جامعة الجزيرة الخاصة
ALJAZEERA PRIVATE UNIVERSITY

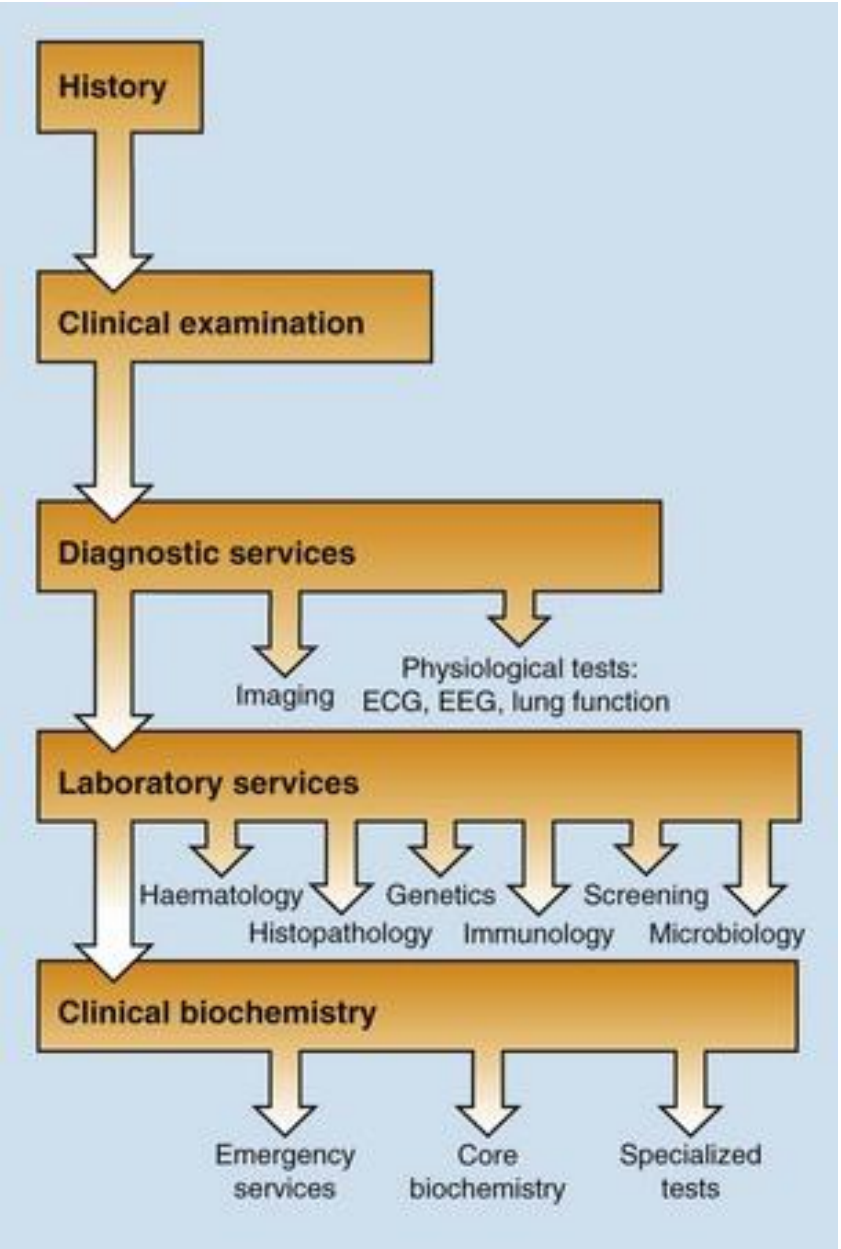
1 INTRODUCING CLINICAL BIOCHEMISTRY

Clinical Biochemistry.Dr.Iman Bakir

I The clinical biochemistry laboratory

Introduction to Clinical Laboratories

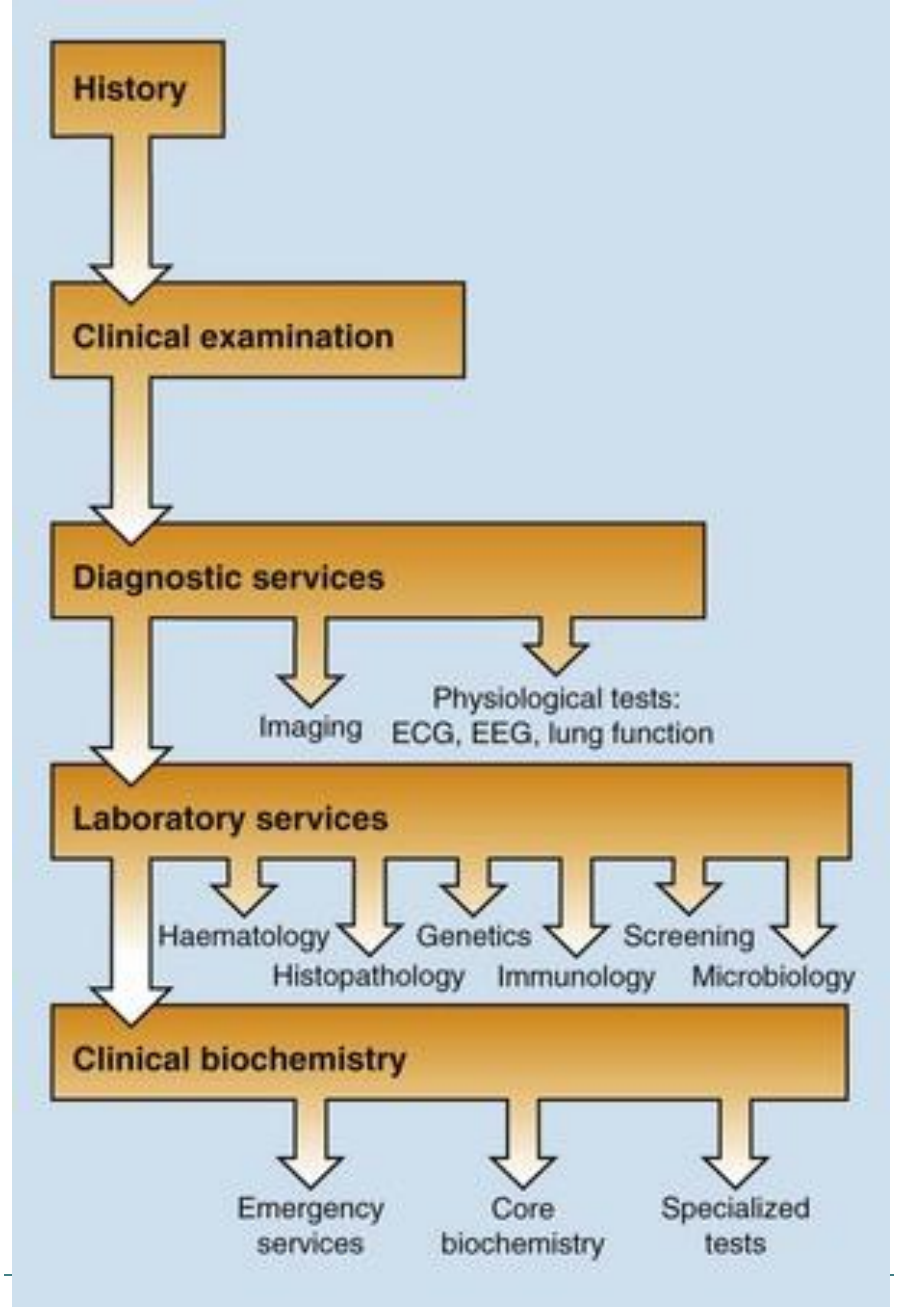
- Diagnosis begins with **physical examination** by a doctor.
- **Diagnostic tests** are important steps to confirm a suspected diagnosis



Introduction to Clinical Laboratories

❖**Clinical biochemistry**, **chemical pathology** and **clinical chemistry** are all names for the subject of this book, that