Medical Laboratory

NTQF Level III

Learning Guide -39

Unit of Competence: -	**Prepare Laboratory Solutions**
Module Title: -	**Preparing Laboratory Solutions**
LG Code:	**HLT MLT3 M08 LO1-LG-39**
TTLM Code:	**HLT MLT3 TTLM 1019v1**

LO 1: Prepare a working solution

Instruction Sheet	Learning Guide #-

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 e***quipment***, 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 – ---**

Information Sheet-1	Introduction to solution preparation

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 dissolves 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.
  - ✓ **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, it is 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: _____

Information Sheet-2	Equipments and materials for solution preparation

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 composion

1. **Glass with high thermal resistance** – borosilicate glass can resist about 500^{o}c 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
- ✓ 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^{o}_{C} and this is marked on the pipette.

2.2.1.1. Micropipettes

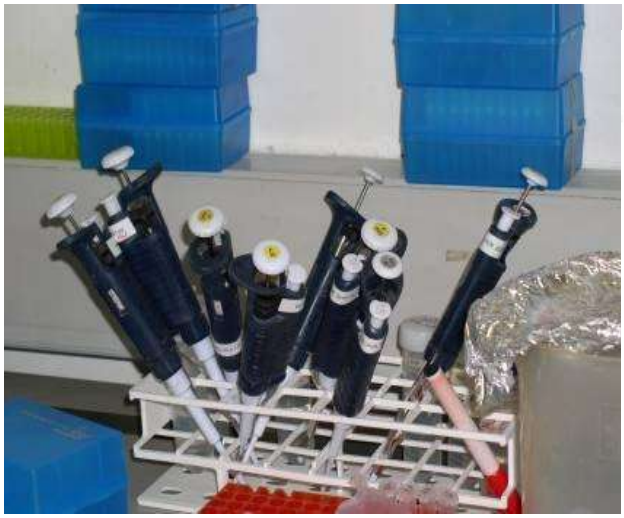


Fig.2.1 automatic pipette

- 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.*

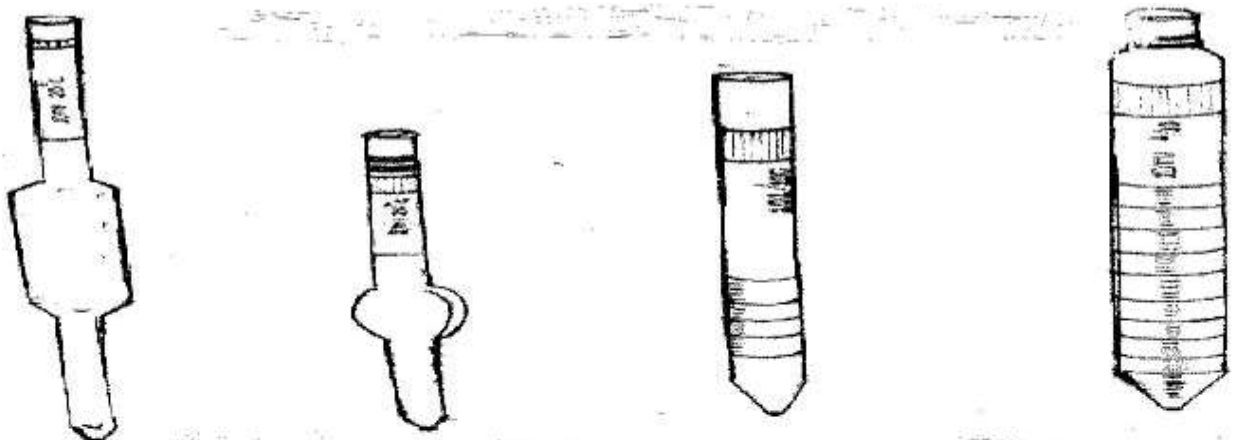


Fig 2.2 :- A. Volumetric (transfer) B. Ostwald folin (transfer). C. Measuring (Mohr) D. Serological (Graduated)

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 to100 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 flaks 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 with stand 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)

