MLS 2301(JKUAT)	CLINICAL CHEMISTRY II
Year/	THREE/
CONTACT HOURS	42
PRE-REQUISITE	CLINICAL CHEMISTRY I
PURPOSE/ OBJECTIVES	**Purpose:** This course unit is intended to equip the trainee with attitudes, knowledge and skills in carrying out medical laboratory chemical techniques. **Objectives** At the end of the course, the students should be able to: ▪ **Explain** the concepts of Clinical Chemistry ▪ **Explain** the application of theories and principle techniques in clinical chemistry ▪ **Discuss** collection of specimens and application of clinical chemistry techniques in research ▪ **Apply** Clinical Chemistry in research ▪ **Perform** the various diagnostic techniques
CONTENT	***General laboratory techniques and procedures; analytical procedures and instrumentation; Spectral techniques, chromatography; isotopes in clinical chemistry****, electrophoresis, Immunochemical reactions and techniques, electrochemical techniques, automation in clinical laboratory. Quality assurance. Specimen collection, handling and processing. Acquisition, management and application of laboratory data. Statistical procedures, evaluation of methods. Microprocessor and computers in clinical chemistry laboratory. Non-protein nitrogen and their determination* .Haemoglobin and haemoglobin-derived pigments and their determination. Tumour makers: CA 125, CA19-9,CA 153, CEA, TPSA, β HCG and α-FETO PROTEIN .Analysis of tumour makers. Therapeuticdrug monitoring and analysis. Drug of abuse and their analysis.
LEARNING AND TEACHING METHODOLOGIES	Lectures, Tutorials and Laboratory practicals.
ASSESSMENT	• Continuous Assessment Test (CATs) 10% • Practical 20% • End of semester examination 70% Total = 100%

CORE TEXT	**Title**	**Author**	**Publisher**	**ISBN**	**Year**
	1. Clinical chemistry in diagnosis and treatment	Phillip D. Mane	Oxford Uni. Press	0 340 70080 7	1994
	2. Clinical chemistry 4th. ed.	William J. Marshall	Mosby	0-7234-3159-0	2000
SUPPORT MATERIAL AND RESOURCES	Equipped clinical chemistry laboratory Equipment: auto analyzer, Colorimeter, analytical balance, flame photometer, centrifuge, refrigerator, ph meters, Mechanical mixers, electrophoretic kit, hot air oven, distiller, deionizer, ion selective electrodes etc				

CLINICAL CHEMISTRY II FOR BSC STUDENTS

TOPIC ONE—
GENERAL LABORATORY TECHNIQUES AND PROCEDURES

INTRODUCTION

The function of the clinical chemistry laboratory in the delivery of quality health care services is to perform qualitative and quantitative analysis on body fluids such as blood, urine, cerebral spinal fluid as well as faces, tissues, calculi and other materials.

- If the results have to be useful to the health provider in the diagnosis and management of various pathological disorders, the tests must be performed as accurately as possible. To achieve this goal, the instruments, reagents, analytical methods must be of good state. Qualified personnel and good infrastructure also plays an important role in achieving this goal of provision of quality laboratory results

(A) ANALYTICAL TECHNIQUES IN CLINICAL CHEMISTRY

-There are two important techniques used in clinical chemistry laboratory. These techniques are:

(1) QUALITATIVE ANALYSIS
(2) QUANTITATIVE ANALYSIS

(1) QUALITATIVE ANALYSIS

- This is a technique of analysis which is based on detecting the presence or absence of an analyte in a sample/specimen.
- The results of qualitative analysis are given as either POSITIVE (indicates presence of an analyte) or NEGATIVE (indicates absence of an analyte).
- Dry chemistry which involves the use of reagent strips is widely used in this technique.
- The constituent reagents used for a specific analyte are immobilized on the reagent strip and can be used directly by the health provider or indeed by the patients themselves.
- Comparatively the reagents strips are expensive but easy to handle and usually gives results in a very short time. That is they are very fast.
- Among the most commonly employed reagents strips are those designed to detect glucose in body fluids. The wafer thin plastic strips contain a narrow pad at one end, to which the sample is applied. The sample material diffuses inwards into the body of the pad which houses the appropriate diagnostic reagents.
- The reagents strips are designed such that one of the products is a chromogen thus coloured.
- Most systems are based on visual examination of any colour development on the pad after the sample has been applied. The intensity of the colour produced reflects the concentration of the metabolite in the sample applied.
- These reagent strips are also widely used in routine examination of urine for the detection of the presence or absence of proteins, bilirubin, blood, glucose, ketones, nitrites etc.

(2) QUANTITATIVE ANALYSIS

- This is the type of a technique which is based on determination of the actual concentration of an analyte in a sample.
- The results are given as a concentration followed by the unit of measure eg x mmol/l,x mol/l, x g/l, x g/dl, x g%, x µiu/ml, x µmol/l etc.
- The procedures used in quantitative analysis can either be wet or dry chemistry. Wet chemistry procedures involve reactions whereby the participating reactants reacts with each other in a certain ratio.
- Currently there are other procedures in dry chemistry that give the actual concentration of an analyte under investigation.
- The reagents used in wet chemistry procedures are comparatively less expensive.

- Several instruments are involved in wet chemistry analysis eg simple calorimeters, spectrophotometers, automated chemistry analyzers, flame photometer.

(B) SOLUTIONS PREPARETIONS AND DILUTIONS PROCEDURES

(B1) SOLUTIONS

- Solutions are made of solutes and solvents.
- The concentration of a solvent in a solution may be expressed in different ways. Traditionally, in clinical chemistry concentration has most often been expressed as mass of a solute per volume of solution (ie mass concentration)
- The system international (SI) recommends the use whenever possible of moles of solute per volume of solution (ie substance concentration) , and the use of a litre as the reference volume.
- Atomic weights of elements are always considered whenever such reagents solutions are being prepared.

EXAMPLES

(1) How many grams of NaCL are required to make 1 litre of 0.05 M NaCL solution?

(mwt: sodium=23, chloride= 35.5)
23 + 35.5= 58.5 gms
58.5 g dissolved in a litre of distilled water = 1 molar solution of NaCL
58.5 G = 1M
? = 0.05M
0.05M/1M×58.5G = 2.925 G NaCL

(2) How many grams of NaCL are required to make 1500ml of 2M NaCL
1M= 58.5 g dissolved in (1L) 1000ml
2M= 58.5 g×2=117 g dissolved in a liter(1000ml)
1500ml=1.5 L
117 g = 1L
? = 1.5L
1.5L/1L×117g
= 175.5 g of NaCL

(B2) DILUTIONS

- Dilutions involve adding more solvent to a given solution. For instance: A 1:10 dilution results when 1 volume of a given solution is diluted to a total volume of 10 with solvent. Generally it would be acceptable to add 9 volumes of a solvent to obtain a total volume of 10. The concentration of the solute in the final solutioin then is 1/10 of its [ ] in the original solution
- If two successive 1:10 dilutions are carried out, the [ ] of the final sol. is 1/10 × 1/10= 1/100 times the [ ] of the original solution.
- It is useful to carryout successive dilutions not only to avoid diluting very small volumes (which are difficult to measure accurately), but also to avoid diluting into large volumes (which require large containers and excessive amount of solvent) eg

1;5000 (1 in 5000) dilution can be carried out by successive 1:50 and 1:100 dilutions

(1 ml of the original solution diluted to 50ml , then 1 ml of this solution diluted to 100 ml), rather than a straight 1:5000 dilution , which would require a 5 litre container and nearly 5,000 ml (5litres) of solvent if 1ml of the original solution were taken for this solution.

SAMPLE PROBLEMS

(1) What is the final concentration when 5N solution is diluted successively first 1;5, then 2:15 and finally 3:25

1⁄5 × 2⁄15 × 3⁄25 ×5N = 2⁄125 N=0.016 N

(2) How many mls of distilled water must be added to 5ml of a 2M NaCL solution in order to obtain a final solution of 0.1 M

- First calculate the fractional dilution

0.1 M⁄2M = 1⁄20

So the original solution must be diluted 1:20, therefore the total final volume

If 1 ml of the original solution is added to 20 ml then 5 ml of the original solution is diluted to?

5/1 × 20 = 100ml

Therefore total volume is 100 ml. So 95 ml of distilled water will be added to 5 ml of the original solution to obtain a final volume of 100 ml.

(B3)A DILUTION FORMULA

- Consider that a solution designated as A is diluted to give a solution B. If volume V_A of solution A is diluted to give volume V_B of solution B and the concentrations of solutions A and B are C_A and C_B respectively, then the following very useful relationship is true whenever the same volume and [ ] units are used for both.

$$V_A \times C_A = V_B \times C_B$$

This formula is derived from the fact that the amount of solute in a volume V_A is the same as the amount of solute in volume V_B .The amount is equal to the [ ] times the volume in each case , that is :

Amount of solute in $V_A = V_A \times C_A$

Amount of solute in $V_B = V_B \times C_B$

Example

What would be the final concentration of a NaCL solution if 10 mls distilled water is added to 10 mls of 2mg/ml NaCL solution

$V_A \times C_A = V_B \times C_B$

10 ml x 2mg/ml = 20 ml x C_B

1 mg/ ml =_C_B

(B4) MAKING DILUTED SOLUTIONS FROM STANDARD (std)SOLUTION

- A standard solution is one which has a specific known concentration.

- Std (primary (std) are made from highly purified chemicals (ANALAR) that can be directly weighed or measured to produce a solution whose concentration is exactly known.
- The analar reagents (chemicals) that make the std solutions must be stable substances of definite composition that can be dried preferably at 104-110 ^{o}c without a change in composition.
- The analar reagents must not be hygroscopic (must not absorb water during weighing

- The following formula is used to prepare standards solutions from a given MASTER STD solution. (note that the stds made must be of lesser concentration than that of the original (master)std solution:

RC= required concentration
RV= required volume
OC= original std concentration

RC X RV/ OC will give the actual amount of the original STD to be used to make the desired standard solution.

(B5) STANDARD / CALIBRATION CURVE

- Std/ calibration curve are used to replace mathematical calculations to find [ ] of the test.
-Formula

Optical density (OD) of test / optical density (OD) of STD x [std}.

- The reading of the test is located on the appropriate graph and its corresponding [ ] is determined from the curve.
- A plot of OD versus [ ] for a set of std solutions constitutes a calibration or a standard curve
- Three steps are involved in the construction of a std curve/ calibration curve:-
(a) preparation of std solution
(b) obtaining the optical values of the std solutions
(c) plotting of OD versus [std]
- STD curves must always be constructed whenever new standards, fresh reagents or a new instrument has been introduced or a machine has been repaired.

SPECTRAL TECHNIQUES

SPECTROPHOTOMETRY

INTRODUCTION
-Most quantitative analytical methods in clinical chemistry and some in haematology are based on a colour compound (chromophore) produced when a sample containing the substance to be measured is mixed with appropriate reagents and subjected to certain reaction conditions. Such methods usually are referred to as colorimetric methods and measurements are made with instruments called photoelectric colorimeters or spectrophotometers.

COLOR AND WAVELENGTH

-Daylight, or white light, is a combination of all colors. If white light is dispersed or partially absorbed , certain of its component colors become visible .The colors seen depends on the wavelength of the emergent light eg droplets of moisture in the air disperse the suns rays to create a spectrum of colors known as rainbow.
- Many solutions have a characteristic of absorbing certain wavelengths of light and transmitting others. These solutions appear to have certain characteristic colors, which are determined by the wavelength of the light that is permitted to pass through (transmitted). Eg a solution of haemoglobin appears to be red because it absorbs blue green light and transmits the complementary color of red.
- Every color belongs to a particular wavelength region of the visible spectrum. Light travels in a wave like manner. The wavelength of a band of light is defined as the distance

between the wave peaks. The visible spectrum extends from about 400 nanometers (nm)=violet light to about 700nm= red light. Below 400nm is the ultraviolet and above 700 nm is the infrared both of which are invisible to the human eye

LIGHT INTESITY BAND BEERS LAW

-Besides wavelength the other property of light that is of principle interest in spectrophotometry is intensity. When light is absorbed , its intensity is reduced . The amount of light which is absorbed by a given solution depends on the concentration of the absorbing substances in the solution and on the thickness or depth of solution through which the light travels. Therefore beers law states that the concentration of a substance is directly proportional to the amount of light absorbed or inversely proportional to the logarithm of the transmitted light. The mathematical relationship between absorption of radiant energy and the concentration of a solution is shown by BEERS law

$-A = alc$

where A=absorbance which is a measure of the amount of light absorbed by a solution

a=constant (extinction coefficient)

l= light path of the solution in cms.

C= molar concentration of the absorbing substances.

-Another measure of change of light intensity is transmittance. Whereas absorbance (A) measures the amount of light absorbed by a solution , tranamittance measures the amount of light which passes through the solution.The relationship of A AND T is logarithimic in nature ie

$A = - \log T$

NB / T (transmittance is defined as the ratio of transmitted light (I) to incident light (Io) ie I/Io where I=%T and Io=100%

$A = -(\log I/Io)$

$A = -(\text{Log}(\%T/100\ \%)$

$A = -(\log \%T - \log 100)$

$A = -\log \%T + 2$

$A = 2 - \log \%T$

NB/ Most of the photometers and spectrophotometrers are calibrated on the reading scale upto 100% light transmittance and absorbance upto to 2.

DEVIATION FROM BEERS LAW .

-Not all colorimetric reactions follow beers law. Theoretically , the law applies to substances whose chemical environment is constant as their concentration changes and which they are being measured by a narrow band of light.Deviations occur for instance :-

(a) when more than one absorbing substance is present.

(b) when the chemical nature of the medium changes.

(c) when more than one wavelength of light(stray or uncontrolled light) is incident on the sample.

COMPONENTS OF A SPECTROPHOTOMETER

-THIS IS AN INSTRUMENT THAT MEASURES THE ABSORBANCE OF A SOLUTION AT ONE OR MORE WAVELENGHTS.

- When absorbances are being made for the purpose of quantification, the wavelength of choice is generally the one that at which the greatest absorbance occurs. This provides the greatest sensitivity since the substance being measured is irradiated with the wavelength of light which it can absorb most strongly.

- The component of a single beam spectrophotometer are.

(1)Light source

(2) entrance slit

(3)monochromator

(4) exit slit
(5) cuvette
(6)detector
(7) meter

COLORIMETRY OR SPECTROPHOTOMETRY SUMMARIZED

This method involves measuring the intensity of a color in a solution and relating it to the concentration of the analyte. While some materials of interest are already colored, most of these analyses require the analyst to add some chemical *reagents* (reacting chemicals) to a sample to produce a characteristic color.
The simplest type of measurement is visual comparison of the intensity of the color to a set of color standards which represent various concentrations of the analyte. While this is method does not require any expensive equipment, color perception is rather subjective--and many people have some degree of color-blindness.

A more precise measurement can be made using a *colorimeter.* A colorimeter is a device consisting of 1) a light source, which can be as simple as tungsten-filament light bulb; 2) some optics for focusing the light 3) a colored filter, which passes light of the color which is absorbed by the treated sample; 4) a sample compartment to hold a transparent tube or cell containing the sample, 5) a light-sensitive detector, like the light meter on a camera, which converts the light intensity into an electric current, and 6) electronics for measuring and displaying the output of the detector. Some colorimeters may be designed to read out directly in concentration units, while others may show the results in units of light absorbance which need to be compared to a calibration curve. (An interesting point is that the filter is not the same color as the solution being tested, but rather the complementary color. We want to use a filter which transmits light of the color which the solution absorbs. A yellow solution looks yellow because it absorbs blue light, so a blue filter would be used.)

If we want to get more precise and more interference-free measurements, we can use a *spectrophotometer*. This is very similar to a colorimeter, except that instead of using a filter to select the color of light to pass through the sample, we instead break the white light up into a rainbow (*spectrum*) of colors using a prism or a *diffraction grating*. The light is passed through a narrow opening (slit) before reaching the sample. By rotating the prism or grating, the color {"wavelength") of light can be selected more precisely and we can better match the color with that absorbed by the sample. The principle is shown in the diagram below. Needless to say, spectrophotometers cost more than colorimeters, and are likely to be more delicate and less portable, as well. While many tests are done using visible light, some analyses also make use of the invisible ultraviolet (wavelength < 400nm) or infrared (>700nm) portions of the spectrum. Scanning spectro photometers can also be used to identify some types of analytes by the wavelengths or colors of the light they absorb..

ANALYTICAL FORMULA RELATED TO SPECTROPHOTOMETRY

In the calibration of a particular analytical procedure, a standard solution is used since it has known concentration. The standard concentration value is used in the determination of the test value which is unknown. **T**he following formula gives the relationship between the calibrator/standard and the analyte under test.

OD test divide by OD of the standard multiply by concentration of the standard.

OD TEST/OD STD X [STD]

OD= optical density

ASSIGHNMENT ONE(

Sampled questions

(1) (a) If a solution of [ ] 10 mg/dl is diluted 1:10, what is the final [ ].

(b) if the above resulting solution is re-diluted 1:5, what is the final [ ].

(2) What is the final [ ] of an X molar solution that is diluted 5:50, re-diluted10:100 then re-diluted5:25?

(3) Given a series of ten tubes, each of which contains 4 ml of diluent. 1 ml of fluid is added to the first tube and a serial dilution using 1 ml is carried out in the remaining tubes. What is the dilution of the fluid in the ***fifth tube*** and in the ***tenth tube***

(4) How much 70% (v/v) alcohol can be made from 200ml of 95% alcohol?.