branch of laboratory medicine in which **chemical and biochemical methods are applied to the study of disease**.

❖Clinical biochemical tests comprise over **one-third** of all hospital laboratory investigations



Introduction to Clinical Laboratories

❖usually, though not exclusively, confined to studies on **blood and urine** because of the relative ease in obtaining such specimens. Analyses are made on other body fluids, however, such as **gastric aspirate** and **cerebrospinal fluid.**

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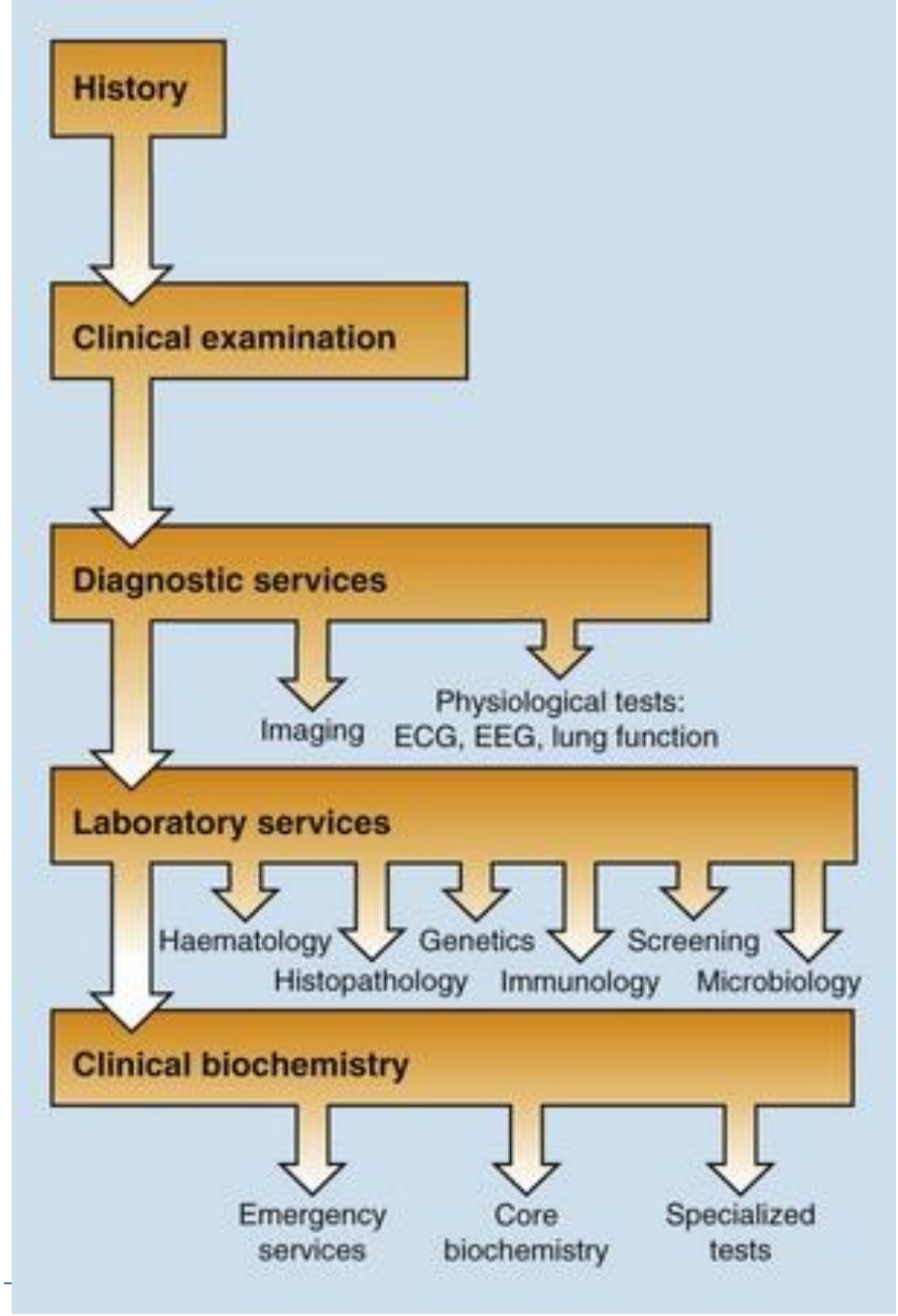