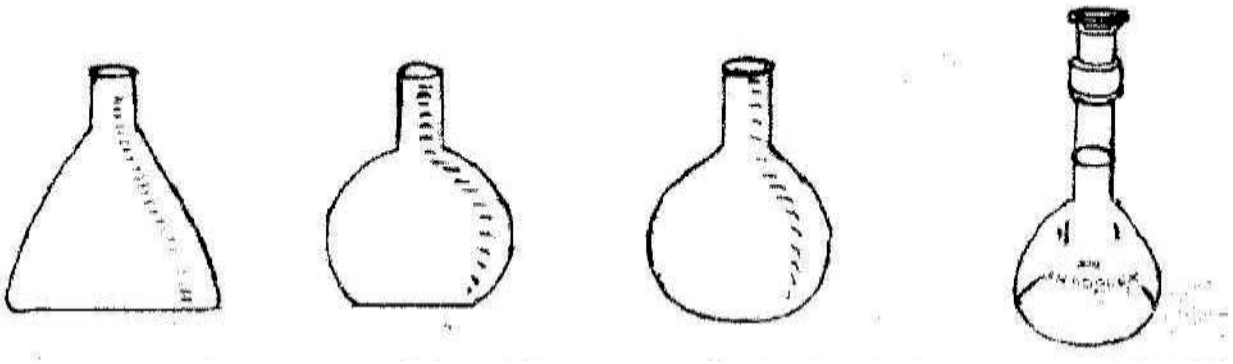


Fig2.4:- A. Conical B. Flat bottomed C. round bottomed D.Volumetric

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, vaccutainer tubes. Test tubes can be with or without rims (lips)

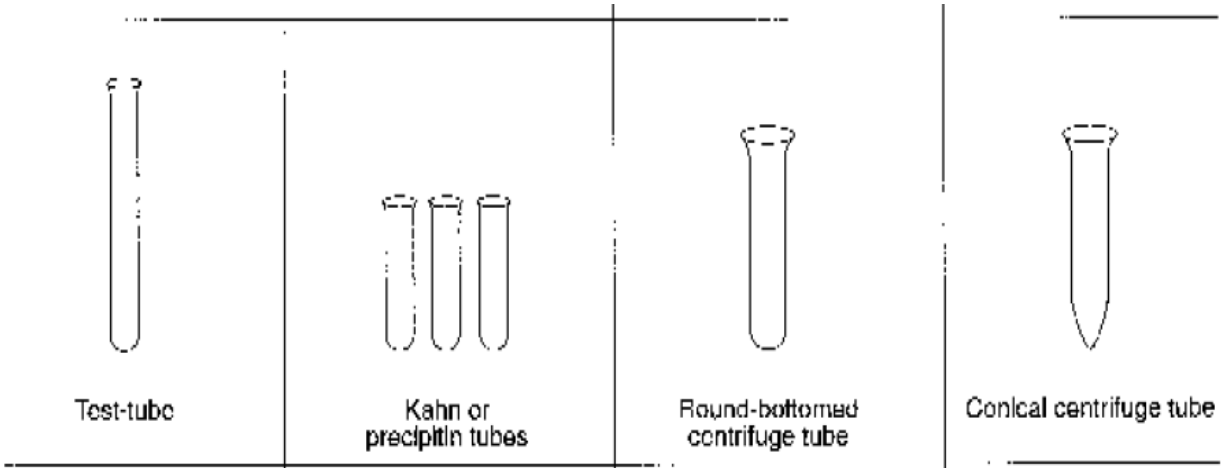


Fig 2.7:- test tubes

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

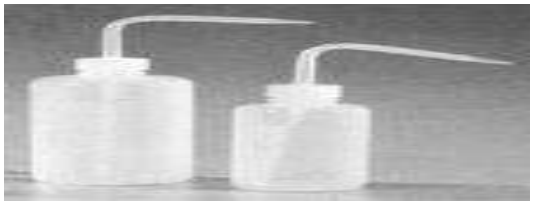


Fig 2.8:- Reagent bottles

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.

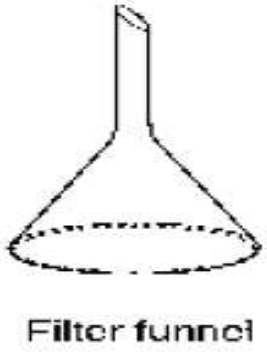


Fig. 2.9:- funnels

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

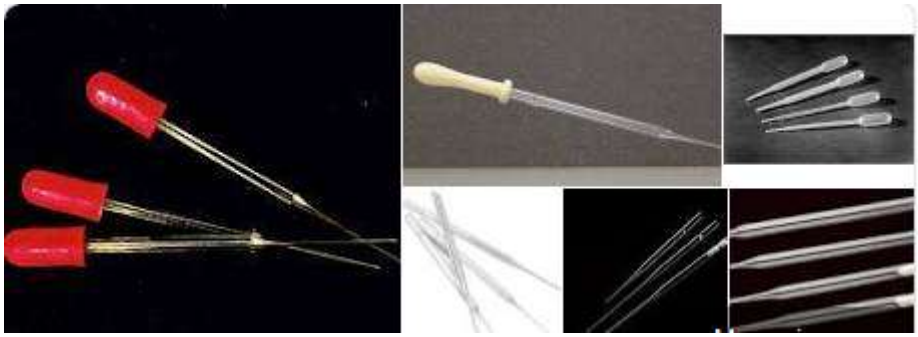


Fig 2.11:- pasture pipettes

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.

