontamination and laundering of protective clothing
- Preventing laboratory acquired infection including regulations to avoid the accidental:
 - Ingestion of pathogens
 - Inhaling of pathogens

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- Inoculation of pathogens
- What to do when there is a spillage of a specimen or liquid culture
- Safety rules concerning the handling and storage of chemicals and reagents that are flammable, oxidizing, toxic, harmful, irritant, and corrosive, and how to manage chemical spillages
- What to do when there is a glass breakage
- How to pipette and dispense safely
- Safe operation of manual, electrical, and battery operated laboratory equipment
- Working tidily, use of racks, and rules to prevent the floor and benches from becoming cluttered and exits obstructed
- Use of protective gloves, goggles, face shield dust mask, eyewash bottle
- How to control noise levels and other causes of loss of concentration

Safe laboratory working environment

- The safety of the working environment must take into consideration:
- Type of work being performed, ie specimens which the laboratory handles and pathogens which may be encountered
- Working practices including the procedures and equipment used
- Number of staff and workload
- Laboratory's location, climatic conditions, and security of premise

The following are important in making the workplace safe:

- Laboratory premise that is structurally sound and in good repair with a reliable water supply and a safe plumbing and waste disposal system. Drainage from sinks must be closed and connected to a septic tank or to a deep pit. *Note:* If there is a shortage of piped water, provision must be made for the storage of water, e.g. collection of rain water in storage tanks. It is not safe for a laboratory to function without an adequate water supply

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- Adequate floor and bench space and storage areas. The overall size of the laboratory must be appropriate for the workload, staff numbers, storage and equipment requirements
- Well constructed floor with a surface that is nonslip, impermeable to liquids, and resistant to those chemicals used in the laboratory. It should be bevelled to the wall and the entire floor should be accessible for washing. The floor must not be waxed or covered with matting. Floor drains are recommended
- Walls that are smooth, free from cracks, impermeable to liquids, and painted with washable light colored paint
- When practical, a door at each end of the laboratory so that laboratory staff will not be trapped should a fire break out. Doors should open outwards and exit routes must never be obstructed. Where fitted, internal doors should be self closing and contain upper viewing panes. External doors must be fitted with secure locks
- Adequate ventilation supplied by wall vents and windows that can be opened. The windows should not face the prevailing winds to avoid excessive dust entering the laboratory in the dry season and the wind interfering with work activities. Windows should be fitted with sun blinds and insect proof screens, and when indicated secure window bars
- Sectioning of the laboratory into separate rooms or working areas. The area where blood samples are collected from patients must be away from the testing area of the laboratory. Seating should be provided for patients outside the laboratory. The specimen reception area must be equipped with a table or hatchway which has a surface that is impervious, washable, and resistant to disinfectants. There should also be a First Aid area in the laboratory containing a First Aid box, eyewash bottle and fire blanket
- Bench surfaces that are without cracks, impervious, washable, and resistant to the disinfectants and chemicals used in the laboratory. Benches, shelving, and cupboards need to be well constructed and kept free of insect and rodent infestation. Benches should be kept as clear as possible to provide maximum working area and facilitate cleaning
- Suitable storage facilities, including a ventilated locked store for the storage of chemicals and expensive equipment

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- Where required, a gas supply that is piped into the laboratory with the gas cylinder stored in an outside weatherproof, well-ventilated locked store
- A staff room that is separate from the working area where refreshments can be taken and personal food and other belongings stored safely. Near to the staff room there should be a separate room with toilet and hand-washing facilities. There should be separate toilet facilities for patients.
- A hand basin with running water preferably sited near the door. Whenever possible, taps should be operated by wrist levers or foot pedals. Bars of soap should be provided, not soap dispensers. Ideally paper towels should be used. If this is not possible small cloth hand towels that are laundered daily should be provided
- Provision of protective safety cabinets and fume cupboards as required and when feasible
- Safe electricity supply with sufficient wall electric points to avoid the use of adaptors and extension leads
- Fire extinguishers sited at accessible points. These need to be of the dry chemical type. Several buckets of sand and a fire blanket are also required
- As good illumination as possible. Low energy tube lights are recommended. Window screens must be fitted to protect from direct sunlight and glare but these should not make the working areas too dark

Provision of *separate* labeled containers for the decontamination of infected material, discarding of needles, syringes, lancets, glassware for cleaning, broken glass, and general laboratory waste. A warning symbol such as a red triangle can be used to mark containers in which infected material is placed.