The use of biochemical tests

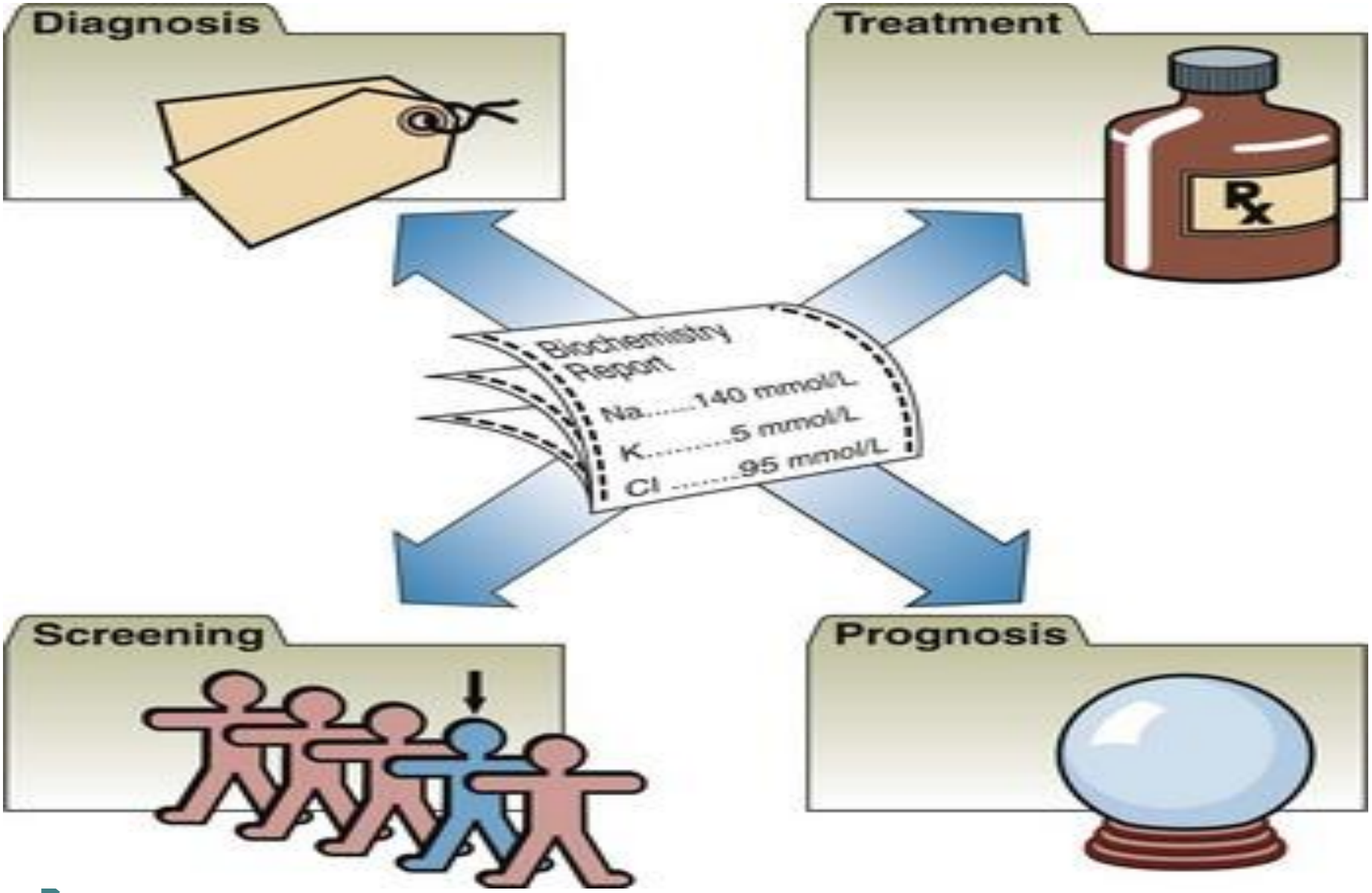
Biochemical investigations are involved in every branch of clinical medicine.