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 mili 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 weighs. 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 37^0c). 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 - 37Oc 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^{o}_{C} to 100^{o}_{C}. The heating coil may be of immersion type or enclosed in a case, some models have propellers to help to circulated 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

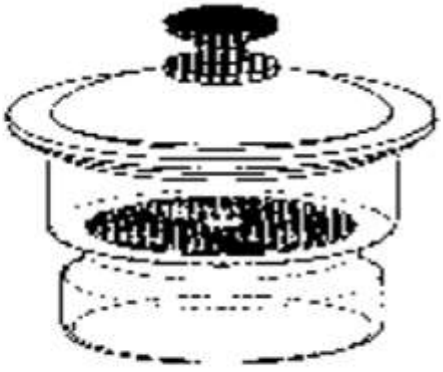
2.7. Mixers

- 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 dessicators, they sometimes absorb water. The chemical is dried in an oven at 110^{o}_{c} for 1 hour, and then it is placed in a desecrator over night before weighing on the analytical balance.
- The purpose of the oven is to remove the water and that of the desicator is to store the chemical at an ambient temperature where it cannot reabsorb water.
- A desiccators 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 desiccators.



Dessicator

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.

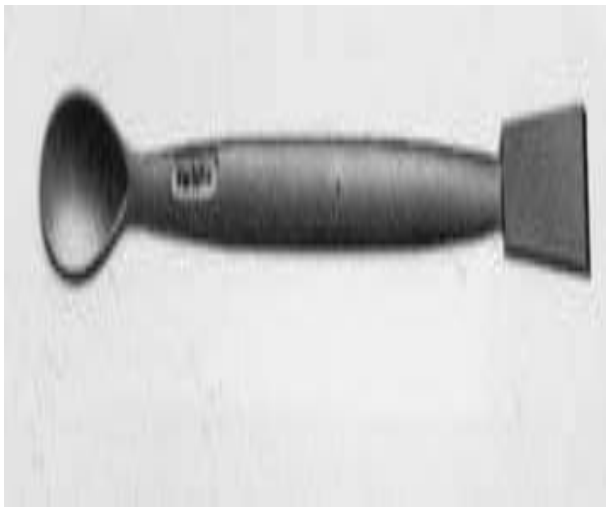


fig 2.22. Spatula

Self-Check -2	Written Test

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

1. When comparing glassware with plastic wares, plastic wares are?
   A. Cheaper and safer to use.
   B. Re-usable and autoclavable.
   C. Heavier, more costly
   D. easily broken
2. _____ is/are calibrated to deliver a constant volume of liquid?
   A. Micropipettes
   B. Volumetric pipettes
   C. Graduated pipettes
   D. measuring pipettes
3. Glass ware not calibrated to hold a particular or exact volume, but rather are available for various volumes, depending on the use desired
   A. Volumetric glass wares
   B. Non- volumetric glass wares
   C. Semi-volumetric Glass wares
   D. Volumetric flasks
4. _____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: Mach the following types of laboratory glass wares listed on column A from their correct composition listed on column B.

A	B
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: _____

Short Answer Questions

Information Sheet-3	Measurement

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		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	°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	103	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

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
   B. square meter
   C. newton
   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

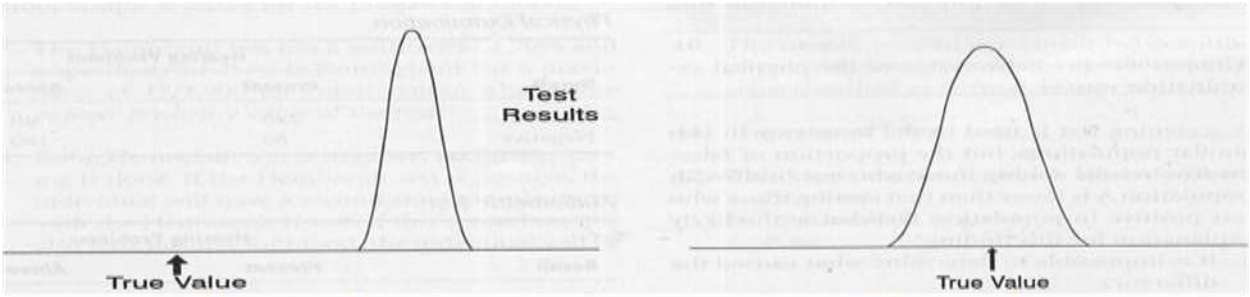
4.1. Accuracy and precision of measurement

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

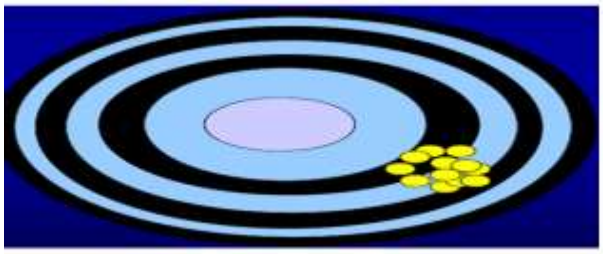
4.1.2. Accuracy (Validity)

- 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.