(5) A 1M solution of NaOH is diluted 5:25, then re-diluted 3:100. what is the final molarity?. How many grams of NaOH are present in 100ml of the final dilution?.

(6) 80 gms of $CuSO_4$ are dissolved and diluted to 1000ml. This solution is diluted 1:10, then re-diluted 5:25.

(a) How many grams of $CuSO_4$ would be present in 1 litre of the final solution?.

(b) What is the [ ] of the final solution in terms of molarity and percentage (w/v).

ASSIGHNMENT TWO

DISCUSS THE **The Diagnostic Importance** *OF Chromatography AND Isotopes In Clinical Chemistry*

MAKING SOLUTIONSFROM CHEMICALS/SOLVENTS/REAGENTS WITH GIVEN PERCENTAGE(%) PURITIES

- The solutions are mostly prepared from concentrated acid eg HCL, H_2SO4, HNO_3 etc.
- The information given by the manufacturer includes:-

(a) molecular weight(mwt)
(b) specific gravity
(c) % purity
(d) Manufacturing date
(e) Constituent chemicals

- The most important thing when making these solution is to determine the molarity of the acid. .Once the molarity has been determined the formula for making dilutions then follows.
- Eg: Prepare 1500mls of 2M HCL acid from concentrated HCL with the following characteristics:- % purity= 36%, specific gravity(spgr)= 1.18, molecular weight(mwt)=36.5.

NOTE/ The first step here is to determine the molarity of the given neat concentrated acid

***STEP ONE*:**

(a)The spgr gives the equivalent weight of 1 ml HCL acid ie

1ml=**1.18gms**

(b) To make a molar solution a certain number of grams must be contained in 1 litre of a solvent(1 L). therefore

1ml=1.18gms
1000ml= ? gms
1000ml/1ml x 1.18gms=**1180gms**

(c) The given acid is only 36% pure, therefore the number of grams contained are

36/100 x 1180gms = **424.8 gms**

(d) So the neat acid contains 424.8 gms. To make 1 molar HCL acid solution , 36.5gms(mwt) are dissolved in a litre of diluent. Therefore

36.5gms= 1 M
424.8 gms= ? M
424.8 gms/36.5 gms x 1 M = **11.6 M**

The molarity of the given neat acid is 11.6 M

STEP TWO

(a) To prepare 1500mls 2 M HCL acid from the above neat conc HCL acid, the following formular is used to get the actual volume of the neat acid to be used

$$\frac{RC \times RV}{OC}$$

RC=Required concentration, RV= required volume and OC= original concentration

$$\frac{2M \times 1500 \text{ mls}}{11.6 M} = 250 \text{ mls}$$

250 mls of the neat HCL(11.6 M) acid in a volumetric flask are diluted with 1250 mls of distilled water to make 1500 mls 2M HCL acid solution.

FACTORS CONSIDERED IN CHOOSING THE BEST INSTRUMENT (EQUIPMENT) FOR ANALYTICAL PURPOSES

- Selection of the instrument is governed by the type of the tests being carried out in a specific laboratory.

- The choice of instrument is governed by the number of tests analyzed in a specific laboratory.
- The reliability and safety of the instrument must be assured.
- The laboratory should have enough space for the placement of the instrument.
-The cost of the instrument should be considered and the laboratory should source for funds to purchase the instrument.
- The climatic conditions of the country of origin and the country of use should be considered. This will take care of any variation that might be there that can affect the overall working of the instrument.
- The warranty period of the instrument should be well understood and signed by the buyer and the seller.
- Availability of spare parts must be assured. It has to be stated clearly whether they are locally available or have to be imported.
- During the acquisition of the instrument the buyer should ensure that the following are provided by the seller : (a) parts manual (b) operators manual (c) service manual
- Staff training on how to operate the instrument is important. This enables the staff to familiarize themselves with the instrument. The training can be organized locally or from the instruments country of origin.
- Operation procedures and instruments maintenance (periodical-daily,weekly or monthly) must be emphazised during training.

FACTORS CONSIDERED IN SELECTING AN ANALYTICAL METHOD

- The surveying of the technical literature with an aim of deciding which procedures best fulfills the test requirements. The literature includes professional journals, manufactures reports and brochures. A preliminary judgment can be made from a review of this information.
- The principle of the assay with original references is an important factor.
- Reagents composition, quality and quantity. The storage temperatures and the stability after reconstitution
- Specimens of choice, volume or the anticoagulant to used.
- Anticipated analytical performance ie accuracy, precision, analytical range, linearity range and specificity.
- Reference ranges of the tests with information on how it was derived.
- Complete and detailed protocol for the test performance.
- Instrument requirements, limitations and the availability of the technical support, supplies and services.

SPECIMEN COLLECTION ,HANDLING AND PROCESSING

-Health providers use laboratory test results to diagonize pathological disorders, to evaluate its severity, to monitor its progress or response to treatment and to screen for disease in the healthy individuals.

- Many factors besides disease affect the composition of the body fluids , these factors are pre-analytical based whilest those that affect the quality of result produced by a laboratory may be analytical or post analytical based.

- The variability of test results due to biological factors is often greater than the analytical factors and the control of these biological factors should begin with proper preparation of the client/ patient prior to specimen collection.

PRE-ANALYTICAL ERRORS

NB/ These errors are known to occur before the specimen reaches the analytical area. They include:-

(a) Specimen from the wrong patient
(b) Incorrectly labeled bottles
(c) Blood taken from a vein close to intravenous infusion
(d) Prolonged venous occlusion of the upper arm
(e) Transferring blood from one tube to another , eg, EDTA to heparin to make up for a shortfall.
(f) Haemolysis of blood specimens
(g) Delay in a blood specimen reaching the laboratory
(h) In function tests a common source of error is the failure to empty the bladder completely or an incomplete or inaccurately timed collection of urine or faeces.
(i) Some drugs may interfere with certain analysis. If this is suspected the laboratory should be informed immediately.
(j) Failure of the requesting person to give a brief history about the patients illness.

ANALYTICAL ERRORS

NB/ These errors are known to be contributed by the laboratory personnel.
They include:-

(a) Use of wrong analytical procedure
(b) Failure to calibrate the test
(c) failure to run a QC specimen
(d) Use of wrong specimen
(e) Use of a faulty equipment
(f) Failure to observe the incubation temperature where appropriate
(g) Wrong calculation of test results . Use of inappropriate formula
(h) Use of incorrect volumes for the sample and reagents.

POST-ANALYTICAL ERRORS

NB/ These errors can originate from the laboratory or other sections of the health facility. They include:
(a) Transposition (interchanging) of results ie giving the patient the wrong results.
(b) Use of the incorrect units of measure
(c) Errors can arise when results are given by telephone. Results should always be read back to check them.
(d) Delay to dispatch results to the intended destination on time.
(e) Incorrect interpretation of laboratory results due to the use of wrong reference values.

SPECIMEN COLLECTION

(A) BLOOD.

Blood for analysis may be collected from veins, arteries and capillaries. Veinuos blood is the most commonly and usually the specimen of choice and is collected by a method referred to as venipunture. In young children, skin puncture is frequently used to obtain what is predominantly capillary blood. Arterial blood is collected for blood gases analysis.

An evacuated blood tube, needle and a syringe are usually used for collection of blood specimens. Before bleeding , the phlebotomist should verify the patients identity, the patient should be seated or supine if seating is not feasible and should be in that position 20 min before the specimen is drawn. Standardization of the patients position minimizes differences in [ ] of blood constituents caused by postural variations in blood volume(hemoconcentrations).

-The skin is cleaned with 70% isopropanol and allowed to dry, a tourniquet is applied (10-15) cm above the intended site of puncture so as to obstruct the return of blood to the heart band in the process distend the vein.

- Items for the collection of blood are—(1) vacuatainer tube (2) tube holder (3) a needle for use with multiple tubes intended for same patient..

- There are two classes of vacuatainer tubes ie those with a serum separating material and those without. Various anticoagulants and preservatives may be added to these tubes. The stopper colour denotes the type of the additives eg

COLOUR	USE	ADDITIVES
Gray	Plasma, whole blood (glycolysis inhibitor)	Fluoride(Na+), oxalate (Na+, K+) or iodoacetate (NA+)
Yellow	Sterile	None
Green	Plasma ,whole blood	Heparin (Na+, Li or NH4)
Red	serum	None
Blue	Plasma or whole blood	Citrate (Na+)
Lavender	Plasma or whole blood	EDTA

Serum separator tubes contain an inert , thixotropic polymer polymer gel material with a spgr of approx. 1.04. Silica or glass particles that accelerate clotting may be associated with it. Aspiration of blood into the tube and subsequent centrifugation displaces the gel which settles like a disk between the cells and the plasma when the separation is done. RCF(relative centrifugal force) of at least 1,100 x g for gel release and barrier formation. The release of intracellular components into the serum is prevented by the barrier for several hours or some cases for several days.

Before venipunture the phlebotomist selects the appropriate tubes and needles (gauges 19-22) and they must be sterile and sharp.

-VENOUS OCCLUTION ----- It is rarely necessary to leave the tourniquet in place for more than 1 min. When the flow of blood in the large vein is obstructed by a tourniquet, the filtration pressure across the capillary wall is increased. This increase causes fluids and compounds with molecular weight less than 5000 mwt to pass through the capillary wall. Slight changes might occur if left for 1 min but marked changes have been observed after 3 min. EG protein will increase by 4.9 g/l, ast(9.3), total choleterol(5.1), bili(8.4), iron(6.7) and potassium (< 6.2). The composition of blood drawn first will be the most representative of the circulating blood.

- Stress associated with blood collection can have affects in patients at any age. Cortisol and growth hormone concentration in plasma may increase. Particularly in young children who are frightened , struggling and held in physical restraint, collection may cause adrenal stimulation leading to an increase in plasma glucose concentration increase or increase in activities of enzymes that originate in the skeletal muscles.

- Vigorous sunction on a syringe during collection or forceful transfer from the syringe to the receiving vessel may cause haemolysis of blood.

(B) SKIN PUNCTURE

This is done when only a small amount of blood is required. However this procedure is time consuming and the risk of infection is higher than that of venipunture since the finger tip is more difficult to sterilize than the anticubital fossa. . Care should be taken when prick to avoid reaching the bone. The depth should be less than **2.5 cm** . . The finger should be held in such way that gravity assists the collection of blood on the finger tip . Massage of the finger to stimulate blood flow should be avoided. .For an infant less than 1 hear old , the lateral and medial plantar surface of the foot should be used for skin puncture. For the collection of blood on filter paper for neonatal screening, the skin is cleaned and punctured. Then the filter paper is gently touched against a very large drop of blood and allowed to soak and fill the marked area.

(C) ARTERIAL PUNCTURE

This procedure requires considerable skills and it is usually performed by the clinician, or specially trained nurse technician. The preferred site puncture are:- radial artery at at the wrist, brachial artery in the elbow, and femoral artery in the groin.

ANTI COAGULANT AND PRESERVATIVES FOR BLOOD

-If whole blood or plasma is required for analysis, an anticoagulant must be added to the specimen during collection time.

- Whole blood is rarely required for clinical chemistry tests, only for blood gases, lactate and glycosylated haemoglobin. Serum from coagulated blood is the choice for many analytes

- The use of plasma is usually required for emergency cases since the blood has to wait for 15-30 min for completion of coagulation before centrifugation.

- The other advantage is that plasma yield from a given volume of whole blood is always greater than the yield of serum.

- The only disadvantage is the formation of fibrous clots or fragments during plasma storage and subsequent risk of clogging the sample probe of autoanalyzer.

-***HEPARIN-***----is the most widely used anticoagulant for clinical chemical tests and causes least interferences . It acts by preventing the conversion of prothrombin to thrombin and thus the formation of fibrous fibrinogen. Types of heparin anticoagulant are ammonium and lithium heparin .

- ETHYLENEDIAMINE TETRA-ACETIC ACID (EDTA)---- This is a chelating agent and in concentration of 1-2 mg/ml of blood . It is used for haematological examination since it preserves cellular components of blood. It is used as disodium or dipotassium salt and the later is more soluble. It prevents coagulation by binding calcium , which is essential for the clotting process.. In clinical chemistry is used to collect blood for GLYCOSYLATED HAEMOGLOBIN (HBA1C) analysis.

- SODIUM FLUORIDE--- This is the best preservative of blood glucose, together with an anticoagulant such as potassium, sodium, ammonium or lithium oxalate, . It also acts as a weak anticoagulant. It exerts its preservative action by inhibiting the enzyme system involved in glycolysis. It is also an inhibitor of urease and many other enzymes. Glucose in blood specimen without antiglycolytic agent will decrease as much as 10 mg/dl(0.56 mmol/l) per hour at 25 ◦ C. The decrease is even more in infants due to their increased metabolic activities of rbcs and in leukemia pts due to their high metabolic activity of the leucocytes. **Sodium iodoacetate** at a concentration of 2 mg/ ml of blood is an effective antiglycolytic agent and a substitute for sodium fluoride . It has no effect on urease and can be used when urea and glucose are requested in the same specimen.. It inhibits only creatine kinase and has no significant effect on the other parameters.

HAEMOLYSIS

This is the breakdown of rbcs. Serum or plasma shows haemolysis when the [hb] exceeds 20 mg/dl. Slight haemolysis has little effect on most of the values. Severe haemolysis causes a slight dilutional effect on the constituent present at a lower concentration in the rbcs than in the plasma. However a marked effect may be obtained on those constituents that are present at a higher concentration in the erythrocytes than in the plasma. Eg potassium, alp, ldh, mg, aldolase and INpo4 . IF an electrophoresis is done on an heamolized specimen, an additional band due to hb appears= fibrinogen band..

CAUSES OF HAEMOLYSIS

- use of wet blood containers.
- Excess preservative
- Pushing through the neddle when transferring it from the syringes into blood container.
- Spending a lot of time during bleeding..
- Shaking the blood container vigorously.
- Separating the blood immediately after bleeding..

URINE COLLECTION

-The type of urine specimen to be collected is dictated by the tests to be performed.

- Generally urine for quantitative analysis must be collected over an interval of time eg 1,4, or 24 hours.

- Untimed or random urine specimen are suitable for only very few biochemical tests. A clean, early morning specimen is generally the most concentrated and this is prepared for routine examination.eg testing for unusual compounds such as beta chorionic gonadotropin.

- A clean timed urine specimen is the one obtained at specific times of the day or during certain phases of micturition. A double voided specimen is the urine excreted during a timed period following a complete emptying of the bladder . It is used to assess glucose excretion during a glucose tolerance test procedure. Its collection must be timed in relation to the ingestion of glucose.

- When specimen must be collected over a specified period of time, the patient must adhere closely to the instructions with regard to diet or medication , to avoid interference of ingested compounds with analytical procedures.

- Appropriate information regarding the collection including warnings with respect to handling of the specimen, should appear on the bottle label.
- Urine cannot be collected at the same time for two or more tests for which different procedures are required.
- The removal of aliquots is not permissible even when the volume removed is measured and corrected for , since the excretion rate of most compounds varies throughout the day and the effect of the removal of some specimen on the total concentration cannot be corrected.

URINE PRESERVATIVES

-Preservatives of urine specimens have different roles but are usually added to:-

- (i) reduce bacterial action or chemical decomposition.
- (ii) solubilize constituents that might otherwise precipitate out of solution.
- (iii) decrease atmospheric oxidationof unstable cpds.

Some specimens should not have any preservative added bcoz of the possibility of interference with analytical methods.

-NB/ one of the most satisfactory form of urine preservation is refrigeration during collection. Other preservatives are :-

(i) concentrated HCL ACID (10ML)=6 mol/L
(ii) sulfamic acid
(iii) thymol
(iv) chloroform
(v) toluene----- this is the only organic solvent that is still in use as a preservative.

- When timed collection is complete the specimen must be delivered to the laboratory without delay where the volume is measured using a graduated cylinder.

FAECES (STOOL)

-Small portions of faeces are frequently analyzed to detect the presence of occult blood (hidden) , which is recognized as one of the most effective clues to the presence of a bleeding ulcer or malignant dse in the gastro-intestinal tract. The patient is instructed to abstain from eating proteinous foods three days prior to the collection of the specimen.

- 72 hour faeces specimen is collected for the estimation of faecal fat (to diagonize malabsorption disorders).

CEREBRAL SPINAL FLIUD (CSF)

-Obtained from the puncture of the lumber region
- Lumber puncture are done by the physicians.
- it is requested where there is suspicion of a cerebro-vascular accident, meningitis, demyelinating dse, or menengial involvement in malignant dse.
- The csf specimen is collected in fluoride bottles for the analysis of micro protein and glucose.

SYNOVIAL FLUID

- Obtained from the joint to aid in the characterization of arthritis and differentiation of non- inflammatory effusions from inflammatory effusions. Normally only a small amount is present in any joint , but the fluid is increased in the presence of inflammatory conditions.. The technique of removing the fluid from the joints is referred to as arthrocentesis which is done by a physician.. The fluid is examined for proteins and glucose.
- Amniocentesis is the procedure for removing amniotic fluid for prenatal diagnosis of congenital disorders, to assess fetal maturity or detect intrauterine infection.

PLEURAL/ ASCITIC/PERICARDIAL FLIUD COLLECTION

-Pleural, pericardial and peritoneal cavities normally contain a small amount of fluid that lubricates the opposing parietal and visceral membrane surfaces.
- Inflammation or infections affecting the cavities causes the fluids to accumulate.
- The tests analyzed are proteins and glucose.
- The removal of the specimen is done by the physician and the procedure is referred to as paracentesis but when specially applied to pleural cavity is called thoracentesis and for pericardial cavity is referred to as cardiocentesis.