The results of biochemical tests may be of use in:

1. **Diagnosis** .
2. **Monitoring of treatment**.
3. **Screening for disease** .
4. Assesing the **prognosis.**
5. **Reseach** into the biochemical basis of disease
6. **Clinical trials of new drugs**

Biochemical investigations hold the key for the diagnosis and prognosis of diabetes mellitus, jaundice, myocardial infarction, gout, pancreatitis, rickets, cancers, acid-base imbalance etc. Successful medical practice is unimaginable without the service of clinical biochemistry laboratory.

The use of biochemical tests



Core biochemistry

Core biochemistry: Most biochemistry laboratories provide the "core analyses", commonly requested tests which are of value in many patients, on a frequent basis.

Core biochemical tests:

1. Sodium, potassium, chloride and bicarbonate
2. Urea and creatinine
3. Calcium and phosphate
4. Total protein and albumin
5. Bilirubin and alkaline phosphatase
6. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST)
7. Glucose
8. Amylase…….

Core biochemistry

Specialized tests

Table 1.1 **The clinical biochemistry repertoire**

Core biochemical tests

- Sodium, potassium and bicarbonate
- Urea and creatinine
- Calcium and phosphate
- Total protein and albumin
- Bilirubin and alkaline phosphatase
- Alanine aminotransferase (ALT) and aspartate aminotransferase (AST)
- Free thyroxine (FT_4) and Thyroid Stimulating Hormone (TSH)
- γ-glutamyl transpeptidase (γGT)
- Creatine kinase (CK)
- H^+, PCO_2 and PO_2 (blood gases)
- Glucose
- Amylase

Specialized tests

- Hormones
- Specific proteins
- Trace elements
- Vitamins
- Drugs
- Lipids and lipoproteins
- Intermediary metabolites
- DNA analyses

Specialized tests

Not every laboratory is equiped to carry out all possible biochemistry requests.

Large departments may act as reference centres where less commonly asked for tests are performed.

Specialized tests:

1. Hormones
2. Specific proteins
3. Trace elements
4. Vitamins
5. Drugs
6. Lipids and lipoproteins
7. DNA analyses

Urgent samples

The emergency lab

✓An urgent test is designated as one on which the clinician is likely to take immediate action.

✓The main reason for asking for an analysis to be performed on an urgent basis is that immediate treatment depends on the result.

electrolytes
Blood gases
Urea
Glucose
Amylase

The use of the laboratory

The use of the laboratory

The use of the laboratory

❑Each biochemistry test request should be thought of as a question about the patient; each biochemical result as an answer.

❑**Request forms** and specimens must be **correctly labelled** to ensure that results can be communicated quickly to the clinician.

Lab request and lab report forms

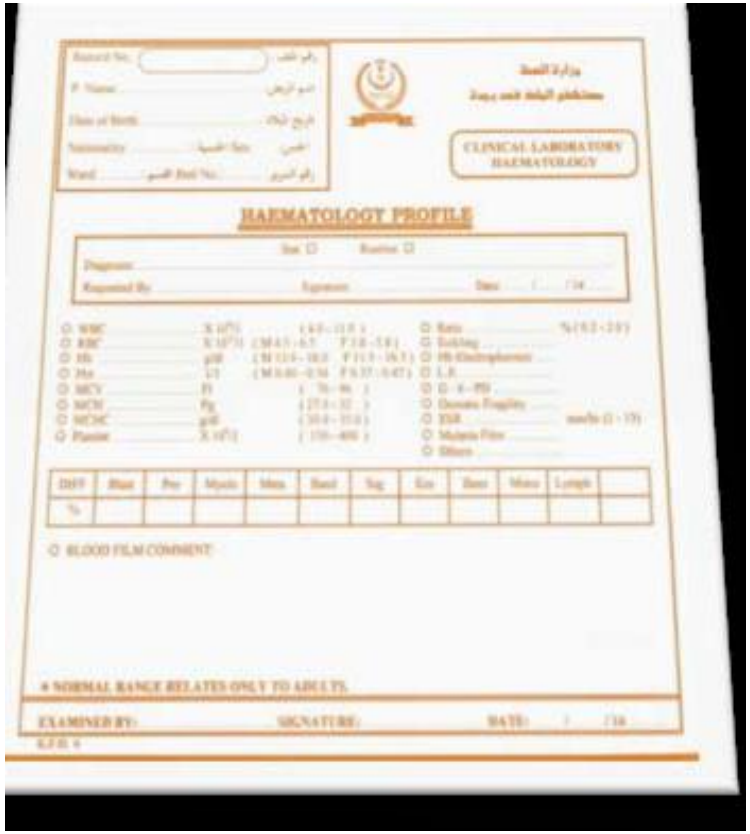
Lab request form: it fills computerize or paper filled by the doctor then send it to the lab. The lab request contains a list of tests to be performed on specimen of patient. Each lab has its specific request; for example, chemistry request, hematology request… etc.

Lab report form: it contains the result of patient.