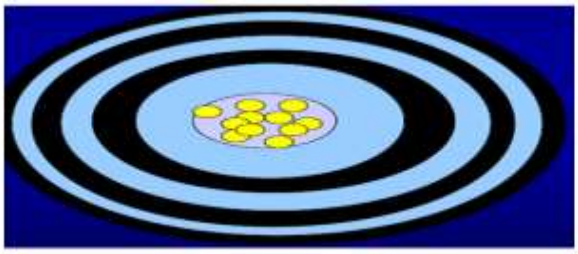


Results reliable but NOT valid

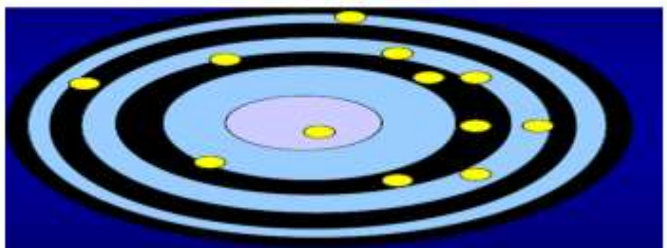
Results reliable and valid



Reliable, not valid



Both reliable and valid



Unreliable and invalid

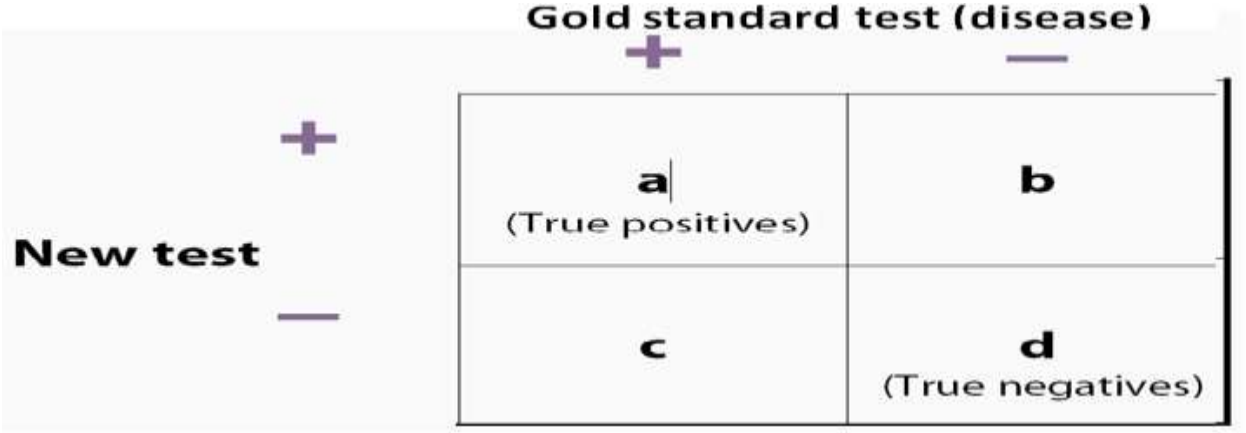
4.2. Key measures of validity

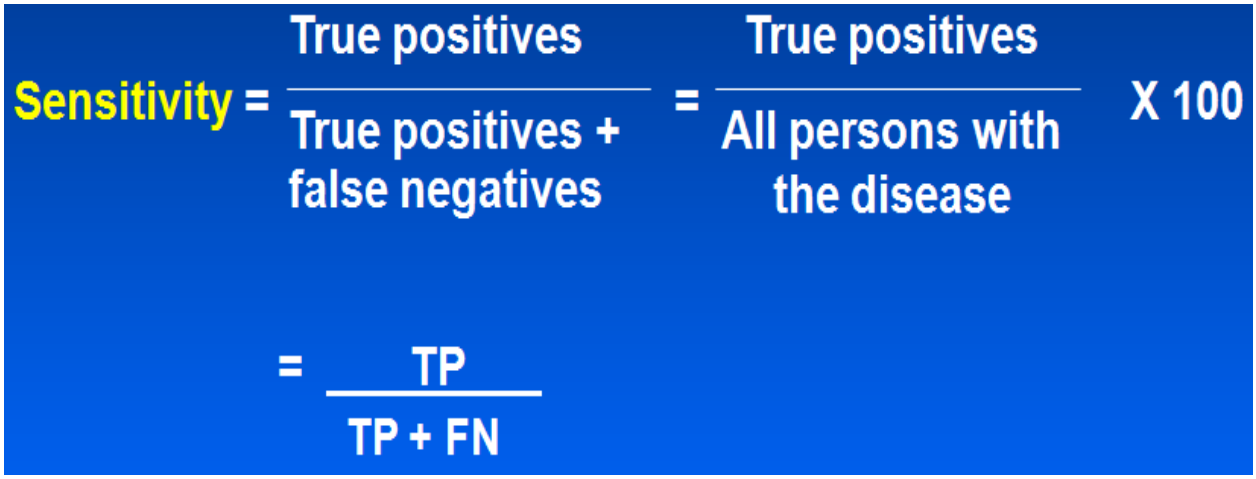
1. Sensitivity
2. Specificity
3. Predictive value