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3.5 Introduction

- **Laboratory Waste management**

- The practice of collecting and disposing of the waste produced by laboratory activities

- **Why manage hazardous waste?**

- To protect human health & the environment
- To minimize the generation of hazardous waste
- To meet compliance with National and/or local Regulations
- To prevent contact with human **blood or blood products** or with certain **chemicals used in the lab**

- **Waste Management Processes**

1. Segregation
2. Packaging
3. Labeling
4. Handling, and storage of waste products
5. Transportation
6. Disposal

- **Waste minimization**

- Significant reduction of the waste generated in health-care establishments and research facilities
- These include:
 - Source reduction
 - Reuse/Recyclable products
 - Good management and control practices
 - Treat

- **Waste segregation**



- Grouping waste into different categories according to the **specific treatment** and **disposal requirements**
- Ensure that waste will be treated according to the **hazards of the waste** and the **correct disposal routes** are taken and that the **correct transportation** equipment will be used.
- Without effective segregation system, the complete waste stream must be considered as hazardous
- The correct segregation relies on a clear identification of different **categories of waste** and separate **disposal system**
- Must be done at point of generation
- **Waste Collection**
- **Color coding** of waste container for collection
 - **Yellow/Red**-infectious waste
 - **Brown**-for chemical and pharmaceutical waste
 - **Black**-for General waste
 - **White or yellow sharp container**- sharp waste

Solids waste Collection and labeling

Primary Containment:

- Collect dry, solid waste in a “**biohazard bag**”.
- The bag must have the international **biohazard symbol** and the word “**biohazard**”



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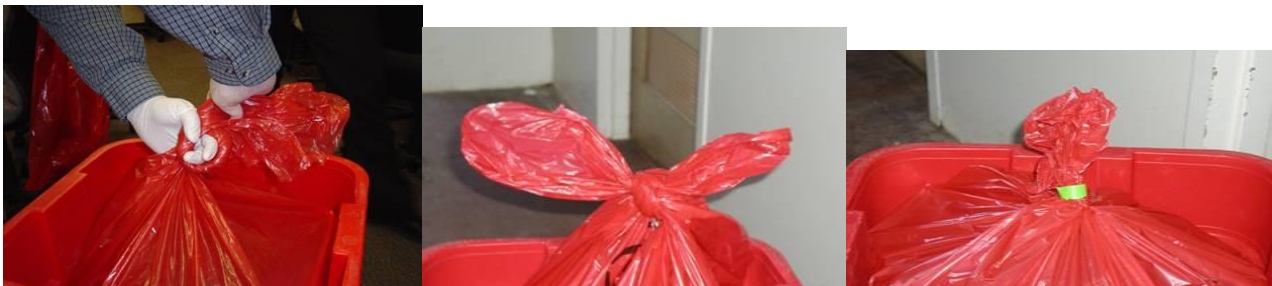
Secondary Containment:

- Rigid container with a lid that is resistant to leaks and punctures.
- The primary bag must be kept in the secondary container during use, storage, and transport.



Packaging (Bag tying)

- **Correct bag tying**
 - Twist bag into single braid.
 - Use the braid to tie single knot.
 - Tighten knot
- **Wrong bag tying**
 - Tied into bunny ears
 - Tape tied



Common problems



Bag on floor



Bag on bench

Full bags must be in secondary containment at all times

- Collect in a rigid, puncture & leak resistant and properly labeled container with the word “**Biohazardous waste**”
 - **Biological Hazard symbol**
 - Placed near workspace
 - Replace sharps containers when 3/4 full
- No mercury thermometers
- Generator information-Lab Name, Initial, Date