HANDLING OF SPECIMENS

-A quality result will depend on a quality specimen which has been well collected, preserved, transported and well identified. The information on the label should have the following:-

(i) pt name,
(ii) location
(iii) identity number
(iv) date and time of collection
(v) type specimen.

SEPARATION AND STORAGE

-Serum or plasma should be separated from the cells as soon as possible and certainly within 2 hours from the collection time. I f this is not possible the specimen should be kept at room temperature rather than at 4^{o} C to avoid haemolysis.
- Separated serum or plasma can be placed at 4^{o} C for later analysis. If analysis is to be carried out in two weeks time , the specimens can be stored at - 20^{o}C. or at - 70^{o}C if it is to be analyzed in future.
- Specimens are supposed to be centrifuged with the stoppers (caps) in place to reduce evaporation and to prevent aerosolization of infectious particles.
-

TRANSPORTATION OF SPECIMEN TO REFERENCE LABORATORY

-It should be assumed that transport from a referring lab. To a referral lab. may take as long as 72 hrs. Referring lab. should retain some specimen under optimal conditions for retesting should an unanticipated problem arise during shipment.
- for transportation of frozen or refrigerated specimen, a Styrofoam container should be used . Dry ice is the most convenient refrigerant material for maintaining specimen frozen temperature as low as -70^{o}C can be achieved. The amount of dry ice required depends on the size of container, insulation efficiency and time for freezing.

CRIETERIA USED FOR ACCEPTING OR REJECTING SPECIMEN.

(1) All specimens must be brought to the laboratory in the correct container.
(2) The must be accompanied with a duly filled request form. The following information must be given:-
(i) patient/ client name -- identification
(ii) type of specimen
(iii) location of patient/ client (address)
(iv) time and date of collection.
(v) signature and name of the person ordering the tests
(vi) urgency of the tests
(vii) the request / report form should be clean ie not soiled (if soiled reject the specimen.
(viii) specimen which is grossly haemolyzed should be rejected .

NB/ ALWAYS REMEMBER THAT QUALITY SPECIMEN MEANS QUALITY RESULTS

AUTOMATION IN CLINICAL CHEMISTRY/REAGENT KITS

-Automation in clinical chemistry is a representation of mechanization of conventional manual technics.
- The first practical automation was introduced in the clinical laboratory in mid last century.
- Factors that necessitated the introduction of automation were:-

(a) Increase in number of patient in health institutions

(b) Clinicians started ordering panel of tests in an effort of making conclusive diagnosis and this became increasingly difficult to handle using the manual techniques.

(c) Invention of new analytical methods which replaced the older methods.

- Several types of automated systems have been since mid last century. Although these systems may differ from each other in many ways, they all represent mechanization of conventional methods.
- The operating details for each instrument is best found in manuals supplied by the manufacturers.
- So far there two types of automated instruments that have been developed:-

(1) CONTINUOUS FLOW AUTO ANALYZER--obselete
(2) DISCRETE AUTO ANALYZER

(2) DISCRETE AUTO ANALYZER

- Advancement in technology has come up with this new type of automation which has virtully replaced the continuous flow auto-analyzer.
- The discrete auto-analyzer involves the treatment and measurement of samples individually.
- This system is made up of four major compartments namely:- (**a) *reagent (b) specimen (c) reaction and (d) data manager.***
- The sample and reagents are measured and delivered to the reaction compartment by the use of ***sample and reagent probes*** respectively.
- The reaction tray is composed of a multiple of cuvettes contained in the reaction compartment. This reaction tray is either detachable or inbuilt depending on the type of the instrument. Those instruments with inbuilt reaction tray wash them automatically when the analysis is over. The detachable reaction trays are removed when the specific reaction has taken place and are either discarded or washed manually.
- The sample and reagent probes will pick the exact amount of sample and reagent according to the application manual installed in the micro processor of the system.
- A delay time is set for that analysis that requires two reagents i.e. reagent two added after a specified period of time e.g. calcium.
- The start up of the auto-analyzer involves the priming, setting the standards (calibration) and running the daily internal quality control of the analytes.
- The reading of the concentration for the analytes, is achieved through spectrophotometer and ion-selective electrodes (for electrolytes analysis only.
- The results are either printed or read straight from the data manager screen.

ADVANTAGES AND DISADVANTAGES OF AUTOMATION

(1) ADVANTAGES

(a) Large number of samples can be analysed within a very short time.
(b) Two or more analytical methods may be performed simultaneously.
(c) Precision and accuracy is very superior to manual methods (techniques).
(d) Cost of reagents is minimal compared to manual methods.

(e) Very little amount of sample is required for analysis.
(f) It is very fast (improve turnaround time) therefore saves the time the health provider uses to manage a patient.
(g) Automation also helps relieve stress on technologists brought about by increased workload
(h) The manual handling of samples that may be infectious constitutes a hazard to laboratory staff, hence robotic system improves safety for laboratory staff by eliminating the exposure to pathogens
(i) boost productivity of the laboratory which could not be achieved through manual method.

(2) DISADVANTAGES
(a) The automatic nature of the machine discourages technicians from making observations regarding potential problems.
(b) These systems are only economical in busy laboratories. Small laboratories will always require the use of manual or semi automated methods of analyzing samples.
(c) These systems contribute to unemployment in developing countries
(d) The systems require placement in big rooms ie occupies huge spaces in the laboratory.
(e) The systems are very expensive to purchase and maintain.

REAGENT KITS
-There are many methods available commercially as kits. A kit is a package which contains either some or all the reagents and components necessary to carry out an analytical procedure
- Some of these reagent kits are well designed and reliable while others are not and should be discarded.
- A good reagent kit should have the following characteristics (information)
(1) The name of the reagent which corresponds to the intended analysis.
(2) An insert which should have the following information:-
(a) Clinical significance of the analyte to be investigated using the reagent kit
(b) Test principle
(c) The specimen/s of choice to be used (either whole blood, serum, plasma,urine,csf etc)
(b) Methodology
(c) Instructions on the reagent reconstitution if necessary
(d) Linearity range of the test ie the lowest and the highest value that can be achieved during analysis.
(e)Reference range of the analyte for the population used during the development of the kit
(f) Statement regarding accuracy and precision to be expected
(*g) Description of any formula to be and a sample calculation*
eg OD TEST/OD STD x [STD]
(h) Should have unit conversion factors (if indicated)
(i) A performance evaluation from a reputable laboratory
(j) The expected number of tests which can be achieved with the kit
(3) The date of the manufacturing of the kit.
(4) The expiring date of the kit
(5) The production batch number
(6) Good and user friendly packaging
(7) The storage conditions
(8) The exact volume of the contained reagents

ACQUISITION, MANAGEMENT AND APPLICATION OF LABORATORY DATA

ACQUISITION

There are several ways of acquiring clinical laboratory data. The most commonly used ways are (1) analysis of specimens using reagents and various instruments (2) physical measurements.

(1)Analysis of specimens using reagents and various instruments
This is achieved through two ways ie quantitative and qualitative analysis.The basic requirements of these methods of analysis are (a) instruments of measure (b)specimen of choice (c)appropriate reagents (d) specific analytical procedure (e)laboratory qualified personnel to carry out the analysis.
-The data produced by the quantitative analysis gives the actual amount of the analyte which is accompanied by the unit of measure.Some of the analytes requires the use of formulas to calculate the amount of the analyte in a specimen.

-The data produced the qualitative analysis gives the presence or absence of an analyte

(2)Physical measurements.
Some of the data acquired through this method are example weight, size, height.These measurements sometimes are required as factors to calculate the amount of a certain analyte eg calculation of creatinine clearance.

APPLICATION OF LABORATORY DATA
The data produced in the laboratory are used by the health provider in the following ways:-

1) **to make a diagnosis of particular pathological disorder,**
2) **gives a guideline in the treatment of a disorder,**
3) **indicates the prognosis of a disorder,**
4) **determine the prevalence of a disease,**
5) **used by a researcher to come up with research findings,**
6) **helps health planners to determine the requirements of a clinical laboratory**

To achieve the above, it is the duty of the laboratory personnel to ensure that the data generated in the clinical laboratory is accurate and precise. This is achieved through the use of quality control measures.

MICROPROCECESSOR'S AND COMPUTERS IN CLINICAL CHEMISTRY LABORATORY

The computers have completely revolutionized the handling of clinical laboratory specimens data. The increase in the number of laboratory tests performed in addition to the trend to centralize laboratory services, has lead to significant management problems in handling and identifying specimens submitted for analysis. Furthermore the critical nature of the data involved requires an accurate flow of laboratory information between other areas of the hospital, including wards, doctors office, medical record room and the accounts office. Thus it is necessary to consider the entire patient-care process and the effect the computer has on all associated areas, as well as the place it occupies in the entire hospital communication network.

COMPUTERIZED LABORATORY INFORMATION SYSTEM
In a computerized laboratory information system(LIS), the flow of information passes through the central processing unit and then into the memory, where it is available for inquiry and reports. All information is entered into the computer through terminals or machine readable forms. In this interactive mode the computer is used to manage the data

transactions through appropriate soft-ware –controlled prompts and edits. Requisitions, logs and workbooks are used as backups on those occasions when the computer is inoperative for prolonged period of time.
In an LIS , entry of information related to patient's identification, admission, transfer, discharge ,test requests and specimen collection is a function performed by non-laboratory personnel.In institutions with hospital information system (HIS), this information is automatically transmitted
To LIS through an electronic interface and communication protocol. Usually test results are returned through LIS to HIS for inquiry and reporting in the ward, wheras billing information is transferred to the hospital financial management system(FMS).

PLANNING A LABORATORY INFORMATION SYSTEM

LIS integrates all aspects of the laboratory, medical staff and hospital that are concerned with laboratory testing. Since the installation of LIS is major commitment of efforts and money, a feasibility study should first be conducted to determine the technical and economic practicality of replacing the existing manual system with a computer system. The feasibility study may show that improvements in the manual system are sufficient to correct existing problems and bring about enhanced services, in which case the plans for an LIS should be abandoned. On the other hand , if an LIS appears preferable , the next step is writing specifications for LIS . These specifications must be detailed and specific, addressing the needs of the laboratory as well as the rest of the hospital and medical staff. If there is an existing HIS , a computer interface specification is included in the planning process.

The Laboratory Database

A major advantage of the LIS in a medical facility is that it establishes standardized laboratory nomenclature and standardized information processing procedures. The basic unit of information in an LIS file is called a ***data element.*** Example of data in a patient's data element:-number, name, date of birth, sex, admission date, admission status, location, bed/room, physician name, diagnosis, discharge date, financial status, guarantor's name and billing number.

STATISTICAL ANALYSIS IN CLINICAL CHEMISTRY

INTRODUCTION

Statistical methods are required to quantitate some of the aspects of quality assessment i.e. precision and accuracy. The fundamental basis for any statistical analysis is variation, and there is plenty of that in laboratory values. The variation may be due to decomposition of standards or reagent batch differences in reagents, instrument failure, technical errors or error in calculation or transcription of results. Sometimes the control materials itself may change. Fortunately a limited number of straight forward statistical concepts substantially deal with most of the problems of variation in clinical chemistry. These concepts are mean, standard deviation and coefficient of variation.

MEAN

The mean or arithmetic average $(\bar{x})$ of a set of individual measurements (x_i) is given by

$$\bar{x} = \frac{\Sigma X}{n}$$

where n is the number of the measurements
$\bar{x}$ is the mean
Σ is the sum of the measurements
x_i is the individual measurements

STANDARD DEVIATION

Standard deviation (SD) measures precision. It is essentially an expression of average error or average variation. A small SD indicates that the measurements are grouped closely about the mean, whereas a large SD indicates that there is a wide variation among the individual measurements. It is calculated as follows;

$$SD = \sqrt{\Sigma \frac{(x_i - x)^2}{n-1}}$$

STANDARD ERROR OF THE MEAN

Standard error of the mean is the SD of the distribution of the mean. For example, if we select several samples from the same population , measure the same quantity in all cases, and compute, eg the mean for each sample, we obtain several estimates with some variation around the unknown population mean. A statistical measure of this sampling variation of the means is provided by the standard error of the mean. It is denoted by $\mathbf{S}_x$ OR SE and calculated using the following formulae:-

$$\mathbf{S}_x = \frac{SD}{\sqrt{n}}$$

$\mathbf{S}_x$ = divide the sample standard deviation by the square root of the sample size

COEFICIENT OF VARIATION

The ratio of the SD to the mean is called the coefficient of variation. Usually it is expressed as percentage by multiplying by 100

$$CV = \frac{SD \ X \ 100}{Mean}$$

The CV is a useful working guide to the precision of a test

CONFIDENCE INTERVAL OF THE MEAN=(CI)

The approximate 0.95 confidence interval of the mean is bounded by a lower limit($x-2\mathbf{S}_x$) and an upper limit ($x+2\mathbf{S}_x$). The population mean is located within these two limits with a confidence of approximately 95%

EXERCISE

(1)The following is internal QC for albumin in the octomber 2010 (20,20,21,20,20,19,19,20,17,16,21,18,18,18,17,20,22,20,22,20,18,19,17,18,20,20,21,21,22, 21,20,19) mmol/l. calculate the mean(x), standard deviation(std) and CV for the whole month.Compare your results with the assigned mean=19 mmol/l, assigned STD=1.5 and assigned CV=7.8%

(2)Using the following data of urea obtained from a given population, calculate, the mean, standard deviation using the two formulaes, SE,CV, CI(Lower and upper limits). (4,2,2,4,2,4)mmol/l

NON-PROTEIN NITROGEN

The term non-protien nitrogen in blood is used to include the nitrogen from all nitrogenous substances other than protein. In urine not having protein, it is equal to the total nitrogen. Urea is the most important of the substances which make up the non-protein nitrogen others are uric acid, creatine and creatinine, amino acids, ammonia and some peptides.

EVALUATING AN ANALYTICAL METHOD

Evaluation studies on an analytical method are performed to test critical reliability characteristics. In general, a preliminary period should be allowed for familiarization with the analytical method, equipment and procedural steps. This may involve trial runs to determinine within-run precision and analytical range. Once the analyst is satisfied that the method can be performed without any procedural difficulties, studies on recovery and interference can be started. Day to day precision should be determined by using at least a four week period for the experiment. Accuracy should be tested by a comparison of methods experiment, using a reference method with established and satisfactory accuracy and precision. The acceptability of the new method is determined from the experimental results by estimating the observed analytical errors, then comparing these errors with the defined allowable limits for the errors. When the observed errors are less than the defined allowable errors, the method can be implemented for routine services.

STEP BY STEP OUTLINE OF A METHOD EVALUATION STUDY

(**1**).Several analylists (technologists), not just the most capable individuals should be assign to carry out the testing procedure.

(**2**).Reagents kits, instruments and all necessary materials and supplies should be obtained. The instructions on the kits reference literature should be followed. Tests are done using quality control specimens and some clinical specimens. This step is repeated several times for several days for familiarization purpose.

(**3**).determine within run precision by analyzing 10-20 aliquots of control material or a single clinical specimen. Contols or clinical specimen within extreme concentrations should be used ie high or low concentrations.

(**4**).Determinine the analytical ranges ie lower and upper analytical ranges and compare this with what has been indicated in the kit. A set of standards or a series of dilutionsof either control or an elevated specimen pool can be used.

(**5**). Determine recovery strength by adding known amounts of the analyte in concentrated form to actual clinical specimens to prepare a new test specimen. Analyse the new specimen and compare the results with the results of the original specimen.

(**6**). Test the interferences of common substances such as bilirubin, heamoglobin, lipids, anticoagulants, common drugs kwown to cause problems during the determination of the particular analyte being measured.

(**7**).Determine other specific characteristics that may be critical for a particular test method , such as sample interaction due to inadequate washout of pippeting divices or cuvetes on certain analytical automated instruments. This is tested by introduction of a known high concentrated specimen followed by a known low concentrated specimen. Results are compared with the original known results to find whether there is any difference in the result for the known low concentrated specimen.

(**8**). Determine day to day precision by analyzing serum pools or control materials each working day over a period of one month.

(**9**). Compare the results of the evaluated method with those of the reference analytical method. The difference in the two methods is considered as the analytical errors. The lower the percentage the better the method.

(**10**). Prepare a report that summarizes the experimental results and data analysis. Carefully review and interpret the data and determine whether or not the method is acceptable. Incase of no previous method of analyzing the particular analyte in the laboratory, a conference with clinicians may be useful to help assess the clinical utility of the method.

(11) Prepare a standard operating procedure for the method. Prepare in-service training to inform the others about the performance of the test method.

(**12**) Inform the institution about the availability of the test.

(**13**) Encourage staff feedback during the introductory phase of the new test. Monitor performance carefully during the first several months of use.

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QUALITY ASSURANCE IN CLINICAL CHEMISTRY

-All laboratory staff must be conscious of how the quality of their work affects medical diagnosis and treatment of patients.
- The staff should ensure that the product of the laboratory ie tests, are of good quality and beneficial to the clients (patients) and health providers.
- Erroneous laboratory data (results) can lead to unnecessary medical treatment, prolonged hospital stay and missed or wrong diagnosis.
- In order to avoid all these, it is important therefore to introduce a system which will control the quality of data (results) produced in the laboratory.
-The system put in place is referred to as ***quality assurance system*** .
- Quality assurance system is a broad spectrum of plans, policies and procedures that together provide an administrative structure for laboratory efforts to achieve quality goals.
- A quality assurance program involves virtually everything and everybody in the laboratory. An error in any step during acquisition, processing and analysis of specimens and the reporting of a laboratory test result can invalidate the quality of the analysis and cause the laboratory to fall short of its quality goals.
- There are several essential elements of a good quality assurance program. These are:- ***commitment, facilities and resources, technical competence, quality assurance procedures, problem solving mechanism.***

(1) COMMITMENT

- Dedication to quality service must be central. Quality must be a major consideration in all management decisions because any single decision may compromise other plans and practice for attaining quality goals.