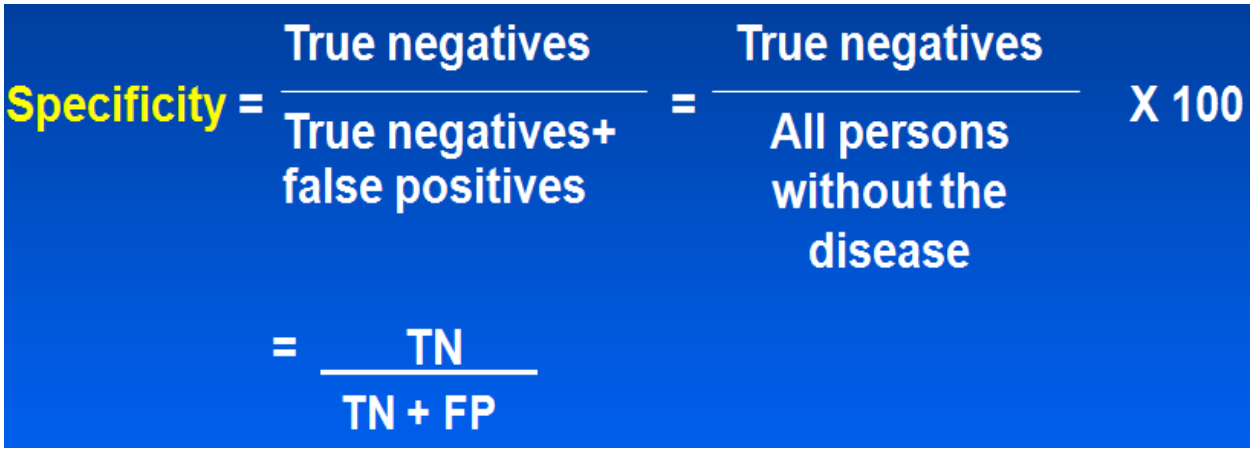
- **Sensitivity** is the ability of the test to identify correctly those who have the disease from all individuals with the disease
- **Specificity** is the ability of the test to identify correctly those who do not have the disease from all individuals free from the disease.
- To determine the sensitivity 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.

A. **Positive predictive value**

B. **Negative predictive value**







- **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

Predictive Value of a Positive Result (%) = __TP___ X 100

TP + FP

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

Predictive Value Negative Result (%) = __TN___ X 100

FN + TN

- **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

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: _____

Short Answer Questions

Information Sheet-5	Chemicals

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, it is 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 = Vs/Tv

  - Where:
  - **D**$_{=}$ dilution
  - **V**$_{s=}$ volume of solute(sample)
  - **Tv**$_{=}$ Final volume
- In the performance of dilution, the following equation is used to determine the volume (V2) needed to dilute a given volume (V1) of solution of a known concentration (C1) to the desired lesser concentration (C2).

  C1xV1=C2V2
- 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 = 70mL 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

  100mg/mL x 1/10 = 10 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 C2 = 30% V1 = C2V2

    V2 = 45ml, C1 = 70% V1= 30 X 45 = 19.3 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.

    C1xV1=C2V2 =>20xV1=1x8V1 (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.
  - ✓ C1xV1=C2V2 => 2xV1=1x4 =>V1 (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.

    Total volume of blood and diluting fluid 50 + 50 = 100 µ l

    Sample: total 50:100 1 in 2 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 µ l => 0.5: 9 = 1 in 18 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 suppl
  - ✓ 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 0.1mL 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 serialy transferred through tube 5. finally, 0.5 mL is discarded from tube 5.

- 1/Y = 0.5/1.0
- Y x 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?

Y= ½ x (½) (Y-1)