- **Liquid waste collection**

Storage:

- Label and secure bulk vessels if not disposed immediately

Treatment:

- Chemical disinfection



Disposal:

- Flush to sewer
- Use proper PPE!
- **Bench top, and equipment disinfection**
- For bench tops, external surfaces and laboratory equipments a freshly prepared **0.5%** sodium hypochlorite disinfectant solution is used.
- HIV is inactivated by 10 minutes exposure to 0.5% bleach and HBV by 2 minutes exposure to the same concentration.
- Depending on the concentration needed, dilutions can be made from the concentrated solutions.
- **Bleach dilutions**

$$= \frac{\% \text{ concentrated solution}}{\% \text{ diluted solution}}$$

E.g.

- To make a 0.5% dilution from 5 % concentrated solution
- Part of water to be added= $5\%/0.5\% -1=9$
- **Personal Decontamination**

Wash hands for 20-30 seconds after:

- Handling infectious materials or animals
- Removing gloves
- Before leaving lab
- **Storage, transport and spills**
- Plastic bags with sufficient strength for the collection and storage
- Sharps container for sharp wastes

- Biohazardous waste spills must be cleaned up immediately unless unsafe to do so.
- Protect personnel handling waste



Wrong waste bag handling

- **Biological Waste Treatment**

Autoclaving

- Highly infectious waste should autoclaved before transported for disposal
- Sterilize by high pressure saturated steam at 121 °C for around 15–20 minutes
- In resource limited setting can be used for sterilizing reusable materials

Chemical Inactivation/Disinfection

- Kill or inactivate the pathogens
- Is most suitable for treating liquid waste such as blood, urine, stools, or hospital sewage

Incineration

- Can destroy pathogens and toxins by high temperatures
- Reduce volume of original waste by 95+%
 - Significantly reduces amount of waste sent to landfill
- Waste converted into ash, flue gases, and heat
- Flue gases may be required to be cleaned of pollutants before released to atmosphere

Waste types not to be incinerated

- Pressurized gas containers
- Large amounts of reactive chemical waste



- Silver salts and photographic or radiographic wastes
- Halogenated plastics such as polyvinyl chloride (PVC)
- Waste with high mercury or cadmium content, such as broken thermometers, used batteries, and lead-lined wooden panels
- Sealed ampoules or ampoules containing heavy metals
- **How to Dispose of Sharps and Sharp Containers**
- Use puncture-resistant sharps containers and work practices that minimize the unnecessary handling of sharps.
- When container is three-quarter full, remove from the procedure area for disposal.
- Dispose of the sharps and sharp containers by burning, burying or encapsulating.
- Always put on a heavy duty gloves when handling sharps containers.
- **How to Dispose of Liquid Waste**
- Wear PPE including utility gloves, protective eyewear and plastic apron when handling and transporting liquid waste.
- Pour waste down a utility sink drain or a flushable toilet and rinse with water. Avoid splashing.
- If no sewage system available, dispose of liquid in a deep, covered hole, not into open drains.
- Decontaminate containers by placing them in a 0.5% chlorine solution before washing them
- Remove utility gloves, wash and dry hands or use antiseptic hand-rub as described in the guidelines.
- **How to Dispose of Solid Waste**
- Wear heavy duty or utility gloves when handling and transporting solid wastes.
- Dispose of solid wastes by placing them in a plastic or galvanized metal container with a tight fitting cover.



- Collect the waste containers on a regular basis and transport the burnable ones to the incinerator or area for burning.
- Remove gloves and wash and dry hands or use an antiseptic hand-rub.
- **How to Dispose of Hazardous Waste**
- All hazardous waste material—chemical, pharmaceutical and one containing heavy metals— should be incinerated or buried if the quantity is very small.
- The large quantity of such materials should be sent back to the original supplier.
- **Final Disposal of Wastes**
- Open site of waste should be avoided because they:
 - Pose infection risks and fire hazards
 - Produce foul odor
 - Attract insects
 - Are unsightly
- Use heavy duty utility gloves and appropriate personal protective equipment when handling wastes.
- Decontaminate and clean gloves between use.
- Handle wastes carefully to avoid spills or splashes.
- Always wash hands after removing gloves and handling contaminated wastes.
- Avoid transferring contaminated waste from one container to another.
- Incineration is the preferred method for waste disposal, as the heat will generally be sufficient to destroy infectious microorganisms and will also prevent scavenging and reuse of discarded items.
- If incineration is not possible, then careful burial is the next best alternative.
- Dispose of used toxic chemicals or medicine containers properly:
 - Rinse glass containers thoroughly with water; glass containers may be washed with detergent, rinsed, dried and reused