Lab request:

1. Full name: middle name should be included
2. Location: inpatient, room, unit, outpatient, address.
3. Patient's identification number: this identification can be very useful for instance in the blood bank.
4. Patient age and sex: disease prevalence may be age- or sex-linked.
5. Name(s) of the physician(s): name all of the physicians on the case; "panic values" should be called to the attention of the physician ordering the test; a physician may have some specific test guidelines for his patients.
6. Name of the test.
7. Possible diagnosis: essential for evaluating laboratory results and selecting appropriate methodology; (media selection in microbiology).
8. The date and time the test is to be done: some tests must be scheduled by the laboratory; patient preparation and diet regulations need to be considered.
9. Special notation: provide relevant information to assist the laboratory--e.g., medications taken; for hormone assay, the point in the menstrual cycle when the specimen was obtained

Lab request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SIGNATURE:
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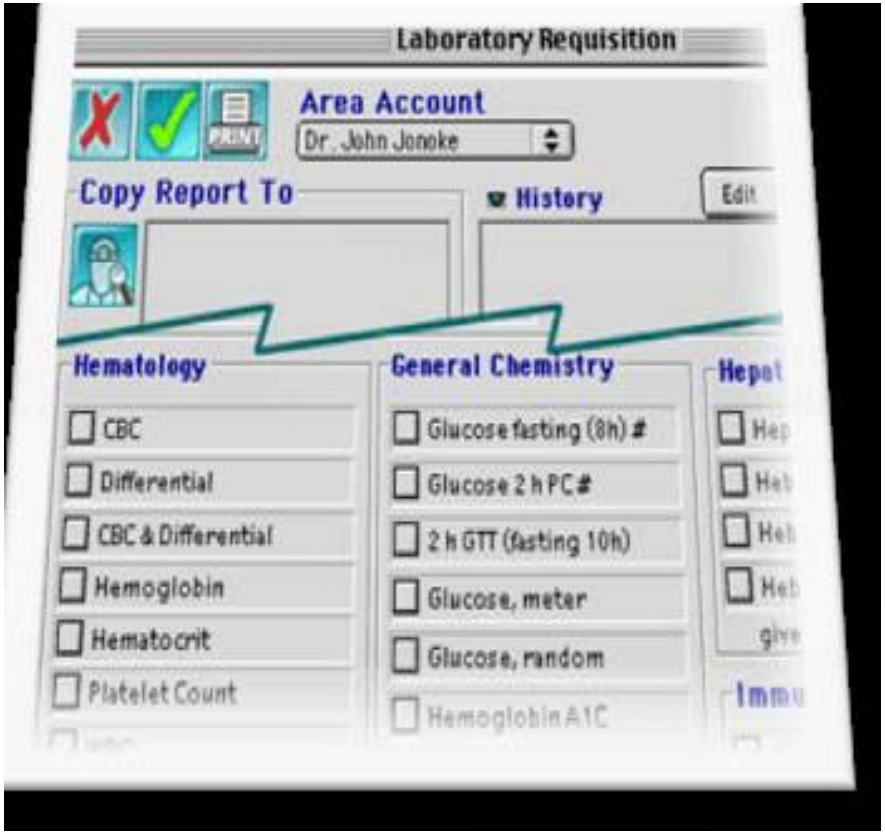
Laboratory Requisition
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CBC & Differential
Hemoglobin
Hematocrit
Platelet Count
General Chemistry
Glucose fasting (8h) #
Glucose 2 h PC #
2 h GTT (fasting 10h)
Glucose, meter
Glucose, random
HemoglobinA1C

Table 2.1 **Specimens used for biochemical analyses**

- Venous blood, serum or plasma
- Arterial blood
- Capillary blood
- Blood spot on a filter paper (Guthrie Card)
- Urine
- Faeces
- Cerebrospinal fluid (CSF)
- Sputum and saliva
- Tissue and cells
- Aspirates, e.g.
  - pleural fluid
  - ascites
  - joint (synovial) fluid
  - intestinal (duodenal)
  - pancreatic pseudocysts
- Calculi (stones)

Phlebotomy

Phlebotomy or blood collection:

The term phlebotomy refers to blood draw from a vein, artery, or the capillary bed for lab analysis or blood transfusion.

The phlebotomy equipments:

For specimen collection, the following materials will be required:

The phlebotomy equipments

1) Disposable syringes or vacutainer systems
2) Disposable lancets
3) Adsorbent cotton
4) Tourniquet
5) Alcohol
6) Waste container

Specimen collection

COLLECTION OF BLOOD:

Venous blood is most commonly used for a majority of biochemical investigations.

It can be drawn from any prominent vein (**usually from a vein on the front of the elbow**).

Capillary blood (<0.2 ml) obtained from a finger or thumb, is less frequently employed.