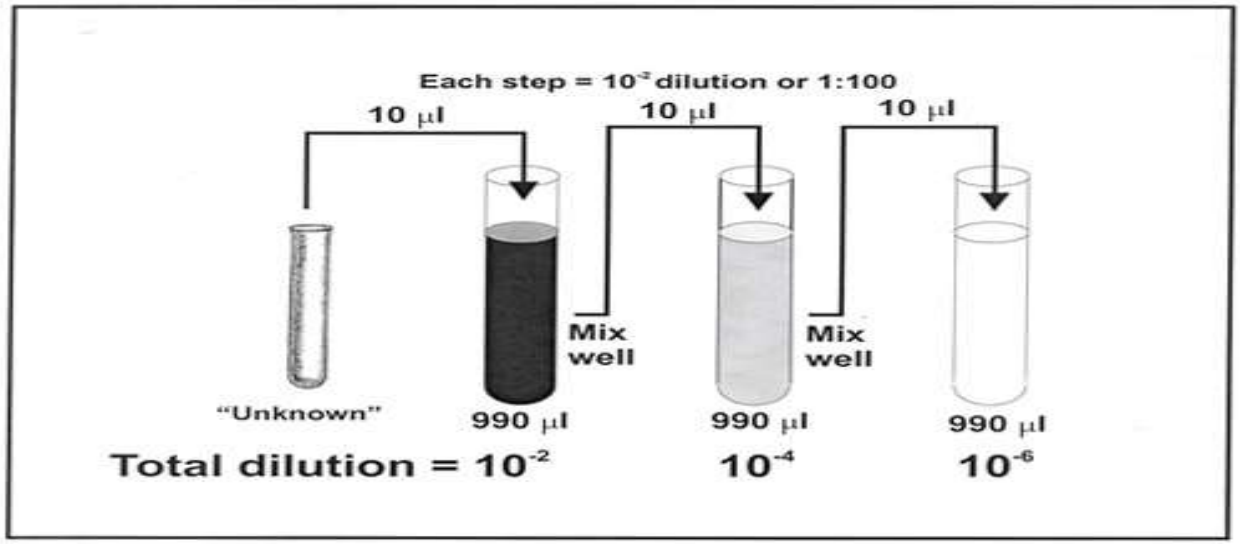
= ½ x (½)2

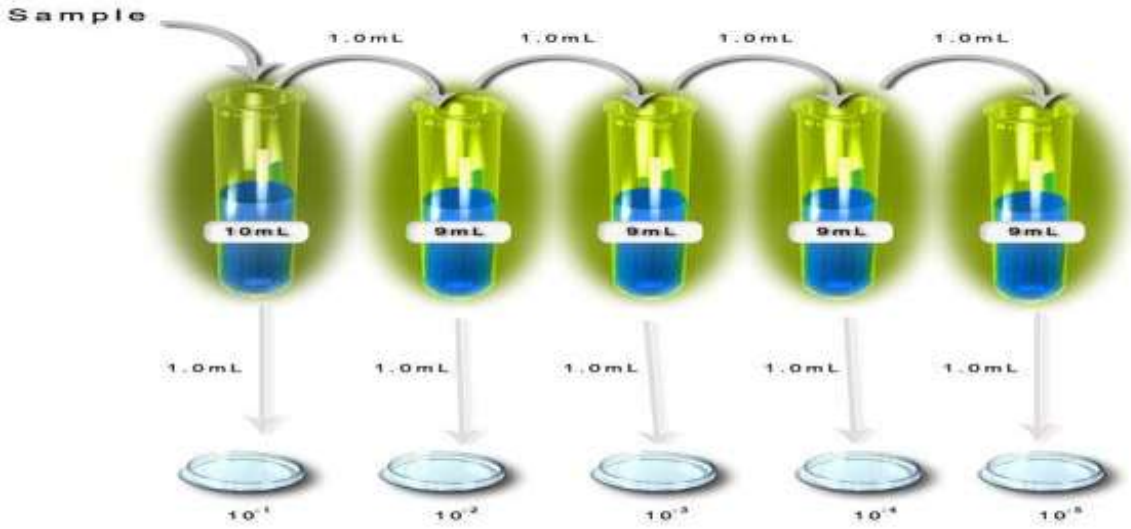
= ½ x ½ x ½

= 1/8 ;

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 ul) with 99 unit volumes of broth (990 ul) = 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.


Each step = 10^{-2} dilution or 1:100
10 µl
10 µl
10 µl
Mix well
Mix well
"Unknown"
990 µl
990 µl
990 µl
Total dilution = 10^{-2}
10^{-4}
10^{-6}


Sample
1.0mL
1.0mL
1.0mL
1.0mL
10mL
9mL
9mL
9mL
9mL
1.0mL
1.0mL
1.0mL
1.0mL
1.0mL

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 serialy 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

- 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 stai

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^{O}c.

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
  - ✓ 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_2O (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_2O (solvent).	Sucrose (table sugar) in water; sodium chloride (table salt) in water, NaCl (solute) dissolved in liquid H_2O (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