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- For plastic containers that contained toxic substances such as glutaraldehyde, rinse three times with water and dispose by incineration and/or burial; these containers may be used for sharp disposal containers, but do not reuse them for any other purpose
- Equipment that is used to hold and transport wastes must not be used for any other purpose in the clinic or healthcare facility, and contaminated waste containers should be labeled clearly.
- Contaminated waste containers should be cleaned each time they are emptied and non-contaminated ones when they are visibly soiled.
- **Material safety Data Sheet ([MSDS](#))**
- Definition:
- Health-related, chemical- and brand-specific information
- MSDS Should be immediately accessible to laboratory workers.
- This contain
- Chemical Product and Company Identification
- Composition and Information on Ingredients
- Hazard identification
- First aid measures
- Fire and Explosion Data
- Accidental Release Measures
- Handling and Storage
- Exposure Controls/Personal Protection
- Physical and chemical properties
- Stability and reactivity Data
- Toxicological information
- Ecological information

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- Disposal considerations
- Transport information
- Regulatory information
- Other information



3.6 Introduction

■ Care of glassware

- All glass ware must be handled carefully.
- Breakage can some times be dangerous and may result in the loss of valuable and irreplaceable materials.
- Flasks and beakers should be placed on a gauze mat when they are heated over a Bunsen flame. Gauze mat is made from asbestos and its function is to distribute the heat evenly.
- Test tube exposed to a naked flame should be made of heat resistant glass such as Pyrex.
- If liquid are to be heated in a bath or boiling water the glass contents should be heat resistant.
- When diluting concentrated acids, thin walled glassware should be heat resistant. Because the heat evolved by the procedure often cracks thick glassware.
- Containers and their corresponding ground glass stopper should be numbered.
- pipettes should never be left lying on the bench.

Cleaning of glass wares

- It is clear that volumetric glass wares and glass apparatus must be absolutely clean, otherwise volumes measured will be inaccurate and chemical reactions are affected adversely.
- Checking cleanness-fill the vessel with distilled water and then empty it and examine the walls to see whether they are covered by a continuous thin film of water.
- Imperfect wetting or the presence of discrete of droplets water indicates that vessel is not sufficiently clean.
 - Wide varieties of methods have been suggested for the cleaning of most glassware.
 - In all cases, glassware for the clinical laboratory must be:



- Physically clean
- Chemically clean
- Bacteriologically clean or sterile

General cleaning procedure

1). Preliminary rinsing

- Rinse all glassware immediately after using cold or warm water.

2) Soaking in detergent solution

- Place in detergent solution (2%).

3). Scrubbing

- Scrub thoroughly with good quality brush

4). Washing

- Wash each glassware one by one under running water 5 times or more.

5). Rinsing

- Rinse each glassware with distilled water or deionized water at least three times.

6). Drying

- Place in a wire baskets and dry glassware completely.

7). Plugging

- The clean dry glassware should be put away in a cup board to protect it from dust.
- It is recommended that containers should be plugged with non – absorbent cotton wool or the mouth covered with little cups made from wrapping paper or preferably thin sheeting of paraffin wax.
- If the glassware becomes highly spoiled (dirty), it must be cleaned with acid cleaning solution.
- Potassium dichromate and sulphuric acid are both power full corrosive solutions and the mixture makes it even more so=> used to remove coagulated organic matter.