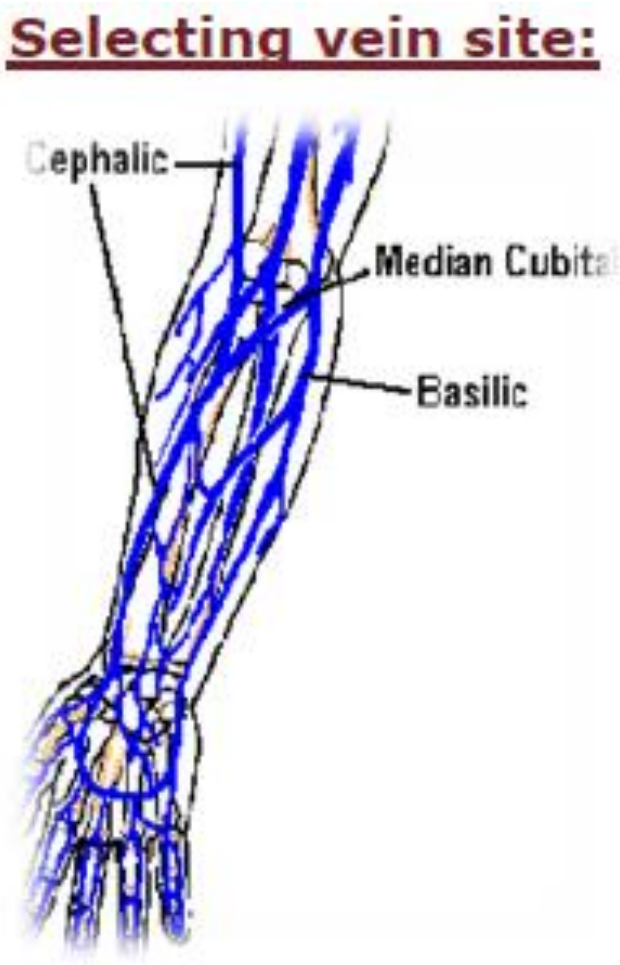
Arterial blood (usually drawn under local anesthesia) is used for blood gas determinations.

Precautions for blood collection : Use of sterile (preferably disposable) needles and syringes, cleaning of patients skin, blood collection in clean and dry vials/tubes are some of the important precautions.

Blood collection

Venous blood

The median cubital vein is the one used for the patient.



Biochemical investigations can be performed on 4 types of blood specimens – whole blood, plasma, serum and red blood cells. The selection of the specimen depends on the parameter to be estimated.

1. ***Whole blood*** (usually mixed with an anticoagulant) is used for the estimation of hemoglobin, pH.

2. Plasma obtained by centrifuging the whole blood collected with an anticoagulant, is employed for the parameters—fibrinogen, glucose, bicarbonate, chloride, ascorbic acid etc.

3. Serum is the supernatant fluid that can be collected after centrifuging the clotted blood. It is the most frequently used specimen in the clinical biochemistry laboratory. The parameters estimated in serum include proteins (albumin/globulins), creatinine, bilirubin, cholesterol, uric acid, electroylets (Na+, K+, Cl-), enzymes (ALT, AST, LDH, CK, ALP, ACP, amylase, lipase) and vitamins.

4. Red blood cells are employed for the determination of abnormal hemoglobins, glucose 6-phosphate dehydrogenase, pyruvate kinase etc.

Preparation of Blood Sample

One of three different specimens may be used:

- **whole blood**
- **serum**
- **plasma**

First: Whole-blood specimen:

It must be analyzed within limited time (why?)

- Over time, cells will lyse in whole-blood which will change the conc. of some analytes as potassium, phosphate and lactate dehydrogenase.
- Some cellular metabolic processes will continuo which will alter analytes conc. like glucose and lactate.

Blood serum:

- Serum is the same as plasma except that clotting factors (such as fibrin) have been removed.
- No coagulation factors
- It is obtained by letting a blood specimen clot prior to centrifugation.

Serum

Second Serum:

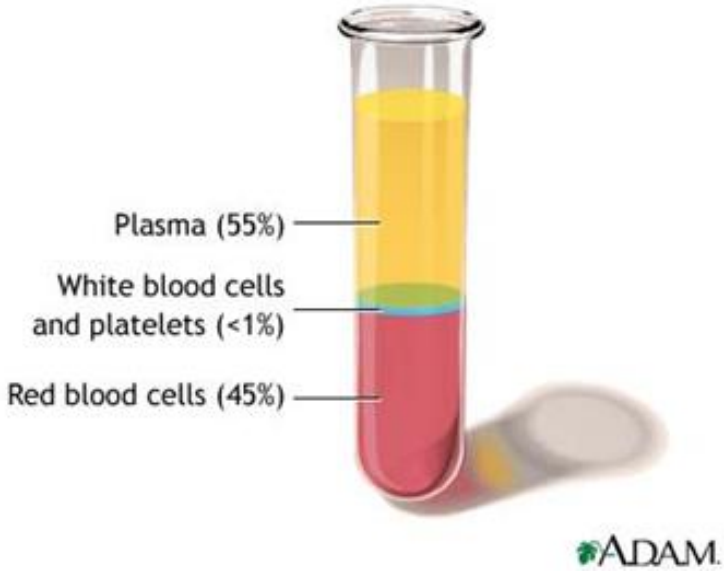
Difference between Serum and plasma:

- Serum is the same as plasma except it doesn't contain clotting factors (as fibrin).
- Plasma contains all clotting factors.
- So, serum and plasma all has the same contents of electrolytes, enzymes proteins, hormones except clotting factors
- Serum is mainly use in chemistry lab & serology.