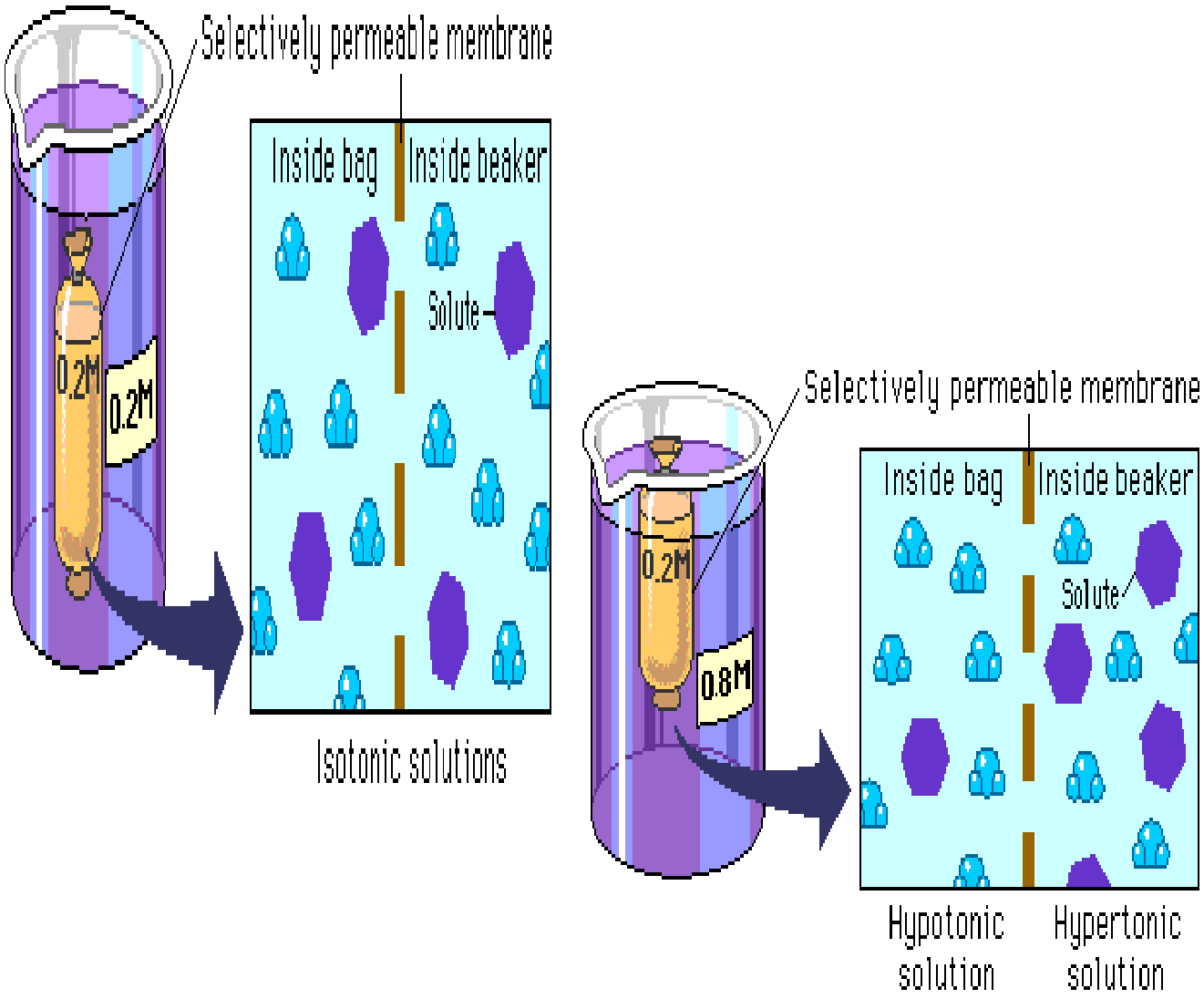
- **Aqueous solutions:** contain water as the solvent. example, sugar in water, carbon dioxide in water, etc.
- **Non-aqueous solution:** contain a solvent other than water. Ether, benzene, petrol, carbon tetrachloride etc., are some common solvents. example, sulfur in carbon disulphide, naphthalene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

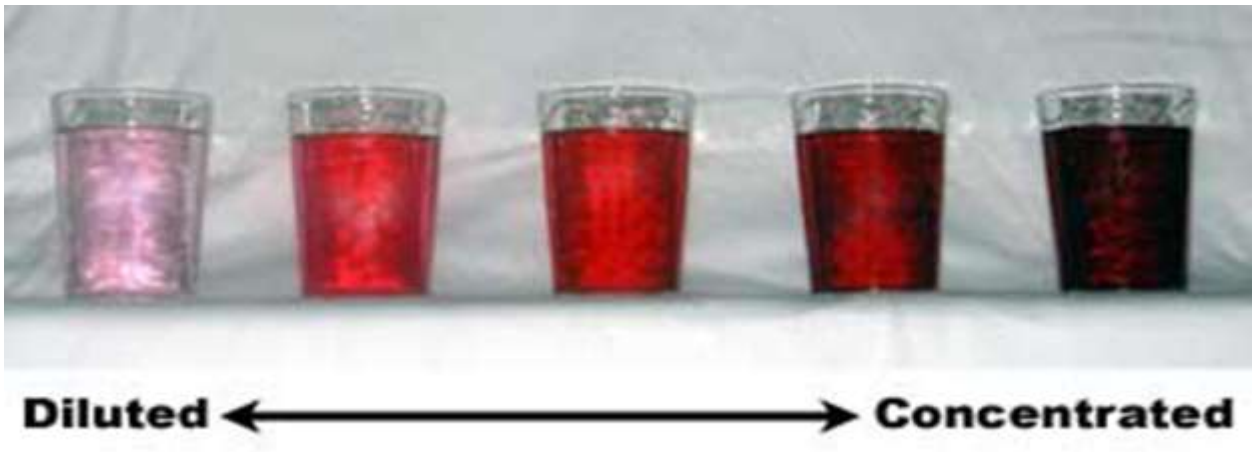
- Stains (Dyes) are coloured chemical compounds that are used to selectively give (impart) colour to the colourless structures of bacteria or other cells
- Staining reactions are made possible because of the Physical phenomena of capillary osmosis, solubility, adsorption, and absorption of stains or 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