- Diluted HCl- 50% in water removes iron stains.
- nitric acid for stains due to Nessler's reagents (iodine).
- boiling with weak alkali solution remove grease
- acetone and ether remove ordinary grease
- Note: All the cleaning reagents must be washed away and the glassware rinsed finally with distilled water or Deionized water.



Information Sheet-8

Reagent storage according to OHS standard

3.7 Introduction

- storing reagents incorrectly are important causes of unreliable test results
- Reagents should be stored in a clean, cool, dry location to maximize shelf life.
- Refrigerate reagents only if indicated on the label.
- Reagents that are stored at elevated temperatures or in humid locations may experience accelerated degradation and reduced shelf life
- Storage requirements are to be indicated on container labels:
 - **a. Temperatures**
 - 1. Store reagents at required temperatures indicated by manufacturer
 - 2. Reagents with temperature storage requirements shall have these listed on the labels.
 - 3. Store reagents that must be refrigerated or frozen in freezers or Refrigerators with the required temperature ranges.
 - **b. Light sensitivity**
 - 1. If indicated store in cabinets or in a dark container.
 - Secure storage areas against unauthorized removal of chemicals.
 - Where possible, storage areas should have two separate exits.
 - Maintain clear access to and from the storage areas.
 - Do not store chemicals in aisles or stairwells, on desks or laboratory benches, on floors or in hallways, or in fume hoods.
 - Use an appropriate "Acid Cabinet" for any acid solutions of 6 M concentration or higher. Nitric acid needs to be isolated.
 - Label storage areas with a general hazard symbol to identify hazardous chemicals and indicate correct fire fighting procedures.
 - File a Material Safety Data Sheet (MSDS) for every chemical stored in the laboratory.



Store all reagent chemicals in compatible family groups. Do not alphabetize.

- Store all chemicals at eye level and below. The preferred shelving material is wood treated with polyurethane or a similar impervious material.
- All shelving should have a two-inch lip. If you use shelving with metal brackets, inspect the clips and brackets annually for corrosion and replace as needed.
- Store chemical reagents prepared in the laboratory in plastic bottles (if possible and appropriate to the chemical) to minimize the risk of breakage.
- Date containers upon receipt and again when opened.
- Attach chemical labels with all necessary information to all containers.
- When opening newly received reagent chemicals, immediately read the warning labels to be aware of any special storage precautions such as refrigeration or inert atmosphere storage.

Test peroxide-forming substances periodically for peroxide levels; dispose of these substances after three months unless the MSDS for the substance indicates a longer shelf life.

- Check chemical containers periodically for rust, corrosion, and leakage.
- Store bottles of especially hazardous and moisture-absorbing chemicals in chemical-safe bags.
- Maintain a complete inventory in the room where the chemicals are stored, and make a copy available to fire fighters.
- Keep storage areas clean and orderly at all times.
- Have spill cleanup supplies (absorbents, neutralizers) in any room where chemicals are stored or used.
- Limit the amount of flammable and combustible materials stored to that required for one year of laboratory work.
- Use only metal flammables cabinets to store flammable and combustible liquids. Label the cabinets *FLAMMABLE - KEEP AWAY FROM FIRE*.



3.8 Components/Classification/Types/parts

3.9 purpose/use/Importance/advantages and disadvantages

3.10 OHS hazards and suitable PPE

3.11 Necessary tools and equipments

3.12 Rules to follow

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Information Sheet-4

Cleaning of splashes of chemicals

3.13 Introduction

- Spills of blood and body fluids occur frequently in the laboratory.
- Blood and body fluids spills can harbor infectious agents such as Hepatitis B, HIV or Hepatitis C and as such pose a potential risk.
- Management of blood and body fluid spills is vital due to:
 - 1) Spread of infectious diseases
 - 2) Risk of falls
 - 3) Promotes sense of uncleanness, foul odor and unpleasant appearance
- When surfaces are contaminated by biological spills, the appropriate actions to take are:
 1. Define/ isolate the contaminated area
 2. Alert coworkers
 3. Put on appropriate PPE
 4. Remove glass/lumps with forceps or scoop
 5. Apply absorbent towel(s) to the spill; remove bulk and reapply if needed.
 6. Apply disinfectant to towel surface.
 7. Allow adequate contact time (**20 minutes**)
 8. Remove towel, mop up, and clean the surface with alcohol or soap and water.
 9. Properly dispose of materials.
 10. Notify the supervisor, safety officer, and other appropriate authorities.
- **Disinfectant:**
 - For most spills, use a **1:50** solution (1 g/l chlorine) of household bleach (sodium hypochlorite solution containing 50 g/l chlorine)-0.1% bleach solution