(2) ***FACILITIES AND RESOURCES,***

-laboratories must have adequate space , equipments , materials, supplies, staff and supervisory personnel and budgetary resources. The facilities and resources are the basis upon which quality services can be developed and maintained.

(3) ***TECHNICAL COMPETENCE***

High quality personnel are essential for high quality services. The educational background and experience of all personnel are important as is the capability for providing in-services training to develop and maintain skills and knowledge.

(4) ***QUALITY ASSURANCE PROCEDURES***

- This can be divided into three groups:-
(a) Control of pre-analytical variables;- such as test request, patient preparation and identification, specimen acquisition and transport, specimen processing and distribution, preparation of work list and record maintainenance.
(b) control of analytical variables:- This includes analytical methodology, standardization/calibration procedures
(c) monitoring of analytical quality by the use of statistical methods and QC charts.

(5)PROBLEM SOLVING MECHANISM

This involves the link between the identification of a problem and the implementation of a solution to solve that problem. In this case of problem solving, teams must be established

ACTIVITIES INVOLVED IN A GOOD AND COMPLETE QUALITY CONTROL PROGRAM

- These interrelated activities includes:- assessment, maintenance and improvements

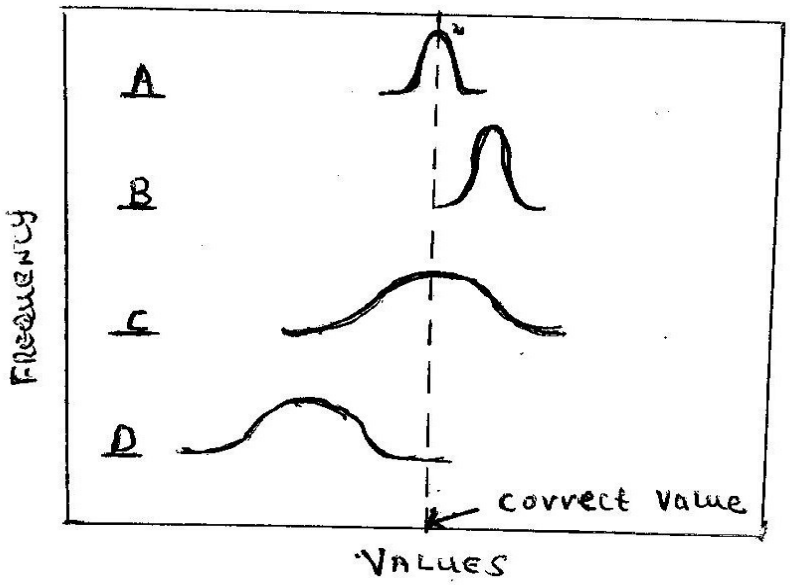
(A) ASSESSMENT

- For an operational laboratory, the quality data being produced must be assessed and evaluated. Two terms that are important in this regard are ACCURACY and PRECISION

ACCURACY
- This refers to how close a measurement is to the true value. This true value is also referred to as the actual, correct, most expected, the target value or the right value.
- The closer the measured value is to the true value, the greater is the accuracy.

PRECISION
- This refers to how close together are multiple measurements of the same sample. The question here is whether the same result will be obtained when the test is repeated.
- Precision can also refer to reproducibility or the agreement between replicate measurements. This means that the results of repeated analysis on the same sample are all very close to each other .
- It is worth to note that demonstrated precision is not a guarantee of accuracy

The concept of accuracy and precision can be illustrated in the figure below:-



In the figure above, curves A,B,C and D are plotted from groups of data obtained from analyzing a single lot of control serum.

A= group A shows data which are tightly grouped with the mean lying on the correct value . Therefore these results are both accurate and precise.

B= In group B , the grouping is also good but since the mean is not close to the correct value , the accuracy is poor.

C= In group C the data are spread over a wide range of values indicating poor precision. However, since the mean value coincides with the correct value, the overall accuracy is good.

D= In group D, the data are widely scattered and the mean is not near the correct value. This indicates a lack of both precision and accuracy

MAINTENANCE AND IMPROVEMENT

-To ensure quality data from the laboratory, accuracy and precision must always be maintained. This is achieved by the use of quality control materials. The quality control material must always be analyzed before any analysis of specimens is commenced.
- Improvement of a good quality control program is achieved by taking the necessary remedy whenever anything that can compromise quality is detected.
- the laboratory must have quality reagents , good working equipments and skilled personnel in order to maintain and improve on the quality of test results produced by the clinical laboratory.

QUALITY CONTROL MATERIAL

-A quality control material is a tool for assessing accuracy and precision in any given laboratory.
- This quality control material is either obtained from well recognized reagents companies working in collaboration with well known and recognized laboratories. A quality control material can also be made from pooled materials in a clinical laboratory.
-Commercial quality control material is usually lyophilized serum or urine from human or animals. Many companies prefer using animal sera to make the QC material since it is easy to make and less expensive. The advantage of using this type of commercially prepared QC material is that the safety of the user is guaranteed.
-Sera from bovine, equine and porcine can be used to make the QC material . Among these sera , bovine sera is commonly used because it is readily available and also less expensive. Although some levels of the analytes are not approximately those clinically useful, the necessary adjustments may be done through additions or simple dilution.
- If the sera is from human, it is usually screened for the blood borne pathogens eg HIV, HEPATITIS, SYPHILLIS ETC. Despite this assurance, the QC material should be handled with care since it is not 100 % safe. It is also difficult ethically to justify the use of human donors to supply large quantities of general purpose control serum for clinical laboratory purposes.
- Another advantage o using the commercially prepared QC sera is that the potency is guaranteed until the expiring date
- Several types of QCmaterials may be prepared depending on the need, cost and purpose (ie for internal or external QC).These are (a) liquid (b) urine based (c) lyophilized (powder form of serum or urine).
-In many cases three levels of concentrations are recommended for QC materials to be used for both internal and external QC programmes. The material is prepared in such way that there are two different formulations of normal and abnormal concentrations (abnormally high or abnormally low). These concentrations are based on the expected values from a given human population. Each of these may be prepared through the necessary adjustment required. High concentration may be obtained by the addition of the respective analytes while the low concentrations may be achieved through dilution.
- A commercially prepared QC material is usually accompanied with a very informative literature which includes the following:-

(a) the name of analyte
(b) method of analysis
(c) target value(mean value)
(d) control range(Lower and upper QC values ie within the +/_2 SD)
(e) date of manufacture

(f) expiring date
(g) reconstitution method

QUALITY CONTROL PROGRAMS

-There are two types of quality control programs that are recommended for every practicing clinical laboratory. These are:- (a) INTERNAL QC program (b) EXTERNAL QC program

-INTERNAL QC PROGRAM (IQC)

DEFINATION

-This can be defined as the set of procedures undertaken in a laboratory for the continual assessment of work carried out within the laboratory and the evaluation of the results of tests to decide whether the latter are reliable enough to be released to the requesting health provider.

CHARACTERISTICS

(a)-must be done always when an analytical test is being carried out.
(b)- must be done before the patients specimens are analysed
(c)-when many specimens are set for analysis, it can be included within the run.
(d) –has a lower and upper value ie has a given range which represents mean +/_ 2 SD
(e)- must be treated the same way as test sample during the analysis.

IQC Results representation

- The results for the day are represented on a ***levy jenning*** *QC chart as demonstrated using the example below*

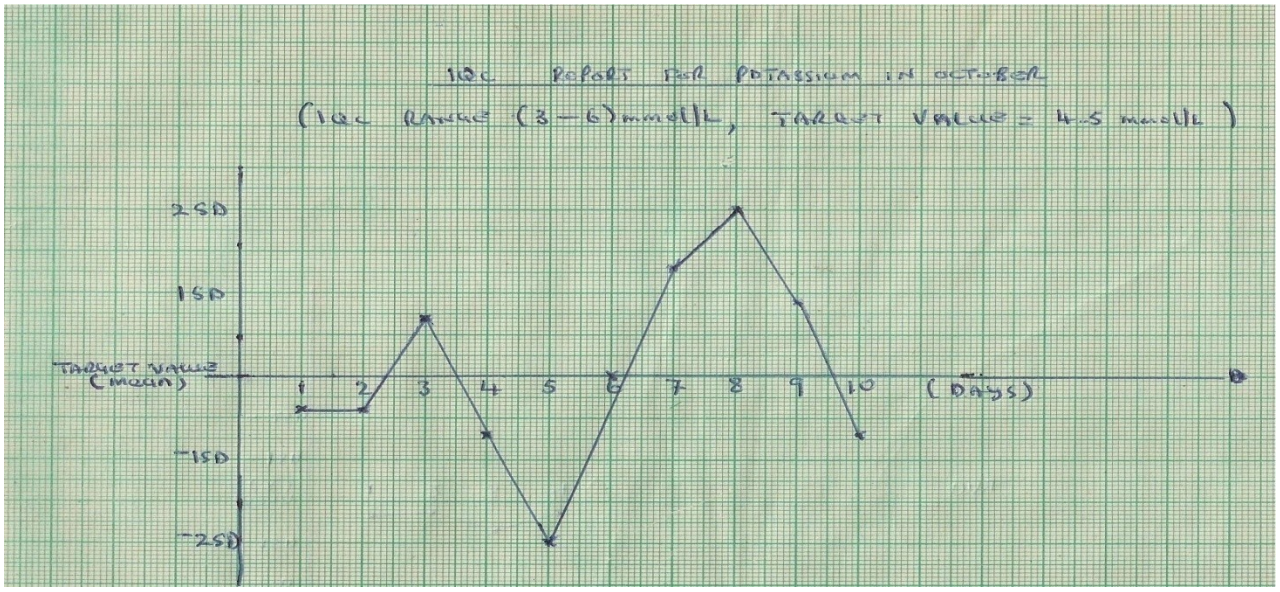
EXAMPLE

The following are the IQC results for potassium a the month of October: day 1=4.2mmol/L, day 2=4.2mmol/L,day 3= 5 mmol/L, day 4=4 mmol/L, day 5 =3 mmol/L, day 6=4.5mmol/L, day 7=5.5mmol/L, day 8=6 mmol/L, day 9=5.2mmol/L, day 10= 4 mmol/L). The IQC target value is 4.5 mmol/l and the control range is (3 up to 6) mmol/L.

Step one-----is to calculate the value of 1SD.

Target value ± 2 SD=X ± 2 SD= 4.5± 2 SD
4SD=(6-3)
4 SD=3
1SD= ¾=0.75
DAY 1 =4.2-4.5= -0.3(NB/ the negative sign indicates which side of the mean (target value) is the QC value)
1SD=0.75
? = -0.3SD
-0.3 divide by 0.75= -0.4 (ON the lower side of the target value)

Nb/SD FOR EACH OF THE OTHER DAYS IS CALCULATED USING THE EXAMPLE GIVEN ABOVE. (The SD are as follows: day 2= -0.4, day 3=0.7 and day 4= -0.7, day 5= -2, day 6= 0, day 7= 1.3, day 8=2, day 9= 0.9,and day 10= -0.7).
-The above SD are represented on a Levy Jennings QC chart given below.



EXTERNAL QUALITY CONTROL(EQC) PROGRAM

DEFINATION

- This is a retrospective comparison of performance with a pre-set criteria or with other laboratories. The aim of EQC is to assess the degree of harmonizationof results between clinical laboratories_

CHARACTERISTICS

(a) This is organized by an external agency that may be nationally or internationally based.

(b)Lyophilized material is suitable for this purpose

(c) the EQC material can be of two forms ie blind or known .Blind material (blind QC serum) means that the QC ranges are not given but are determined by the participating laboratories .It is upon the results given by these laboratories that their performance is determined by the external agent. On the other hand the known qc is similar to the internal QC but the results have to be sent back to the external agent for statistical analysis and interpretation .

(d) –the EQC materials are analyzed periodically eg once in every 3 months

(e) –all participating laboratories are expected to perform all the requested tests and send the results back to the organizing centre(agent)

Xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx29/9/15/

ELECTROPHORESIS

-Electrophoresis refers to migration of charged solutes or particles in a liquid medium under the influence of an **electric field (electric current**).
- The widely practiced type of electrophoresis is the zone electrophoresis which refers to the **migration of charged macromolecules** in a porous supporting medium such as **cellulose acetate paper** or **agarose gel film**.
- The end product of zone electrophoresis is an **electrophoretogram**, which is a display of protein zones, each one sharply separated from neighbouring zones on the electrophoretic support material..
-Solutes analyzed by this technique in clinical chemistry laboratory are mainly macro-molecular in size and colloidal in nature, they include:- protein in serum, urine, csf and other biological fluids as well as protein in rbcs and tissues

THEORY OF ELECTROPHORESIS
- Chemical species carrying an electric charge by virtue of ionization will move either to the cathode or to the anode in an electrophoresis system, depending on the kind of charge on the molecule
- The kind of solutes separated by use of this technique are ampholyte and are said **amphoteric in nature** ie they can either be **positively or negatively charged depending on the ph of the solution they are contained in.** These solutes have an **iso-**

electric point (PI) ie a point where the **solute has no charge**. These types of solutes can also be referred to as **zwitterions.**

TYPES OF ELECTROPHORESIS

There are six types of electrophoresis namely:-

-(1) Paper electrphoresis(PE), (2) Agarose Gel Electrophoresis (AGE), (3)Cellulose Acetate Electrophoesis (CAE),(4) Polyacrylamide Gel electrophoresis (PAGE), (5)Starch Gel Electrophoresis (SGE), (6) Isoelectric Focusing(IEF).

-Among the above mentioned types of electrophoresis the most widely used in clinical chemistry laboratories for routine separation of solutes (particles) are agarose gel and cellulose acetate electrophoresis.

-(1)Paper electrphoresis(PE)

-This was once widely used in clinical laboratories for the separation of serum proteins but has been replaced by cellulose acetate or agarose gel electrophoresis.Significant disadvantage of the use of paper electrophoresis include the long separation time (14-16hrs), excessive background(tailing),and decreased resolution.The advantages of paper electrophoresis were, high tensile strength, low cost, and ease of handling.

(2) Agarose Gel Electrophoresis (AGE)

-This method has been successively applied to the analysis of serum proteins, haemoglobin variants, lactate dehydrogenase isoenzymes and lipoproteins fractions.

-This medium parallels cellulose acetate interms of versatility, convenience and applicability to routine clinical demands.

-Agarose gel has a low affinity for protein and has very little effect on migration rate . Since it is clear after drying, it permits excellent densitometric examination.

-Usually 0.5-1.0 g of agarose /dl of buffer provides a gel with the desired strength and with good migration properties.

-There are two application methods of agarose gel electrophoresis:-

(A)Dissolving serum into warm agarose and applying it directly into precut or precast wells on the agarose gel medium.The precut method has an advantage in that it causes an uneven surface at the sample application points and an artificial peak in densitometry. The precast method is less convenient but it has an advantage in that the agarose –sample solution solidifies to become part of the agarose support.After application, the gel is introduced into the electrophoretic tant for electrophoretic process which takes 30-90 minutes

(B) The other alternative method uses a thin plastic template that has small slots corresponding to sample application points.The template is placed upon the agarose surface and 5 ml samples are introduced to each slot. The serum is allowed to diffuse into the agarose for 5 minutes, the excess is removed by blotting, and the template is then removed from the agarose surface and placed onto the electrophoretic tank for electrophoresis process which takes 30-90 minutes

(3)Cellulose Acetate Electrophoesis (CAE)

-When hydroxyl (OH^-) groups of cellulose are reacted with acetic anhydride, cellulose acetate is formed.This acetylated cellulose forms the raw material for cellulose acetate membranes.The membranes commercially available contain about 80% air spaces in the form of pockets within the interlocking cellulose acetate fibres. The membranes as purchased are dry, opaque, brittle films that crack easily if not handled gently.When the film is placed in the buffer, the air spaces fill with liquid and the film becomes quite pliable. Characteristics of specific membranes vary with the extent of acetylation, the prewashing procedure employed by the manufacturers, and the additives used, as well as the pore size

and thickness of the membrane. Cellulose acetate that has been especially prepared to reduce clectroendosmosis is also commercially available.
NB/The procedure is given below as a representative of electrphoresis process.

4) Polyacrylamide Gel electrophoresis (PAGE)
-Protein electrophoresis on paper, cellulose acetate or some agarose gels yields only five to seven zones owing to diffusion during the electrophoretic procedures. Polyacrylamide , or starch gel , ***electrophoresis commonly yields 20 or more fractions*** and is therefore widely used to study individual serum proteins, particularly genetic variants and isoenzymes.
--PAGE technique employs layers of gel that differ in composition and pore size. Because of the **discontinuities** of the electrophoretic matrix and the **discoid shape** of the separated zones of protein, this method is sometimes reffered to as **disc electrophoresis**.
- Polyacrylamide Gel is thermalstable, transparent, strong, and relatively chemically inert, it can be made in a wide range of pore sizes to optimize particular separations.This gels are uncharged, thus eliminating electroendosmosis.It is also used in isoelectric focusing method.