Plasma

Third Plasma:

- The tube will have anti-coagulation
- After centrifugation the blood sample got separated into three layers

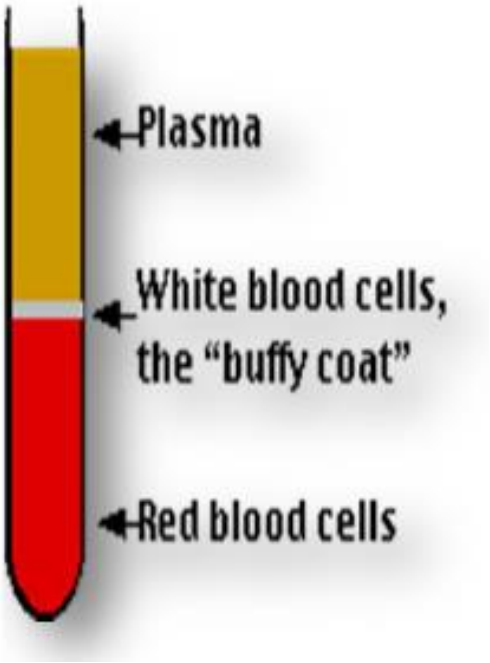


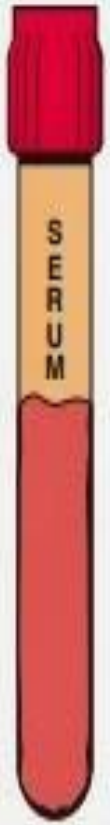
Blood

- After centrifugation of blood, the blood separate into three layers
- Red blood cells(RBCs)

-White blood cells(WBCs)

- -Platelets



Plain tube:no anticoagulant Clot forms	Plain tube: contains SST gel	EDTA anticoagulant	Lithium heparin anticoagulant	Fluoride oxalate	Trace element	Heparinized syringe
						
• General	• General	• Whole blood analysis • Red cell analysis • Lipids and lipoproteins	• General	• Glucose • Lactate • Alcohol	• Copper • Zinc	• Arterial blood sampling

Plasma Separating Tubes (PST)

Top Color	Additives	Principle	Uses
Lavender	**EDTA** Ethylenediamine tetraacetic acid	**-The strongest anti-coagulant** **Ca^{+2} chelating agent -** **- To preserve blood cells components**	**Hematology -** **Blood bank -** **(ABO)** **HbA1C -** **(Glycosylated Hb)**
Light Blue	**Sodium Citrate**	**Ca^{+2} chelating agent**	**- PT: Prothrombin Time** **- PTT: Partial Thromboplastin Time** **(in case of unexplained bleeding and liver disease)**
Green	**Sodium Heparin or Lithium Heparin**	**Heparin binds to Thrombin and inhibits the second step in the coagulation cascade** **(Prothrombin**  **Thrombin)** **Heparin** **Fibrinogen**  **Fibrin**	**Enzymes** **Hormones** **Electrolytes (Na^{+}, K^{+}, Mg^{+}, Cl^{-})**

Top Color	Additives	Principle	Uses
Black	**Sodium Citrate**	**Ca^{+2} chelating agent**	**ESR (Erythrocyte Sedimentation Rate) to test how much inflammation in the patient, unexplained fever, Arthritis, Autoimmune Disorder**
Gray	**-Sodium Fluoride** **-Potassium Oxalate** →	**Glycolysis inhibitor** **Anti-Coagulant**	**Glucose tests**
Royal Blue	**Heparin** **Na-EDTA** →	**Anti-Coagulant** **Tube should not be contaminated with metals**	**Toxicology** **Trace Elements and metals**
Royal blue top tubes Contain either sodium heparin or sodium EDTA anticoagulants, or no anticoagulant. Are used for trace element, toxicology, and nutritional studies.			
Yellow	**ACD (Acid-Citrate Dextrose)**	**Anti-Coagulant**	**DNA Studies** **Paternity Test** **HLA Tissue Typing (Human Leukocyte Antigen)** **The body used this protein to differentiate the self-cells from non-self cells**

Serum Separating Tubes (SST)

Top Tubes	Additives	Principle	Uses
Red	------ Sometimes it has gel or silicon at the bottom of tube to reduce hemolysis	Enhancing the formation of blood clot	Serology -Antibodies -Hormones -Drugs Virology Chemistry Blood cross matching before blood transfusion
Gold	------- It has gel at the bottom of the tube to separate serum from the blood	Serum separating from the blood through the gel in the tube	Serology Chemistry

Hemolysis

- It means liberation of hemoglobin due to rupture of RBCs.
- Due to hemolysis plasma or serum appears pink to red color.
- It causes elevation in: K^+, Ca^{2+}, phosphate, SGOT, SLDH and acid phosphatase.
- Hemolysis is occurred due to sampling, transporting and storage (too hot or too cold).
- According to the degree of hemolysis it is classified as H+, H++ and H+++. H+ may be accepted for some tests that are not affected by RBCs contents as glucose and lactate, H++ and H+++ not acceptable for any test.

Changes in the serum color indicate one of the following:

- **Hemolyzed**: serum appears pink to red due to rupture of RBCs
- **Icteric**: serum appears yellow due to high bilirubin.
- **Lipemic**: serum appears milky or turbid due to high lipid.