- For spills containing large amounts of organic material, use a 1:10 solution (5 g/l chlorine) of household bleach, or an approved mycobactericidal-0.5% bleach solution
- Alcohols are not recommended as surface decontaminating agents because they evaporate quickly, thus decreasing contact time
- If laboratory personnel become contaminated with biological hazards due to splashes or spills, immediate steps to take include:
 1. Clean exposed skin or body surface with soap and water, eyewash (for eye exposures) or saline (for mouth exposures)
 2. Apply first aid and treat as an emergency.
 3. Notify supervisor, safety officer, or security desk (after hours).
 4. Follow appropriate reporting procedures

Report to physician for treatment or counseling

- **Chemical spills**

- A chemical spill is considered to be minor only if:
 - The person who spilled it is familiar with the chemical
 - Knows the associated hazards
 - Knows how to clean up the spill safely
- The recommended steps for dealing with a minor spill include:
 - Alert coworkers, then clean up spill
 - Follow procedures for disposal of materials used to clean up spill
 - Absorb free liquids with an appropriate absorbent, as follows
 - Caustic liquids—use polypropylene pads or diatomaceous earth
 - Oxidizing acids—use diatomaceous earth
 - Mineral acids—use baking soda or polypropylene pads
 - Flammable liquids—use polypropylene pads



- Neutralize residues and decontaminate the area.
- Anything beyond a **minor spill** and that requires help from outside of the laboratory group constitutes a **major spill**.
- Steps to deal with **major spills** include:
 - Alerting coworkers
 - Moving to a safe location and
 - Calling authorities to report the situation

3.14 Components/Classification/Types/parts

3.15 purpose/use/Importance/advantages and disadvantages

3.16 OHS hazards and suitable PPE

3.17 Necessary tools and equipments

3.18 Rules to follow



The following are important hazards that require assessment and management in health laboratories:

- Unsafe premises • Equipment hazards
- Naked flames • Explosions
- Microbial hazards • Infestation by ants,
- Chemical hazards • Glassware hazards
- Unreliable water supply • Sharps hazards

Common causes of accidents in health laboratories

laboratory premises

- When emergency exit routes from the laboratory are blocked by equipment, storage boxes, etc
- -When, in a subdivided laboratory, there is only a single exit and staff Become trapped in one section.
- **Staff are injured by falling on a slippery or damaged floor or from broken glass on the floor:**
- When the floor is not cleaned properly after spillages or glassware breakages
- When wax or other slippery cleaning substance is applied to the floor
- When damaged areas of the floor are covered with matting.
- **Risk of infection to staff and others:**
- When there is no separate hand basin with a reliable water supply for hand washing
- When no separate rest-room is provided for staff and food and drink are consumed in the laboratory
- When laboratory staff do not leave their protective clothing in the laboratory
- When bench surfaces are not disinfected or cleaned properly each day.
- When the working area is not separated from the areas where Outpatients are received and blood samples collected.

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- When the laboratory has no safe systems for decontaminating infective Materials, disposing of waste and washing reusable laboratory ware.
- **Injury from chemicals:**
 - When chemicals with irritating fumes are used in a laboratory with inadequate ventilation.
 - When hazardous chemicals are stored on high shelves or on the floor under benches.
- **Injury from equipment:**
 - When electrical equipment has faulty earthing or insufficient ventilation.
 - When unsafe adaptors or extension leads are used because there are Insufficient electric wall points.
 - When the laboratory has no preventive maintenance schedules and equipment is not inspected regularly for defective insulation, corrosion, And loose connections.