(5)Starch Gel Electrophoresis (SGE)
-SGE, like PAGE seperates macromolecular ions on the basis of both surface charge and molecular size. Partially hydrolyzed starch is used , since native starch does not gel. Starch gel may be used in a horizontal process or with migration taking place in the vertical direction. Proper preparation of gel is relatively difficult and requires considerable skill. The starch concentration is 10-16 g/dl and the ph of the buffer varies according to the specific seperation

(6) Isoelectric Focusing(IEF).
-Isoelectric focusing is a further advance in the electrophoretic separation of proteins into discrete bands. The medium provides a stable ph gradient by distributing carrier ampholytes along its axis. The compounds to be separated (eg proteins) migrate to the zone in the medium where the ph is equal to the isoelectric point (pI) of each compound.In that zone, the charge on the protein becomes zero and migration ceases.With IEF , separated protein zones are very sharp because the pI of a protein is confined to a narrow pH range and because diffusion of the protein is counteracted by acquisition of charge as it moves away from its (pI) position.
- Several support media have been used for IEF including polyacrylamide, agarose and cellulose acetate. PAGE-IEF is widely used in analytical work.

PRINCIPLE OF SERUM PROTEIN ELECTROPHORESIS

-Protein molecules in an alkaline medium (PH 8.6) are negatively charged and when subjected to an electric current will migrate towards the anode (positively charged side) of the electrophoretic system. The migration will progress within a supporting medium, for a standard period of time. The protein molecule will be separated into its various charged particles namely : **albumin, alpha 1-, alpha 2-,beta- and gamma globulins**

FACTORS THAT CONTROL THE MIGRATION OF PROTEIN MOLECULES

(1)*Net electrical charge of the molecule*----molecules with more net electrical charge will migrate faster than the ones with less. The net charge is directly proportional to the electrophoretic mobilityof the protein molecules.

(2) *Size and shape of the molecules*-----protein molecule migration is inversely proportional to the size and shape of the protein molecule. Increase in the size of protein molecule will decrease the migration .Irregular shaped protein molecules will migrate slower than regular shaped protein molecules.

(3) *Viscosity of the medium (electrophoretic buffer)*---- migration of protein molecule will be faster in medium of low viscosity than that of high viscosity.

(4) *Strength of the electrical field*--- The current applied should be enough to cause the migration of the charged protein molecules.. In a low electrical field the migration will be less

(5) *Temperature of operation*-----The temperature in the electrophoretic tank should be enough **not to cause evaporation** of the buffer.

(6) *Buffer properties (supporting medium properties*) ---the buffer used in electrophoresis has two-fold purpose: **a) they carry the applied current**, b) **they fix the Ph at which the electrophoresis is carried out (ie they determine the electrical charge on the solute).**

- The ionic strength of the buffer determines the thickness of the ionic cloud surrounding the charged molecule and thus also the rate of migration and the sharpness of the zones. Increasing the ionic strength by increasing the concentration of the buffer ions causes an increase in the ionic cloud around the charged molecule, and the molecule becomes more hindered in its movement. Buffers of high ionic strength yield sharper separations of bands, but the benefits of sharper resolutions are diminished by the Joule (heat) effect that leads to denaturation of heat-labile proteins.
- The most widely used buffer systems in electrophoretic procedures are **barbital buffers** (ionic strength=0.025-0.075) and **tris buffers** (ionic strength=0.03-0.12).The advantage with these buffers is that the base of both is **monovalent therefore any association between buffer and protein is minimal.**

ELECTROPHORETIC MOBILITY EQUATION

The equation expressing electrophoretic mobility is a general formula that is derived from two formulas, one describing the forward motion towards the electrode of charge opposite to that on the molecule, and the other describing the retarding force due to frictional resistance:

$$m = C/ķrv$$

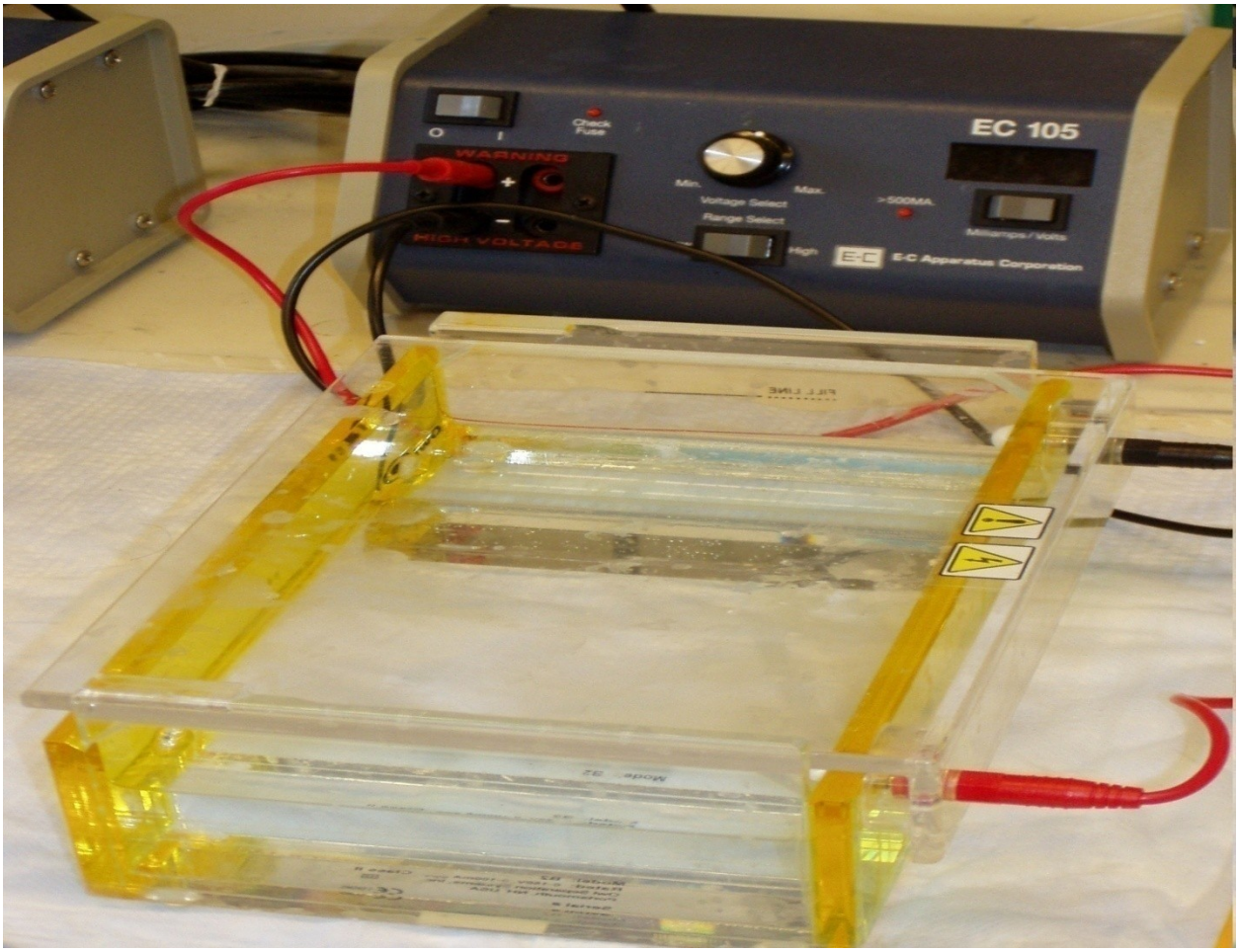
where

m= electrophoretic mobility, cm/(V)(sec)
C= net charge on the molecule
ķ= a constant
r = ionic radius of the migrating molecule
v = viscosity of the solution in which migration is occurring

PROCEDURE FOR PROTIEN ELECTROPHORESIS

(a) materials required:
-sample (serum,urine,plasma,or csf)
- electrophoretic apparatus including the power pack(supply)
- electrophoretic supporting medium(cellulose acetate paper or agarose gel)
-buffer solution (barbital (sodium barbital) or tris buffers

- stain eg ponseau S, methylene blue or silver nitrate



<u>METHODOLOGY OF ELECTROPHORESIS USING CELLULOSE ACETATE PAPER AS AN EXAMPLE</u>

(1) A reasonable size of hydrated support material (cellulose acetate paper) is placed on the electrophoresis chamber. It acts as a bridge between the anode and the cathode chambers of the electrophoretic tank. The hydrating medium is the buffer to be used in the procedure. Note that the excess buffer is removed by placing the cellulose acetate paper (hydrated) on a piece of filter paper.

(2) The two chambers of the electrophoretic tank are filled with the buffer.

(3) filter papers are used as contacts of the buffer (PH 8.6) and the cellulose acetate paper on the bridge.

(4) a capillary tube is used to place approximately 5 ul of sample on the cathode side of cellulose acetate paper.

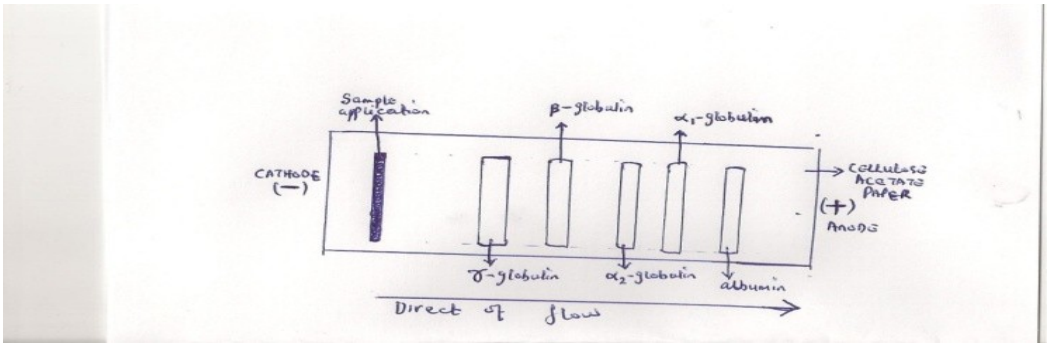
(5) A constant voltage of 100 V is set and a current of 4 m A is applied. The total current will depend on the number of cellulose paper placed on the bridge.

(6) Electrophoressis is conducted for a determined length of time ie (20 min-1 hr)

(7) A cover is placed on the electrophoretic tank to prevent evaporation of the buffer and drying of the cellulose paper
(8) After 1 HR , the current is stoped and paper removed from the tank and placed in a staining trough containing the staining reagent (ponseau S) for 5 minutes.
(8) After 5 minutes the stained paper is placed on a 2nd trough containing 3 % acetic acid solution for 5 minutes to remove excess stain.
(9) After 5 minutes the paper is placed in another trough containing another shade of 3% acetic acid until time desired for reading
NB/ The advantages of this procedure (CAE) are the speed of separation (20 min-1 hr) and the stability of the cleared membranes when stored for long periods.

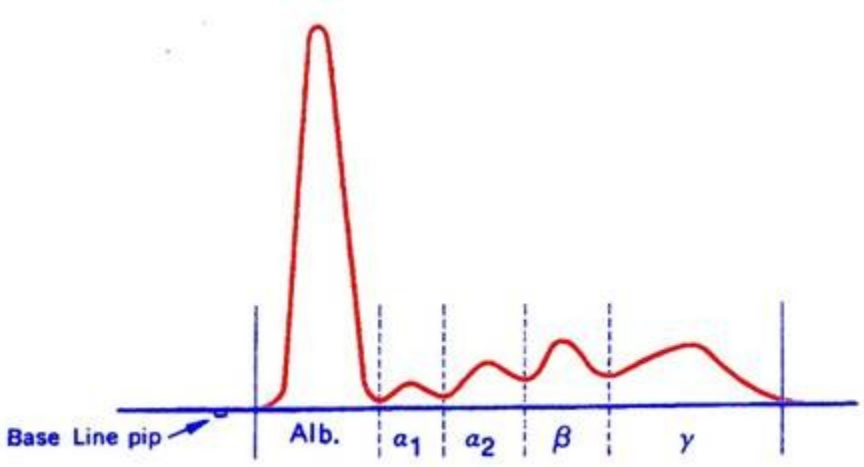
INTERPRETATION.
- The result of protein electrophoresis is an electrophoretogram. In a normal serum , five protein bands are expressed. The expression from the fastest to the slowest is as follows: albumin,alpha 1 globulin, alpha 2 globulin, beta globulin and gamma globulin.
- Another important factor to consider in the interpretation is the intensity of the stain which is directly proportional to the amount of specific protein.
- Densitometry is another method used for interpretation. It involves the use of a densitometer which give the location and intensity of the zones (bands) as successive peaks on a recorder chart. In some densitometer the area under the peak is automatically integrated
- Another method of interpretation is the ELUTION method. This involves cutting of the specific bands and eluting the stain using suitable solvents such as basic buffers or alcoholic solutions.

-Appearance ***of a normal electrophoretogram***



Densitometry

Zones (bands) as successive peaks on a recorder chart



THINGS TO NOTE

(1) urine from patients suspected to have multiple myeloma can be used as a specimen for electrophoresis. In such case , the urine must be centrifuged and the deposits used for inoculation into the support medium. A positive report is indicated by the appearance of only one electrophoretic band called **M band or MYELOMA BAND**.

(2) IF plasma is used instead of serum, a sixth band appears and this is the **fibrinogen band.**

(3) it *IS A GOOD LABORATORY practice TO INCLUDE A CONTROL SERUM IN EVERY ANALYSIS*

LIMITATIONS AND ERRORS IN ROUTINE ELECTROPHORESIS

Limitations and errors in routine electrophoresis

Wick flow

-Heat generated causes evaporation of solvent from the electrophoretic support

-Drying effect causes buffer to rise from both tank compartments

-Flow of buffer from both directions affects protein migration

Electroendosmosis

-Some electrophoretic support media take the negative charge due to adsorption of OH^- when in contact with water

-Since the ions are fixed on the surface of the electrophoretic support they are rendered immobile relative to the other ions in solution

-Positive ions in solution cluster about the fixed negative charge ions sites forming an ionic cloud of mostly positive ions

-application of electric current causes the positive ions in ionic cloud to migrate towards the negative side of the system thus affecting the flow of the intended negatively charged solutes

-electroendosmosis strong medium (eg cellulose acetate paper and agarose gel) and with minimal electroendosmosis(starch,polyacrylamide gel ,highly purified agarose**)**

Buffers storage

growth of micro-organisms when stored at room temparatures and effect of electrolysis of water during electrophoresis

-Amount of stain solution
Recommended volume approximately 100ml for 387cm^2 of cellulose acetate or agarose film, tightly closed to avoid evaporation

Sample application
Amount applied must be optimal and small enough to avoid overloading

ELECTROCHEMICAL TECHNIQUES IN CLINICAL CHEMISTRY

-These techniques make use of electrodes to determine the concentration of an analyte in a sample
- An electrode is a electron or a metallic conductor that is in contact with an electrolyte solution.
- The techniques that fall under electrochemical phenomena includes-POTENTIOMETRY, POLAROGRAPHY, AMPEROMETRY and CONDUCTOMETRY.

DESCRIPTIONS OF THESE TECHINIQUES

(A) POTENTIOMETRY,

-This is the measurement of the electric potential difference between two electrodes in an electrochemical cell. An electrochemical cell always consists of two electrodes (electro or metallic conductors) connected by an electrolyte solution (ion conductors).
-An electrode OR half cell consists of a single metallic conductor in contact with an electrolyte solution.
- An ion conductor (electrolyte solution) can be composed of one or more phases in direct contact with each other or they can be separated by membranes that are permeable only to specific cations or anions. One of the electrolyte solution is the unknown (or test solution), which may be replaced by a standard solution for calibration or quality control solution.
- Some of the electrodes used in potentiometry techniques are as follows:-

(1) REDOX ELECTRODES---These are used in the redox chemical reactions and some of the specific electrodes under these includes: (a) inert metal electrodes eg gold and platinum which make the hydrogen electrode which is used in ph measurement. (b) silver/ silver chloride electrode which is used in the measurement of chloride.

(2) ION SELECTIVE ELECTRODES (ISE)

-These electrodes are made up of membranes which are permeable to selected anions (-ve) and cation (+ve)._

-For analytical application, the membranes are required that possesses a selective permeability to a single ion species. Eg In the estimation of sodium, the ISE used only selects the sodium ions in a forest of other ions. The same will occur with the other ions.

-In application, the selective membrane is usually between two solutions ie one on the left(A) and the other on the right (B) as illustrated below:-

(A)/ MEMBRANE/ (B)

The membranes potential can be defined as the potential on the right minus the potential on the left. This potential difference is compared using a suitable reference electrode The membrane potential is given by the **NERNST EQUATION**

$V=V^1 + N/Z.$ l og a (1) where:- V= MEMBRANE POTENTIAL, V^1= CONSTANT , N/Z= NERNST SLOPE AND a (1)= activity of diffusible ion

-In other words, the membrane potential is directly proportional to the logarithim of the activity of the diffusible ion in the test solution.

- The sensitivity of the electrode is given by the nernst slope (N/Z). Low values of electrolytes indicate poor electrode performance due to malfunction or poor selectivity. Selectivity of an electrode is the capability of an electrode to pick the specific ions in the presence of other ions.

-ISE electrodes come in **different designs eg bulb shape, inverted bulb shape, capillary, needle type, flow through.**

- Available ISE can be classified as (a) glass electrode, (b) solid state electrodes (c) liquid ion exchange.

(a**) glass electrodes**----These are made from specially formulated glass eg silicon oxide with added oxides of other metals. They are used for the estimation of hydrogen, potassium, sodium and lithium ions.

(b) **solid state electrodes**—T hese are made of crystals with embedded active substance on an inert matrix eg silver chloride used for estimation of chloride and fluoride electrodes used for the estimation of fluorine .