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**

A. **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.

B. **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, each100 gm of hydrochloric acid solution contains 30 gm of HCl and the rest 70 gm is the solvent

C. **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 10^2):

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

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 morality 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 (H2SO4) is 98.

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

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

- 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 KNO3 is 101.103 g/mol.)

Mole of KNO3 = 5.7g/101.103g/mol=0.056mol

M=0.56mol/0.233L =0.24 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.
- A normal solution is a solution that contains one-gram equivalent weight of the solute in one liter of solution.

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

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

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

- 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.

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

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

- ✓ E.g. 80 grams of a simple sugar is added to 750 g of water. The sugar is glucose, with the composition $C_6H_{12}O_6$. 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

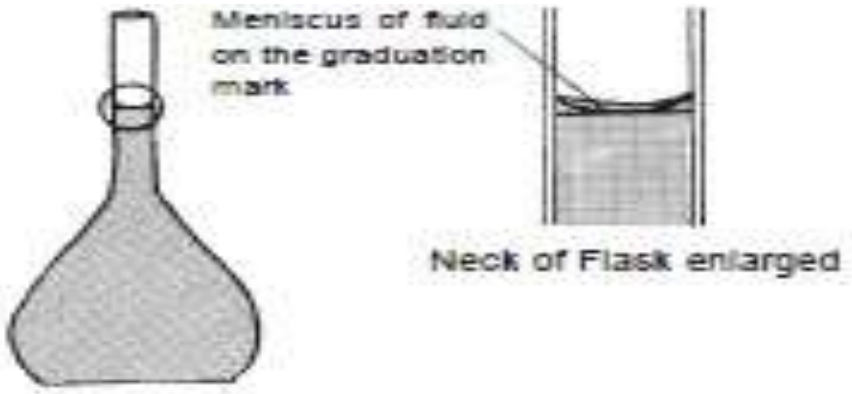
- ✓ Measuring chemicals and solvents
- ✓ Dissolving/ diluting the solution in accordance SOPs
- ✓ Labeling and storage of reagents

- 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
- **nalytical 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.
- **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 Materials &Equipment
  - ✓ 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(PH7.2)
  - ✓ 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 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)
   =10ml× (9/10)
   =9ml
   Volume of 5% bleach=total volume – volume of water added
   =10ml-9ml=1ml
   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 C1V1=C2V2, 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
- 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

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

Mix until all the safranine is dissolved. Transfer the solution to a glass – stoppered bottle. Label the bottle (Safranine 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 (Safranine 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. Carbolfuschin

Solution A (saturated solution of basic fuchsin)

- Basic fuchsin …….. 3gm
- Ehanol ……..100ml

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

- Phenol ……..... 10gm
- Distilled water …… 200ml
- Mix **10 ml** of solution A with **90ml** of solutin B. Transfer resulting mixture to a glass stopperd 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

- Methlene 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 grind 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 Distiied water----------Solution 2

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

Potassium permanganate

Potassium permanganate ($KMnO_4$) ----------0.5g

Dis. Water----------100ml

Dissolve and store in amber bottle

Acridine orange

Acridine orange----------0.01g

Anhydrous dibasic sodium phosphate (NA_2HPO_4) -0.01g

Distilled water----------100ml

7.7.2.8. Lens cleaning solution (Ethyl ether – alcohol)

- Absolute ethanol …….....20ml
- Ethyl ether ……..80ml

- Mix and stopper

7.7.2.9. Boric acid, saturated solution

- Boric acid ……..4.8 g
- Distilled water …….q.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 ,anhydrous .....2.1 g
- Distilled water ……..q.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

- Eosin ……1 g
- Distilled water …….q.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 water …….300ml
- 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) pellets ……20g
- Distilled water ……..q.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 water …….q.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 water ……q.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:

1. Which of the following is solid solution?
   A. alloys
   B. air
   C. normal saline
   D. 70% alcohol
2. 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
3. _____ 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
4. _____ 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
5. 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
6. _____, There is no need to use expensive volumetric glassware when preparing stains
   A. True
   B. false
8. `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
9. 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.

- 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

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: _____

Information Sheet-9	Record working solution details in laboratory register

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 spoilt 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 *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

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 V1 = C2V2/C1 to get initial volume
Step 8 Decide the added volume by V2- V1
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)

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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)

List of Reference Materials

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