Naked flames

- Injury from fire caused by lighted Bunsen burners, spirit burners, tapers, matches, alcohol swabs, ring burners, stoves
- When a lighted burner is placed in sunlight, making the flame difficult to see
- When a Bunsen burner, ring burner, match, or taper is lit too close to a Flammable chemical.
- When a lighted taper is carried across the laboratory close to where a flammable stain or reagent is being used or stored

Microbial Hazards

- **Pathogens are accidentally ingested:**
 - From contaminated fingers when personal hygiene is neglected
 - When hands are not washed after handling specimens or cultures
 - When specimens or liquid cultures are mouth-pipetted
- **Pathogens are accidentally inoculated:**
 - Through needle stick injuries caused by resheathing needles after Collecting blood or careless handling of needles and lancets.
 - Through open uncovered skin wounds Through injury from broken contaminated glassware
- **Pathogens are accidentally inhaled in airborne droplets (aerosols):**
 - When snap-closing specimen containers
 - When vigorously dispensing or pouring infectious fluids
 - When sucking up and blowing out fluids from pipettes
 - When specimens are hand-centrifuged in open containers or when a container breaks in an electric centrifuge and the lid is opened before the aerosols have settled.
 - When infectious material is spilled following the dropping or knocking over of a specimen container or culture.

Chemical Hazards

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- **Toxic or harmful chemicals causing serious ill health, injury, or irritation:**
 - When toxic or harmful chemicals are swallowed by being mouth- Pipetting.
 - When fumes from irritant chemicals are inhaled in poorly ventilated areas of the laboratory
 - When no protective goggles or gloves are worn and harmful chemicals enter the eye or come in contact with the skin
- **Flammable chemicals causing fire:**
 - When flammable chemicals are used or stored near a naked flame
 - When a lighted 'swab' is used to heat stain in the Ziehl-Neelsen method and ignites nearby flammable chemicals
 - When a flammable chemical is spilled near a flame
- **Corrosive chemicals causing serious injury and burns:**
 - When corrosive reagents are ingested by being mouth-pipetted
 - When strong acids are accidentally knocked from shelves or spilled
 - When a corrosive chemical comes into contact with the skin, or the eyes are splashed when opening and pouring a corrosive chemical

Equipment hazard

- **Electric shock:**
 - When equipment is not reliably earthed or electrical circuits are faulty
 - When touching live wires in attempting to repair equipment or replace components, e.g. lamp, without first disconnecting the equipment from the mains
 - When handling electrical equipment with wet hands or standing on a wet floor
- **Fire:**
 - When cables and electrical equipment overheat due to overloading of conductors
 - When there is overheating caused by the overuse of adaptors
 - When insulation is inadequate or becomes damaged



- When thermostats fail and there is no temperature cut-out device to prevent overheating
- When electrical sparking or arcing causes flammable material to ignite
- When preventive maintenance is not carried out to check for corrosion, wear, and loose connections.
- **Injury from moving parts:**
- When an open hand-centrifuge is used in a part of the laboratory where it can easily injure a person.

When a centrifuge is not balanced, resulting in the buckets and trunnions spinning off the rotor, particularly when there is corrosion

General factors that contribute to the occurrence of accidents

- Inexperience and insufficient training and supervision of staff and lack of health and safety awareness by senior laboratory officers
- Untidy working, allowing the bench to become cluttered and not using racks to avoid spillages
- Too heavy a workload for the size of laboratory and number of staff
- Rushing to finish work 'on time'
- Loss of concentration due to a noisy working environment, constant interruptions, and excessive heat particularly in small poorly ventilated outreach laboratories
- Fatigue due to frequent emergency work during night hours.
- **Many of these factors can be remedied by:**
- On-going health and safety training in the workplace
- Good laboratory practice and common sense
- Changing the work attitudes of laboratory staff
- Increasing health and safety awareness in the laboratory by frequent discussions on safety issues and displaying appropriate safety symbols and notices
- Monitoring and improving the working conditions of district laboratory personnel as part of total quality management

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