(c**) Liqiud ion exchange membrane**-------consists of an inert solvent and ion –selective carrier substance dissolved in it eg (i**) potassium** ion selective membrane is made up of dissolved VALINOMYCIN antibiotic in a suitable inert solvent. **VALINOMYCIN** is a neutral carrier that binds K^+ in the centre of a ring of oxygen atoms. This membrane is highly selective to K^+ ions. (ii) **AMMONIUM ION** selective membrane is made up of the mixture of antibiotics **NOACTIN** and **MONACTIN.(both are neutral carriers).**

NB/ (1)The ion selective electrodes are incorporated in the autoanalyzers ISE modules for electrolytes estimation. This can also be seen being applied in simple machines for electrolytes estimation.

(2) These methodology is seen to be replacing the flame photometry and calorimetric methods for the estimation of electrolytes due to its advantage of being very specific .

(d)GAS ELECTRODES—These are specifically designed for the estimation of specific gases in a gas mixture or in a solution. Examples are (CO_2, O_2 and NH_3) electrodes which are based on potentiometric measurement using PH-glass electrode. Usually the gas

electrode is separated from the test solution by a thin gas –permeable membrane eg polyethylene, polypropylene, teflonor silicone rubber.

(e)ENZYME ELECTRODES-These electrodes are covered with a layer of immobilized enzymes that catalyses a chemical reaction that can be monitored by the electrode. The methods of enzyme immobilization are- simple adsorption, entrapment behind a dialysis membrane or by convalent linkage. These principles of enzyme immobilization can be applied in redox electrode, ion selective membranes and also in amperometric electrodes. Examples includes: :-urea electrodes (enzyme used= urease), glucose electodes (enzyme used= glucose oxidase). It should be noted that enzyme electrodes are less durable than eg potassium, hydrogen electrodes , since immobilized enzymes gradually lose their activity owing to surface contamination and aging.

(B) POLAROGRAPHY

- Polarography is based on twin measurement of current flowing through an electrochemical cell and the electrical potential between the two electrodes when the potential is gradually increased at a constant rate by means of an external voltage source . The relationship between the current and the potential is called polarogram.. In this set up, both the anode and the cathode are made of mercury.

(C) AMPEROMETRY

-Based on the measurement of current flowing through an eletrochemical cell when a constant electric potential is applied to the eletrodes eg PO_2 electrodes used in the blood gas analysis machine

(D) COULOMETRY

- Measures the amount of electricity passing between two electrodes in an electrochemical cell . The amount of electicity IS DIRECTRY PROPORTIONAL TO the amount of substance produced or consumed by the redox process at the electrodes. This is said to obey the FARADAYS law. Used in coulometric titration of chloride.

(E) CONDUCTOMETRY

- This is the measurement of the current flow between the non polarized electrodes between which a known electrical potential is established. Used in many kinds of titrations (acid-base, precipitation)

CURRENT LABORATORY APPLICATION OF ELECTROCHEMICAL TECHNOLOGY

-A *variety of electrochemical methods have been incorporated into automated clinical chemistry analyzers. The most widely used electrochemical approach involves ISE. These electrodes have replaced flame photometry and calorimetric methods for the determination of electrolytes eg sodium, potassium and chloride.*

- The application of ISE in auto analyzers is achieved either through indirect or direct ISE Methods. ***The indirect ISE method*** *involves mixing of the sample with appropriate solution and then passing the mixture through the electrodes whilst the direct ISE method does not involve the pre-mixing of the sample with the solution. In direct approach, the sample is introduced to the electrodes directly. The electrodes applied in the indirect ISE method are found to be long lasting as compared to direct ISE method .* ***The direct ISE*** *method electrodes get clogged more often by the proteinous materials present in a sample.Due to the mixing of the sample with electrolytes solution prior to the estimation makes the clogging by the proteinous materials drastically reduced and this prolongs the life span of the electrodes. Comparatively the the electrodes being used in indirect* ***ISE METHOD*** *are expensive and have a longer life span.*

-For the proper maintainance of the electrodes, cleaning an d deproteinizing solutions are recommended to be used after electrolytes analysis.

*-For the success of electrolytes estimation by ISE two important characteristics of the electrodes have to be considered. These characters are (a)**SENSITIVITY and(b) SELECTIVITY**.*
***(A) SENSITIVITY**---This is the power of an electrode to sense a specific ion in an electrolyte solution. The theory of sensitivity of an electrode is given by the NERNST slope. However in practice a sensitive electrode is that which will sense all the presence of a specific ion in a electrolyte solution.*

***(B) SELECTIVITY---**This is the absolute power of an electrode to pick (or sort out) a specific ion in a cloud of different ions .Example : A potassium electrode is only supposed to pick the potassium ions in a solution leaving the other ions behind*
- Commercially prepared solutions are available to test for the selectivity and sensitivity of electrodes.

.ESTIMATION OF GLUCOSE USING ELECTRODES TECHNOLOGY
*The application of electrochemical methods in the quantitative determination of glucose is replacing the optical methods. The optical methods were developed at the early stages and the glucose meters using this methodology are referred to as first generation meters. The electrochemical method = (**BIOSENSOR (ELECTRODES) TECHNOOGY**, are currently being developed and widely being used in the diagnostic field and are seen to replace the optical method type of meters. These electrochemical method type of glucose meters are referred to as 2nd generation and are using biosensor electrodes for the glucose determination. This has improved the estimation of glucose in all body fluids. The methods incorporated are still the conventional ones eg glucose oxidase but they are quick (5-10 seconds), precise and very accurate.*

IMMUNOCHEMICAL REACTIONS AND TECHNIQUES IN CLINICAL CHEMISTRY

Immunochemistry is a generic term to describe all analytical methods that employ the reaction between an antigen and its complementary antibody for the recognition and/or quantitation of an analyte. The analyte may be the antigen or antibody and in each case the complementary species (antibody or antigen, respectively) will be the primary reagent in the analytical system. The analyte may be present in free solution (e.g. in blood, urine, fermentation broth, effluent, etc.) or may be structurally incorporated in a cell (e.g. red cell, bacterium, virus particle) or solid tissue. Our knowledge of the chemical structure and reactivity of the combining sites of antigens and antibodies (epitopes and paratopes, respectively) has meant that antigen- and antibody-like species can now be synthesized. In the former case this may be undertaken to provide an appropriate immunogen or calibration material whilst several antibody mimics have been demonstrated.

The immunoassay format will depend on the purpose for which the assay is to be used. In that the antigen–antibody reaction is unique to two complementary partners, in terms of cells and tissues, there is no other technique that will provide the same specificity – with the exception of DNA. Quantitative assays are usually required for the analysis of molecules in complex fluids. In the research phase, chromatographic techniques, e.g. high-performance liquid chromatography (HPLC), gas chromatography (GC), or capillary zone electrophoresis (CZE) can provide the characterization and quantitation required; however, these techniques are not always applicable when the need for the assay is established and a large workload is envisaged. An immunoassay then provides the necessary specificity and workload capability. In addition, when assays are used outside the laboratory, e.g. at the bedside, then only immunoassays are applicable because they can be encapsulated in microfabricated devices.

EXAMPLES OF IMMUNOCHEMICAL REACTIONS AND TECHNIQUES

USED IN CLINICAL CHEMISTRY

(1)Pregnancy test: This is one of the frequently performed routine test in clinical laboratory either in clinical chemistry or immunology laboratory. A pregnancy test attempts to determine whether or not a woman is pregnant. Modern pregnancy tests look for chemical markers associated with pregnancy. These markers are found in urine and blood, and pregnancy tests require sampling one of these substances. The first of these markers to be discovered, human chorionic gonadotropin (hCG),. Obstetric ultrasonography may also be used to detect pregnancy.

The hormone *human chorionic gonadotropin* (better known as hCG) is produced during pregnancy. It is made by cells that form the placenta, which nourishes the egg after it has been fertilized and becomes attached to the uterine wall. Levels can first be detected by a blood test about 11 days after conception and about 12 - 14 days after conception by a urine test. In general the hCG levels will double every 72 hours. The level will reach its peak in the first 8 - 11 weeks of pregnancy and then will decline and level off for the remainder of the pregnancy.

.**False Positives and Negatives**Most medications, including birth control pills and antibiotics, do not affect the results of pregnancy tests. Alcohol and illegal drugs do not affect the test results. The only drugs that can cause a false positive are those containing the pregnancy hormone hCG in them (usually used for treating infertility). Some tissues in a non-pregnant woman can produce hCG, but the levels are normally too low to be within the detectable range of the tests. Also, about half of all conceptions don't proceed to pregnancy, so there may be chemical 'positives' for a pregnancy that won't progress. For some urine tests, evaporation may form a line that could be interpreted as a 'positive'. This is why tests have a time limit during which you should examine the results. It's untrue that urine from a normal man will give a positive test result.

Although the level of hCG rises over time for a pregant women, the quantity of hCG for one woman is different from the amount produced in another. This means some women may not have enough hCG in their urine or blood right at six days post-conception to see a positive test result. All tests on the market should be sensitive enough to give a highly accurate result (~97-99%) by the time a woman misses her period.

(2)HORMONES AND TUMOUR MAKERS ANALYSIS

Mini-Vidas machine: Hormones and tumour makers were analyzed using a Mini-Vidas machine. Every analyte had a specific master lot data entry card which contained all the specifications i.e analyte identification, factory master calibration curve, control data, and lot number of the reagent.

The Strip

The strip consisted of 10 wells covered with a labeled foil seal. The label comprised a bar code which mainly indicated the assay code, kit lot number and expiration date. The foil of the first well was perforated to facilitate sample introduction. T he last well of each strip was a cuvette in which the fluorimetric reading was performed. The wells in the centre section contained the various reagents required for the assay.

Solid Phase Receptacle (SPR)

Interior of the SPR was coated during production with specific monoclonal anti-(T4, T3, FT4, FT3, TSH, LH, PRG, FSH, PRL, TPSA, CA 19-9, CA125, CEA, CA 15-3) immunoglobulins (mouse) or specific polyclonal anti- (EST, TESTO) immunoglobulins (rabbit). Each SPR was identified by specific analyte code.

Analytical Principle

The assay principle combined an enzyme immunoassay sandwich method with a final fluorescent detection (enzyme linked fluorescent assay (ELFA))

Analytical method

The SPR served as the solid phase as well as the pipetting device for the assay. Reagents for the assay were ready for use and pre-dispensed in the sealed reagent strips. All the assay steps were performed automatically by the machine. The reaction medium was cycled in and out of the SPR several times. The sample was taken and transferred into the well containing alkaline – phosphatase labeled anti- (T4, T3, FT4, FT3, TSH, LH, PRG, FSH, PRL, TPSA, CA 19-9, CA125, CEA, CA 15-3, EST, TESTO) immunoglobulins (conjugate). The sample/conjugate mixture was cycled in and out of SPR several times to increase reaction speed. The antigens binds with the antibodies coated on the SPR and to the conjugate forming a "sandwich". Unbound components were eliminated during the washing steps of the assay.

In the final detection step, the substrate (4-methyl-umbelliferyl phosphate) was cycled in and out of the SPR. The conjugate enzyme catalyzed the hydrolysis of the substrate into a fluorescent product (4-methyl-umbelliferone). The intensity of the fluorescence was directly proportional to the antigen present in the sample.At the end of the assay, results were automatically calculated by the machine in relation to the calibration curve stored in memory and then printed out.

(3) Lipid profile analysis-----HIGH DENSITY LIPOPROTEIN CHOLESTEROL

LATEST METHODS CURRENTLY USED AS DIRECT ASSAYS HAVE INCORPORATED NON-HDL ANTI-B-LIPOPROTEIN IN THE FIRST STEP OF THE REACTION AS EXPLAINED BELOW.

Test principle

Anti human –B-lipoprotein antibody binds to lipoproteins other than HDL (LDL,VLDL. and chylomicrons).The antigen antibody complexes formed block enzyme reactions. HDLC is quantified by the presence of an enzyme chromogen system

Reaction principle

LDL,VLDL and chylomicrons $\xrightarrow{\text{Anti-human-b-lipoprotein antibody}}$ antigen-antiboby complexes

HDLC + H_2O + O_2 $\xrightarrow{\text{CHO and CHE}}$ cholestene-3 – one + fatty acid + H_2O_2

$2H_2O_2$ + 4-AAP + Phenol $\xrightarrow{\text{POD}}$ Quinoneimine (red) dye + $4H_2O$

CANCER

Cancer is one of the leading non-communicable diseases that affect many people in the world today. This pathological disorder is characterised by failure of the body system that regulate normal cell growth and as a result it leads to uncontrollable proliferation of cells with destruction of neighbouring organ tissues and spreading to other section of the body. The spreading of cancerous cells to other body organs is enhanced by the fact that the movement is through blood and lymph.

The presence of cancer can be suspected on the basis of symptoms, or findings on radiology. Definitive diagnosis of cancer, however, requires the microscopic examination of a biopsy specimen. Most cancers can be treated. Possible treatments include chemotherapy, radiotherapy and surgery. The prognosis is influenced by the type of cancer and the extent of disease. While cancer can affect people of all ages, and a few types of cancer are more common in children, the overall risk of developing cancer increases with age. Cancer is a leading cause of death. In 2007 cancer caused about 13% of all human deaths worldwide (7.9 million), and the number of cases is rising as more people live to old age. There are lots of different kinds of cancers. Some of the most common include breast cancer, brain cancer, leukemia, testicular cancer, mesothelioma, and lung cancer

CAUSES OF CANCER

Several factors have been attributed to be the cause of cancer. The main causes of cancer irrespective of the type are either ***genetic or environmental***.

- Factors that are ***environmental*** in nature that contribute in the spread of cancer includes; ***biological carcinogens*** such as certain ***viruses, bacteria and parasites***.
- Exposures to ***ionizing and non-ionizing radiation*** such as radon or ***ultraviolet radiation*** respectively affect the general population.
- C***hemicals*** such as asbestos and dioxins which are the waste products in the various industries are emitted in the air. These air pollutants contribute a great percentage as an environmental cancer risk factors to persons leaving around these industries.
- Other known forms of environmental cancer risk factors that fall under chemical carcinogens are natural constituents and contaminants found in food and drinking water includes ***pesticides residues, arsenic and aflatoxins***. .These types of exposures may occur many times and in different environmental situations during the course of somebody's lifetime. The nature of exposure may be widespread as found in the case of air pollutants or it can be within an enclosed area as in the case of a certain industrial locality.
- ***Smoking and tobacco consumption*** has brought a lot of interest as far as environmental cause of cancer is concerned. Smoking has been proven to be the cause of cancer in many different parts of the human body. Some of the major types of cancer caused by smoking include l***ungs*** and the ***upper breathing systems***. It is not only cigarette smoking alone that is a cause of cancer but also pipe and cigar smoking. It has been reported in some developed country that 23 % of all deaths caused by cancer is due to smoking and tobacco consumption.

The activities that take place in a cell are under the control of ***genes***. These activities includes how fast the cell grows, how even does it divide and how long does the cell lives., including how quickly it grows, how often it divides, and how long it lives.Genes can undergo some changes also referred to as mutations which can either be inherited from one's parent or acquired during a person's life. ***This gene mutation is known to contribute to the growth and development of cancer.***

- Genes that contribute to the development of can are grouped together in different categories but cancer is caused by multiple changes to several different types of genes.

- ***Tumour suppressor genes are considered as protective genes***. They function by limiting cell growth which is achieved by monitoring how quickly cells divide into new ones at the same time controlling the death of a cell. When a suppressor gene is mutated either due to hereditary or environmental factors, cell grow uncontrollably which may eventually result to the formation of a mass called a **tumour**.

- Examples of tumour suppressor genes are; ***BRCA1, BRCA2 and p53***.

- Heriditary mutation of genes ***BRCA1 or BRCA2*** are known to increase the risk of a woman developing ***breast*** or ***ovarian cancers***.

- Among these three suppressor genes, the most commonly ***mutated gene found in people irrespective of gender is p53.*** More than 50% of all cancers cases are due to a missing or damaged *p53* gene.

- The highest percentage of *p53* mutation is ***acquired and rarely inherited mutations***.

- Genes ***HER2*** controls cancer growth and spread and is found in some cancer cells for example breast and ovarian cancer cells.

- The ***ras family of genes*** are involved in the cell growth, communication pathways and death. These are the two types of oncogenes that turn a healthy cell into a cancerous cell.

- The mutations of these oncogenes is ***acquired and not inherited***.

- The other type of genes that is related to cancer development is ***DNA repair gene*** whose function is ***to repair or fix mistakes made when DNA is replicated or copied***. If a person has an error in this repair gene, the mistakes are not corrected. DNA mistakes which are not corrected become mutations and at long run leads to cancer. The mutations of this gene can either be ***inherited or acquired***. .

- Despite all these relationships between and cancers, rarely a specific single gene mutation can contribute to the development of cancer. Mutations in many and different genes is the cause of many types of cancers.

ECOMONIC AND SOCIAL BURDEN OF CANCER

Cancer like any other type of pathological condition contributed heavily to the ***suffering*** and in most cases the eventual cause of ***death to the affected patient***.

- The incidences of cancer is ***increasing due to combination of many risk factors such as changes in lifestyle and diet, high tobacco***

consumption and more people going to old age with cancer being more common in older population.

- Ill health caused by cancer contributed negatively to the economic status and social welfare of both the patient and the family members.
- There is a high motility and disability related to cancer which amounts to expensive treatments.
- Cancer has therefore become an economic and social burden to the affected patients, family members and the country and has heavily affected the world's economy.
- This economic and social burden is significant to the affected client irrespective of whether one is in developed or developing country. The situation on the cost of treatment for cancer case is complicated by the fact that there are limited insurance options and this has forced families to depend on out of pocket spending to finance their healthcare

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MAJOR PROBLEMS ENCOUNTERED IN CANCER MANAGEMENT

- Cancer is a **chronic pathological condition that affects people of all ages** and of different economic and social status in all parts of the world.
- The management of cancer is **very involving and expensive** for the patients, family members and the country.
- Cancer management drains off the income resources for the affected individual and the burden is also transferred to other household members.
- **Labour force participation** both for the **patient and the family members** is affected mostly by the fact that a lot of time is spent in the management centres irrespective of whether the patient is in-patient or out-patient.
- The situation of cancer management is even complicated since the **insurance options are very limited.** Very few insurance companies are willing to insure cancer patients due to the chronic nature of the pathological disorder.

- -The **cost** of managing cancer continue **to rise** despite the various research engagements being undertaken to come with cheap and effective cancer drugs.
- -The cost of managing cancer has become a burden to households since a large portion of the income is spent on the treatment of the affected family member.
- **Very few donor organizations are ready to take up the cost burden** and assist in the purchase of the cancer drugs (chemotherapy treatment) for the needy patients especially in the developing countries where Kenya is member.
- The other modes of cancer treatment ie surgery and radiotherapy are neither cheap. Patients and the affected households continue to suffer silently as they bear this economic and social burden of cancer.
- **Cost of cancer medication may be a barrier to adherence of taking medication on schedule.** Some patients stretch out their supply of drugs such as that instead of taking them as the doctor prescribed, they take them every other day so that they do not run out of their drugs so quickly. When

the patient's share of prescription costs becomes too high, many patients skip doses or stop taking medication entirely.

RESEARCH OPENINGS

- There is need therefore to **establish the economic and social burden of cancer** based on the assessment of the following dimensions namely: out of pocket expenditure, borrowing, family assets sale, loan, time spend out of work to attend treatment sessions for both patient and family members. There is a need to assess how cancer affects the social under takings of the patient, family members and the health care givers.
- What is the **impact of cancer to the economy** of a country and the world as a whole?
- What is the relationship between cancer and other pathological disorder either as a primary or secondary cause?
- How has the **invention and introduction of new drugs coupled with the moderation of treatment regiments** contributed to the improvement of the survival rate of cancer patients.

LITERATURE REVIEW

- Globally, **a**bout ***14.1 million people*** were diagonised with cancer by the end of ***2012*** and about ***8.2 million cancer related deaths were registered that year***. According to World
- Health Organization, the most commonly types of cancer diagnosed worldwide are those of ***lung, breast, and colorectal.*** The type of cancer registering the highest number of deaths are lung, liver and stomach. Low and middle income countries experience more than 70 % of all cancer deaths worldwide which is attributed to the fact economic burden of cancer treatment. Nevertheless, the entire burden of cancer across the world is projected to continue rising, especially in developing countries.
- ***It is estimated that an estimated sixteen million people will be diagnosed with cancer and twelve millions will die of cancer and cancer related ailment by the year 2030.***

- In ***Kenya,*** cancer is considered ***third cause of death after the infectious and cardiovascular diseases***. 7% of all deaths in Kenya are due to cancer and its related complications.
- It is estimated that around 28000 people are diagnosed with cancer with ***22000 dying*** of cancer every year. More than sixty percent of those with cancer are below the age of seventy years.
- In Kenya ***the risk of being diagnosed with cancer before the age of seventy years is fourteen percent, while the risk of dying of cancer is twelve percent***.. The rapid increase in the number of cases of cancer and non-communicable diseases in developing countries has been attributed to increased exposure to environmental carcinogens, tobacco use ,harmful use of alcohol and some risks associated with infectious diseases such as HIV/AIDS, HPV, HEPATITIS and some parasitic infestation such as schistosomiasis
- The total budget of most countries in the world today, a lot is spent in the diagnosis and subsequent treatment of cancer. An increasing trend on the expenditure has been witnessed in the past decades and it is expected to

continue to rise further in the coming years ***due to advances in treatment coupled with population aging***.

- This economic burden of cancer has been found to have a negative effect not only to the health services of a country but also on the patient, family members and the society as a whole.
- The cost of cancer expenditure starts at the ***time of diagnosis***. This includes the cost of laboratory tests, consultation fees and the cost of purchasing the drugs to relief pain and side-effects of cancer drugs.
- Further to this, cancer patients spend ***out of pocket expenses*** in relation to travelling to hospitals for appointments. More expenditure is incurred for the purchase of cancer drugs for those under taking chemotherapy mode of treatment.
- Cancer patient under taking **surgical mode of treatment** equally spend more money on the surgical procedures. A cancer patient recuperating at home spends more than before diagnosis. The additional cost incurred by the patients and their family members are quit substantial.
- A recent ***study in India*** on the out of pocket expenditure by the patients and their family members concluded that most of the households are ***pushed into poverty***. Most of those households affected by the out of pocket expenditure were concentrated among the poor. Protecting households from out of pocket health payments and its subsequent outcome of catastrophic shocks has continued been a major health policy problem
- Cancer management has affected the ***working nature of the patients and their family members.*** A lot of time is spent during the time of diagnosis and there after during treatment. Some employers end up not paying for the time spent seeking medical treatment for the patients and their family members and this contribute to income decrease for the households.
- Lose of income together with the out of pocket health spending has a wide range of consequences for the patients and their family members. ***Increased financial stress*** and strain results with a greater likelihood of developing depression, anxiety and emotional stress to the cancer patients and their family members.
- Many ***patients perceive cancer as a life-threatening pathological disorder*** regardless of its prognosis. Psychosocial adjustment to cancer has been found lacking to most patients. This happens irrespective of the type of treatment, disease severity and prognosis.

Minority of patient are those who accept their cancer condition and find a sense of meaning to what is happening to them. On the other hand cancer can be equally or even more ***distressing to family members and friends of the cancer patients***. Social isolation, family tensions and family loss of income are situations that follow closely after cancer diagnosis . Not only cancer patients and family members are psychologically affected by cancer but also health care providers who care for cancer patients.

- Health care providers who very often are with the sick and dying cancer patients and sometimes they find themselves not able to provide care to the best of their capability are always at a risk of a psychological syndrome known as ***'staff burn out'***. The characteristics of this syndrome are; emotional exhaustion and de-personalization of the patient. This can results

to a health care provider suffer from insomnia and sometimes use of substance of abuse

Tumour markers (Cancer Markers)

Tumor markers are antigens produced by tumor cells or by other cells within the body in response to cancer or certain benign conditions. These markers may be detected within exfoliated or distributed cells, or as circulating agents within the peripheral blood or plasma. Other surrogate biological specimens, typically bodily fluids (e.g., urine, saliva, sputum, cerebrospinal fluid, or effusions) may also carry tumor markers (Sturgeon, 2002). Tumor markers are usually constituents of healthy cells that are produced in greater abundance by cancerous cells; however, normal cells may also produce them in response to the malignancy. In addition, tumor growth may cause an obstruction and/or cellular breakdown of normal tissue either of which could lead to increased levels of an analyte that could serve as a surrogate marker of malignancy. Some of the tumor markers in highest use in clinical practice includes:, cancer antigen 125 (CA 125), cancer antigen 15-3 (CA 15-3), cancer antigen 19-9 (CA 19-9), carcinoembryonic antigen (CEA) and total prostatic specific antigen (TPSA) (Tsao *et al*., 2006).

CA 125 is a surface antigen associated with epithelial ovarian cancer, and to date CA125 is the most sensitive marker for residual epithelial ovarian cancer. CA125 may also be elevated in patients with lung, cervical, fallopian tube, and uterine cancer and endometriosis. CA125 is the only marker that has been accepted for clinical use in ovarian cancer (Rustin *et al*., 2004).

CA 15-3 is a serum cancer antigen whose principal utility is in monitoring therapy; used in the management of patients with breast cancer. It has value for evaluating patients for breast-cancer recurrence, assessing prognosis, and checking for residual disease following surgery. Cancers of the ovary, lung, and prostate may also raise CA 15-3 levels. The literature indicates elevated levels of CA 15-3 may be associated with noncancerous conditions, such as benign breast or ovarian disease, endometriosis, pelvic inflammatory disease, and hepatitis (Goldhirsch *et al*, 2003). Breast cancer is by far the most common cancer affecting women worldwide with approximately one million new cases diagnosed each year (Peto *et al*., 2000).

CA 19-9 is a glycosylated protein produced by adenocarcinomas of the pancreas, stomach, gall-bladder, colon, ovary and lung. Levels of CA 19-9 have also been identified in patients with hepatocellular and bile duct cancer. In individuals who have pancreatic cancer, the literature indicates that higher levels of CA 19-9 tend to be associated with more advanced disease (Rosty and Goggins, 2002). CA 19-9 levels correlate well with the stage of disease and are associated with ability to successfully provide surgical intervention (Neoptolemos *et al*., 2004).

Carcinoembryonic antigen (CEA) is a glycoprotein involved in cell adhesion. It is normally produced during fetal development, but the production of CEA stops before birth. Therefore, it is not usually present in the blood of healthy adults, although levels are raised in heavy smokers. (CEA) is present on virtually all colorectal tumors (Strate and Syngal, (2005). Colorectal cancer (CRC) is the third most common cancer, worldwide with an estimated one million new cases and half a million deaths each year (Desch *et al*., 2005).

Prostate-specific antigen (PSA) is a protein produced by the cells of the prostate gland. PSA is present in small quantities in the serum of men with healthy prostates, but is often elevated in the presence of prostate cancer and in other prostate disorders. A blood test to measure PSA is considered the most effective test currently available for the early

detection of prostate cancer. Rising levels of PSA over time are associated with both localized and metastatic prostate cancer (Hugosson *et al.,* 2004). Prostate cancer tends to develop in men over the age of fifty and although it is one of the most prevalent types of cancer in men, many never have symptoms, undergo no therapy, and eventually die of other causes. This is because cancer of the prostate is, in most cases, slow-growing, symptom-free, and since men with the condition are older they often die of causes unrelated to the prostate cancer, such as heart/circulatory disease, pneumonia, other unconnected cancers, or old age .

ANALYTICAL METHODS FOR TUMOUR MARKERS

The current analytical technique employed in the analysis of all the tumour markers use the principle of immunochemical reaction based on an antiboby/antigen reaction. The set up of the reagent could either have the the antigen to the tumour marker being determined and this will react with the specific antibody (being the tumour markerin the specimen) in the specimen.

Analytical Principle:
The assay principle combined an enzyme immunoassay sandwich method with a final fluorescent detection (enzyme linked fluorescent assay (ELFA))

Analytical method:
The SPR served as the solid phase as well as the pipetting device for the assay. Reagents for the assay were ready for use and pre-dispensed in the sealed reagent strips. All the assay steps were performed automatically by the machine. The reaction medium was cycled in and out of the SPR several times. The sample was taken and transferred into the well containing alkaline – phosphatase labeled anti- (TPSA, CA19-9, CEA, CA 153, CA 125) immunoglobulins (conjugate). The sample/conjugate mixture was cycled in and out of SPR several times to increase reaction speed. The antigens binds with the antibodies coated on the SPR and to the conjugate forming a “sandwich”. Unbound components were eliminated during the washing steps of the assay.

In the final detection step, the substrate (4-methyl-umbelliferyl phosphate) was cycled in and out of the SPR. The conjugate enzyme catalyzed the hydrolysis of the substrate into a fluorescent product (4-methyl-umbelliferone). The intensity of the fluorescence was directly proportional to the antigen present in the sample.At the end of the assay, results were automatically calculated by the machine in relation to the calibration curve stored in memory and then printed out.

SPECIMEN OF CHOICE---HEPERINIZED PLASMA OR SERUM

METHODS OF ANALYSIS

Analytical Principle:
The assay principle combined an enzyme immunoassay sandwich method with a final fluorescent detection (enzyme linked fluorescent assay (ELFA))

Comparison of established Kenyan adult reference ranges for tumour makers with some reference ranges quoted in literature

Parameter(uni	**Gender**	**Kenyan**	**French**	**German**

ts)				
CA125(U/ML)	M/F	0-23	5-35	0-35
CA15.3(U/ML)	M/F	0-20	0-30	0-22
CA 19.9(U/ML)	M/F	0-16	0-27	<37
CEA(ng/ML)	M/F	0-3.5	0-5	<4.6
TPSA(ng/ML)	M (18-28 YRS)	0.1-1.4	0.2-1.7	0-1.3
	M (29-39 YRS)	0.1-2.6	-	-
	M (40-50 YRS)	1.1-3.5	0.3-2.2	0-2
	M (51-61 YRS)	1.1-6.3	0.3-3.4	0-3

POINTS TO NOTE

- In the diagnosis and management of various cancer related pathological disorders, upper reference range limit of specific tumour marker is a major tool for interpretation. Lower reference range limit of any tumour marker is of no clinical significance but a sign of good health.
- It is worth to mention that clinical chemistry quantitative laboratory results alone can not make a conclusive cancer diagnosis. Other tools such as histological tissue examinations and radiological procedures are used in conjunction with the clinical chemistry quantitative laboratory results.
- Currently there are more than 20 different tumor markers that have been characterized and are in clinical use. Some are associated with only one type of cancer, whereas others are associated with two or more cancer types. There is no “universal” tumor marker that can detect any type of cancer.
- Tumour markers are used to determine risk, screen for early cancer, estimate prognosis, predict that a specific therapy will work, or monitor for disease recurrence or progression.
- Total prostatic specific antigen which is the male oriented tumour maker, shows an increasing trend as male advances in age. Interpretation of total prostatic specific antigen report for the diagnosis and management of prostrate cancer would require the provision of the individual’s age.

THERAPEUTIC DRUG MONITORING and LABORATORY DRUG ANALYSIS

(A) THERAPEUTIC DRUG MONITORING(TDM)

-TDM is a rapidily developing area in the clinical laboratory.To be effective TDM requires :-

(a) The acquisition of varied specimens.

(b) Routine, timely and very reliable determinations of drug concentrations in the specimens.

(c) Reporting of the results either individually or in collarated so that in conjunction with the dosing schedule they may be interpreted in a pharmacokinetic context.

-TDM calls for a multidisplinary approach since it relies on the cooperative efforts of the physician, nurse, pharmacologist, pharmacist, and clinical chemist.

- The recent rapid evolution of TDM stems from several factors;-

(a) Development of more numerous and more effective drugs with therapeutic potential and application.

(b) Development of analytical techniques which are accurate, precise, sensitive and specific for analyzing drugs in biological fluids.

(c) Utilization of computers in the analytical technology, in pharmacokinetic data analysis, in the dose regimen design and in the collection and storage of the patients data.

- The mechanism of drug action refers to the actual biochemical or physical process that occurs to elicit the biological response.

- Drug action is generally mediated through a receptor on the cell membrane or within the cell. Cellular proteins such as enzymes as well as structural or transport proteins are important examples of drug receptors. None protein macromolecules may also bind drugs resulting in altered cellular function such as membrane permeability.

- Some drugs are similar to natural endogenous substances and may compete with these for the binding sites. Some drugs may block the formation ,release, uptake or transportation of essential substances. Although the exact molecular interactions remain obscure for most drugs , theoretical models have developed to explain them. One concept postulates that if the drug-receptor complex is sufficiently stable and able to modify the target system, an observable pharmacological response will occur.

-For many drugs a strong correlation exists between the drug concentration and the observed pharmacological effect. Nevertheless one must keep in mind that a drug serum concentration does not necessarily equal the concentration of the drug at the receptor, it may merely reflect it. Changes in serum drug concentration versus time may reflect changes in local concentration at the receptor site or in body tissues. This effect is called ***THE PROPERTY OF KINETIC HOMOGENICITY*** and it is applicable to all pharmacokinetic models in post absorptive and post distributive phases of the time course.

- The property of kinetic homogeneity is an important assumption in the TDM because it is the bases upon which all therapeutic concentration and toxic concentration ranges are established. These ranges collectively defines the THERAPEUTIC WINDOW (the relationship between minimum effective concentration (MEC) and minimum toxic

concentration (MTC). Ideally the trough blood concentration (the lowest concentration achieved just before the next dose) within each dosing cycle should not fall below the MEC and the peak blood concentration (the highest concentration achieved within the dosing cycle) should not rise higher the MTC. This means that the concentration should stay within the therapeutic range.
- Blood drug concentration above MTC put the patient at risk of toxicity and concentration below MEC have the patient at risk for the disorder the drug is supposed to treat.
- Dosages must be planned to achieve therapeutic concentration and this must be monitored to allow adjustment of dosage if necessary.

TERMINOLOGIES USED IN TDM

(1) PHARMACOLOGY-----Is the study of the drug therapeutic effect on living processes.
(2) PHARMACODYNAMICS-----Is the study of the biochemical and physiological effects of drugs and their mechanism of action.
(3) PHARMACOKINETICS----Is the study of the rate processes of absorption, distribution , biotransformation and excretion of drugs. This is the aspect of pharmacology that influence the interpretation of TDM results.
(4) Toxicology-----This is the aspect of pharmacology concerned with the adverse effects drugs in living systems.

PHARMACOKINETICS

Is the study of the rate processes of absorption, distribution , biotransformation and excretion of drugs. Clinical pharmacokinetics applies the principles of pharmacokinetics to therapeutic management of an individual patient.
The chart below shows the factors that affect plasma drug concentration.These factors are:-

(a) Absorption----drug must be formulated to assure bioavailability for absorption from the gastrointestinal tract or other administration site
(b) Metabolism----drug is converted to a more soluble compound (metabolite)that may be pharmacologically active or inactive. Metabolism may occur in the tissue other than the liver.
(c) Excretion-------the more water soluble drugs and their metabolites are usually excreted through the urine. Excretion may also occur via bile, faeces, saliva and expired air.
(d) Tissue storage------the drug may be stored in tissue that exhibit no pharmacologic response to it, side effect may occur from drug reaction with a specific physiological system
(e) Site of action--------Free drug binds to the receptor and produces pharmacologic response. The number and type of receptors to which the drug is bound determine the intensity and duration of the observed response.

PRINCIPLES OF DRUG DISPOSITION(SETTLEMENT)

ABSORPTION-----Most drugs administered chronically to patients are given orally.The absorption process depends on:-

(a)drug’s dissociating from its dosage form
(b) drug’s dissolving in the GIT fliud
(c) drug’s diffusing across biological membranes into the blood stream.

The rate and extent of drug absorption may vary considerably.The fractio of a drog that is absorbed into the systemic circulation is reffered to as its bioavailability.The bioavailability of a particular drug , if the drug is to be useful, must be great enough so that te active component can pass in sufficient amount and in a desirable time from the gut into the systemiv circulation.

DISTRIBUTION----Following the entrance of drug into the vascular compartment , the drug will be carried to all organs and fluid compartments.the overall process is reffered to as **DISTRIBUTION.** The **distribution** process depends on:

(a)Binding of the drug to circulating components or fixed receptors
(b) ability of the drug to pass through membrane barriers
(c) ability of the drug to dissolve in structural or storage lipids

Once a drug enters the systemic circulation it will rapidly distribute and come to equilibrium with many of the blod components. The most significant are the blood proteins. It is believed that only the drug unbound to plasma protein (free) is available for distribution beyond the intravascular space and for elimination.In addition it is only the free drug which is responsible to elicit a biological response.
Therefore, changes in in the protein – binding characteristics of a drug can have a profound influence on the distribution and elimination of a drug as well as on the manner in which steady- state concentrations are interpreted.Each drug has its own characteristics protein binding pattern.

METABOLISM--------the rate of enzymatic processes that metabolize most drugs is usually characterized by the Michaelis-Menten equation and follows first –order reaction kinetics. In other words, the rate of metabolism is proportional to the concentration of the drug. First order kinetics are characteristics of the metabolism of numerous drugs.

BIOTRANSFORMATION----The liver is the organ principally responsible for drug metabolism. Its role is to convert lipophilic nonpolar molecules to more polar, water-soluble forms. The drug molecule can be modified by reactions that alter its chemical structure (oxidation, reduction or hydrolysis), or by reactions that conjugate the drug (glucuronidation, sulfation), or by both. Many environmental chemicals and endogenous bio-chemicals are processed by the same mechanisms.

RENAL EXCRETION-----Is the major pathway for the elimination of drugs and their metabolites. Therefore decreased renal function will cause elevated serum drug concentration and increase the pharmacologic response. Three processes are involved in the elimination of the drug via the kidney: glomerular filtration, tubular secretion, and tubular reabsorption.
Kidney function is readily and reliably evaluated by estimation of creatinine clearance. A strong correlation has been shown to exist between creatinine clearance and the total body clearance and elimination rate constant of those drugs primarily dependent upon the kidney for their elimination. Example of drugs whose therapeutic use is adjusted to account for changes in creatinine clearance include gentamicin, tobramycin, amikacin, digoxin, vancomycin and cyclosporine.

Summary Of Factors Influencing Drug Disposition

(1) Patient's history and adjunct(supplementary) drug therapy is essential at the initiation of drug therapy and therapeutic drug monitoring.
(2) Altered patho-physiological states influence on clearance mechanisms of several drugs frequently monitored by therapeutic drug monitoring programs.Such patho-physiological states are:-(a)renal disease, (b)liver disease (c) cardiovascular disease (d) congestive heart failure and (e)hypothyroidism

CLINCAL UTILITY OF TDM

-TDM is most valuable when the gravity of an acute clinical situation demands aggressive therapy with a drug that has a narrow therapeutic index.

- TDM laboratory data helps the physician to recognize and document the pts with an unusual pattern of drug disposition (absorption, distribution, biotransformation and excretion). The physician is able to modify therapy inorder to reduce risks of over or under medication.
- As the treatment regimen is continued , laboratory reports provide the basis for timely and rational adjustment of drug dosage and scheduling as the pathological effect of the primary or intercurrent disease progress or regress.
- TDM allows the physician to establish the baseline at which the patient responds well. This is important if the same treatment will be reinstituted at later time.
- Continuation of TDM also identify the need for changes in the treatment regimen in response to physiological changes such as puberty, pregnancy, or ageing.
- TDM is useful in the detection of non-compliance drug which is important to avoid failure of treatment , especially in those conditions that are frequently free of pain or unusual discomfort such as epilepsy, asthma, hypertention and mild heart disease.

FACTORS UNIQUE TO THE TDM CLINICAL LABORATORY.

The value and quality of a TDM service are only as good as the data produced. Therefore the TDM clinical laboratory should accomplish the following:-

(1) The TDM laboratory should inform the medical staff as to ---

-Therapeutic concentration ranges of drugs.
- Toxic concentration ranges of drugs.
-Analytical methods appropriate for drugs monitoring.
- Action values of drugs.
- The required specimens and the volumes.
- The specification of the specimens collection tubes.

(2) TDM laboratory should avail the guidelines for ideal sample schedule for each individual drug monitored.

(3) TDM laboratory should be provided with the time and date of sample collection and the last dose should be noted . To asses steady state conditions , the length of time the pts has been on a particular drug (regimen) should be known.

(4) TDM laboratory should device a reporting form that incorporates all the data necessary for interpretation (ie the dosage form, frequency and amount, plasma concentration trough or peak value, time of dosage, time of draw and other drugs co administered).

(5) TDM laboratory should a good quality management programmes (internal and external).

(6) TDM laboratory should have well designed standard operating procedures (SOPS) for all the activities taking place in the laboratory.

(7) TDM laboratory should have a proper waste disposal procedures.

ANALYSIS OF TOXIC SUBSTANCES

Analytical toxicology, effectively performed is an important and frequently essential part of the sequence of events leading from diagnosis to treatment of chronic and acute poisoning.

- In order to fulfill its role in dealing with emergency situations , the toxicology laboratory must provide a 24-hr service and utilize reliable methods with short – turnaround times.
- Diagnosis and treatment of toxic episodes requires an emergency response. This emergency response has three components:- 1)clinical findings, 2)laboratory reports and 3) data coordination.
- Clinical findings-----The patient must undergo a thorough clinical assessment including both physical examination and medical, occupational and social histories by a physician.. For diagnostic purpose, clinical history of the patient is

very important since the information sort includes drugs or chemicals with which the patient may have been in direct contact , those available in the home, work place, or other environment.

- The laboratory does both the specific assays or the screening tests. Many toxic substances can be ruled out or into a differential diagnosis by demonstration of the presence of a particular chemical in specimens removed from the patient or in substances available to the patient.The laboratory results may confirm the previous impression of the clinician or may raise the possibility of a toxic substance not previously suspected..The laboratory findings gives way to the type of treatment to be instituted. The laboratory has another role in the follow-up determinations for evaluation of effectiveness of treatment or for prognosis.
- Toxicology data coordination---This is the link between the laboratory and the clinician and it is usually done by a coordinator. The coordinator correlates a specific patients history and clinical state with information on toxic substances and their known effects. The coordinator interprets the laboratory results in a meaningful way and communicates both the results and the informational context to the physician. In well set institutions with emergency toxicology laboratory, the coordinator the coordinator is usually a a trained and experienced toxicologist. In other laboratories the role can be carried out by a a clinician or a laboratory scientist who has acquired capability through training and experienced.

CHRONIC POISONING (INDUSTRIAL AND ENVIROMENTAL MONITORING)

- The problem of chronic poisoning has always been present, but only in recent years has it received attention
- Most of the data upon which decisions regarding actions levels by regulatory agencies are based on experimental animals studies or retrospective epidemiological studies in which subjects exposed to various concentrations of chemicals develop signs and symptoms of poisoning .
- The nature of toxic substances that give rise to chronic poisoning varies widely , ranging from elements(particularly metals) through complex organic and inorganic compounds.
- These substances may be encountered as drugs, pesticides, industrial chemicals, food additives and environmental chemicals and pollutants.
- ACTION LEVELS-----These are concentration levels of toxic chemicals in food , air and water that may be expected to exert adverse effects after prolonged exposure in human.. The clinical laboratory has an increasing role in detection and follow-up in chronic poisoning.
-

RESPONSIBILIIES OF A CLINICAL TOXICOLOGY LABORATORY

- the provision of reliable and sensitive methods for the assay of biological specimens. These methods should be both qualitative and quantitative.
- the laboratory should put in place a system whereby results are released in the shortest time possible. That is the specimen turnaround time ranging from minutes to hours but not more than 24 hours.
- The laboratory should provide a list of toxic assay that it is able to analyse.
- The laboratory should be well equipped with toxic substance monitoring equipments and well qualified personnel.
- In the reporting the laboratory should use the generic names or chemical names rather than using the trade names.
- The units of reporting should be the standard ones . For EXAMPLE, drugs should be reported in ug/Ml or mg/l and for metals in parts per million (ppm)

CLINICAL LABORATORY DIAGNOSIS OF DRUGS

- It is important to determine the concentration of a drug being used to manage a certain pathological disorder. The aim is to ensure that the patient is getting the benefits of the drug and that the concentration of the drug is within the expected range in the course of treatment.

- The current laboratory approach in the drug concentration determination (quantitative) is based on antibody/antigen reaction under the principles of homogeneous enzyme immunoassay or immuno-inhibition test .

-- The specimens of choice are either serum or plasma

HOMOGENEOUS ENZYME IMMUNOASSAY

Example: (for the quantitative determination of carbamazepine (CBZ) in human serum and plasma)

Summary

Carbamazepine is an anticonvulsant drug , used in particular for the treatment of all form of partial epilepsy. Effective serum concentration of carbamazepine is essential for seizure control.

Test Principle

This assay is based on the bacterial enzyme β-galactosidase, which has been genetically engineered into two inactive fragments. These fragments spontaneously re-associate to form fully active enzyme that , in the assay format, cleaves a substrate, generating a colour change that can be measured spectrophotometrically.

In the assay, analyte(CBZ) in the sample competes with analyte (CBZ) conjugated into one inactive fragment of β-galactosidase for antibody binding site.

If CBZ is present in the sample it binds to antibody, leaving the inactive enzyme fragments free to form active enzyme.

If CBZ is not present in the sample, antibody binds to CBZ conjugated on the inactive fragment, inhibiting the re-association of inactive β-galactosidase fragments, and no active enzyme wil be formed

The amount of active enzyme formed and resultant absorbance change are directly proportional to the amount of CBZ present in the sample.

Reagent constituents

-mouse monoclonal anti-CBZ antibodies

- enzyme acceptor

-enzyme donor conjugated to CBZ

-buffer

IMMUNO-INHIBITION TEST

Example: (for the quantitative determination of Digitoxin (CBZ) in human serum and plasma)

Summary

Digitoxin is a naturally occurring cardiac glycoside that bis used to treat congestive heart failure.

Test Principle

Reagent 1 contains monoclonal antibody specific for digitoxin that binds to microparticles covalently coated with a digitoxin derivatives in reagent 2. The resulting agglutination reaction causes an increase in turbidity, which can be measured spectrophotometrically at 700nm. Digitoxin from the sample competes with the microparticle-bound digitoxin for the antibody, thus inhibiting the agglutination reaction.

Reagent constituents

-monoclonal anti-digitoxin antibody
- digitoxin-derivative coated particles.

HAEMOGLOBIN DISORDERS.

-The clinical consequences of abnormalities of RBCs (erythrocytes) structure,function and metabolism includes anaemia (decreased rbcs or haemoglobin content of blood), polycythemia (greater than normal the number of rbcs or haemoglobin in the blood), cynosis (a bluish discoloration of skin and mucous membrane that results from deoxygenation of hb), and haemolysis (more rapid than normal destruction of rbc).

- *HAEMOGLOBIN.(hb)*

This is the part of rbcs that determines the oxggen carrying capacity of rbcs to various parts of the body systems. The oxygen is very important for the various metabolic activities that take place in the body cells.
- Hb is made up of four sub units ie two part of identical polypeptide chains and a pair of globin chains (alpha and beta).
- There are types of Haemoglobins-:
(a) In normal adults, 96% is Hb A, (2.5-3)% is Hb A2 and less than 1% is Hb F.
(b) Fetal Hb (Hb F) predorminates during fetal life but rapidly diminishes during the first year of post natal life.

-THELASSEMIAS .

This is a condition in which there is a reduction in the rate of synthesis of one type of globin chain.
-If the reduction is in alpha chain synthesis the resulting condition is alpha thelassemia and if the reduction of synthesis is in beta chain the resulting condition is beta thelassemia. Thelassemias occur world wide but with highest frequency in persons of African , Asian or Mediterranian origin. Alpha thelassemia is the most common disorder in man. The terms thelassemia minor and major signifies the severity of the clinical disorder and the term thelassemia tract signifies the heterozygous state for a thelassemia gene.

HAEMOGLOBINOPATHIES

These are disorders of Hb that results from the the structural abnormalities of a globin chain.
(a)SICKLING DISORDER.
When deoxygenated Hb forms long rope like polymers that distorts the shape of the rbcs thus forming sickle cells. The formation of irreversibly sickled rbcs is the cause of severe manifestations that accompany homozygous Hb S disease (Hb SS). In contrast, HbS trait (the heterozygous state is usually not life threatening.

(b) ERYTHROCYTOSIS.
This is the Hb variants with high affinity of oxygen which often cause moderate increase in haemoglobin concentration and haematocrit (pcv) concentration in rbcs content. The Hb variants that causes erythrocytosis do not readily release oxygen to tissues and are thus functionally inert.

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ASSIGNMENT(three)

(1)Discuss the diagnostic importance of chromatography in clinical /research laboratory.

(2) You are a manager of clinical services in a county hospital. Discuss the consideration you would make in purchasing new automated clinical chemistry equipment for the laboratory

(3)Daily Nation Advertisement (iv):DN/AD:001-9-2012

"Government of Japan medical laboratory equipment funding"
The government of Japan in collaboration with the Government of Kenya wishes to fund public health institutions to equip clinical chemistry laboratory. A total of 100million Kenya shillings set aside for this project will be given to the institution with the best proposal.

Discuss the key points in your proposal that will enable your institution attract this funding

ANSWERS

(1) (a) If a solution of [ ] 10 mg/dl is diluted 1:10, what is the final [ ].
(b) if the above resulting solution is re-diluted 1:5, what is the final [ ].

(a) I/10 x 10 mg/dl= **1 mg/dl**
(b) 1/5 x 1 mg/dl = **0.2 mg/dl**

(2) What is the final [ ] of an X molar solution that is diluted 5:50, re-diluted10:100 then re-diluted5:25?
5/50 x10/100 x 5/25 x X M = **(X/500) M or 0.002X M**

(3) Given a series of ten tubes, each of which contains 4 ml of diluent. 1 ml of fluid is added to the first tube and a serial dilution using 1 ml is carried out in the remaining tubes. What is the dilution of the fluid in the ***fifth tube*** and in the ***tenth tube***

1^{ST}_tube=1ml to 4 ml = 1:5=1/5
2^{nd} tube=1/5 x 1/5=1/25
3^{rd} tube=1/25 x 1/5=1/125
4^{th} tube= 1/125 x 1/5=1/625
5^{th} tube= 1/625 x 1/5= $1/5^5$ or 1/3125 or 1: 3125
6^{th} tube= 1/3125 x 1/5= $1/5^6$
7th tube = $1/5^6$x 1/5= $1/5^7$
8^{th} tube = $1/5^7$ x 1/5= $1/5^8$
9^{th} tube = $1/5^8$x 1/5= $1/5^9$
10^{th} tube = $1/5^9$ x 1/5= $1/5^{10}$ (1/9,765,625) or 1: 9,765,625

(4) How much 70% (v/v) alcohol can be made from 200ml of 95% alcohol?.

$V_A \times C_A = V_B \times C_B$
200ml x 95% = V_B x 70%
$$\frac{200\text{ml} \times 95\%}{70\%} = \mathbf{271.4ml}$$

(5) A 1M solution of NaOH is diluted 5:25, then re-diluted 3:100. what is the final molarity?. How many grams of NaOH are present in 100ml of the final dilution?.
(
a) 5/25 x 3/100 x 1M= 0.006 M

(b) NaOH= 23+16+1=40 gm
40 gm dissolved in 1 L = 1M
Then 0.006 M= 0.006M/1 M x 40 gm = 0.24 gm
0.24gm are in 1000ml (1L) then 100 ml of this solution will have ? gm
0.24gm=1000ml
? = 100 ml
100ml/1000ml x 0.24gm= **0.024gms**

(6) 80 gms of $CuSO_4$ are dissolved and diluted to 1000ml. This solution is diluted 1:10, then re-diluted 5:25.

(a) How many grams of $CuSO_4$ would be present in 1 litre of the final solution?.

(b) What is the [ ] of the final solution in terms of molarity and percentage (w/v).

(a) 1/10 x 5/25 x 80 gm= **1.6 gm**

(b1) 1 molar (1 M) = (63.54 + 32.06 + 64=159.6 gm in 1 L
If 160 gm = 1L=1 M
Then 1.6gm=?
1.6gm/ 160 gms x 1 M= **0.01 M**

(b2) 1.6 gm/1000 x 100 = **0.16%(w/v)**

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