Sampling errors

- ***Blood sampling technique Difficulty in obtaining a blood specimen may lead to haemolysis with consequent release of potassium and other red cell constituents.***
- ***Prolonged stasis during venepuncture***.Plasma water diffuses into the interstitial space and the serum or plasma sample obtained will be concentrated. Proteins and protein bound components of plasma, such as calcium or thyroxine, will be falsely elevated.
- ***Insufficient specimen***. *It may prove to* be impossible for the laboratory to measure everything requested on a small volume*Errors in timing.The biggest source* of error in the measurement of anyanalyte in a 24-hour urine specimen is in the collection of an accurately timed volume of urine.
- ***Incorrect specimen container***.
- **Inappropriate sampling site.**

Urine specimens

Urine specimen containers may include a preservative to inhibit bacterial growth, or acid to stabilize certain metabolites. They need to be large enough to hold a full 24-hour collection. Random urine samples are collected into small 'universal' containers

Other specimen types

For some tests, specific body fluids or tissue may be required. There will be specific protocols for the handling and transport of these samples to the laboratory. Consult the local lab for advice

3 The interpretation of results

How biochemical results are expressed

❖Most biochemical analyses are quantitative.

❖although simple qualitative or semiquantitative tests, such as those for the presence of glucose in urine, are commonly encountered methods used for point of care testing.

How biochemical results are expressed

- Many tests measure the amount of the analyte in a small volume of blood, plasma, serum, urine or some other fluid or tissue.
- Results are reported as concentrations, usually interms of the number of moles in one litre **(mol/L**).
- Enzymes are not usually expressed in moles but as enzyme activity in **'units**'.
- Some hormone measurements are expressed as '**units'**
- Large molecules such as proteins are reported inmass units (grams or milligrams) per

litre. Blood gas results (PCO2 or PO2)are expressed in kilopascals (**kPa),** the unit in which partial pressures are measured.

Molar units

Mole	Abbreviation	Definition
Milimole	mmol	*10 -3 of a mole
Micromole	µmol	*10 -6 of a mole
Nanomole	nmol	*10 -9 of a mole
Picomole	pmol	*10 -12 of a mole
Femtomole	fmol	*10 -15 of a mole

Variation in results

Biochemical measurements vary for two reasons.

These are described as

- ‘analytical variation’
- and ‘biological variation

Analytical variation is a function of analytical performance

biological variation is related to the actual changes that take place in patients’ body fluids over a period of time.

Laboratory analytical performance

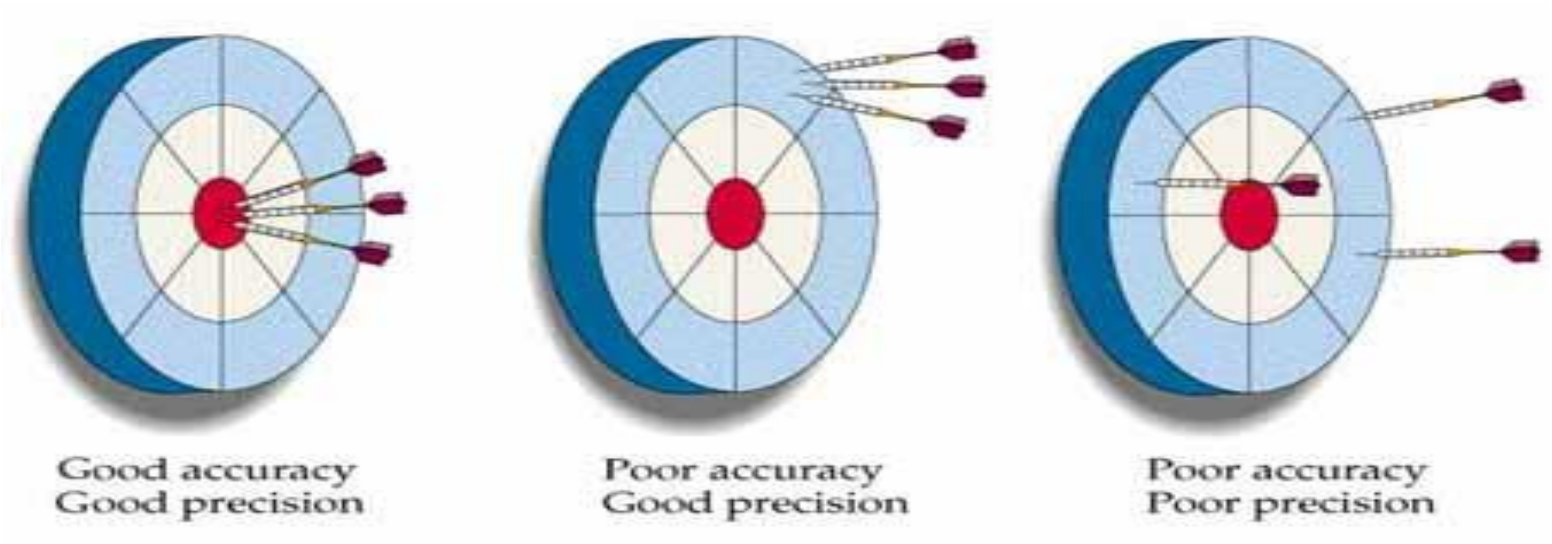
A number of terms describe biochemical results. These include:

- **precision and accuracy**
- **sensitivity and specificity**
- **quality assurance**
- **reference intervals**

1.Precision and accuracy

Accuracy: Defines how close the measured value is to the actual value.

if true blood urea level is 50 mg/dl, the laboratory reporting 45 mg/dl is more accurate than the one reporting 35 mg/dl.

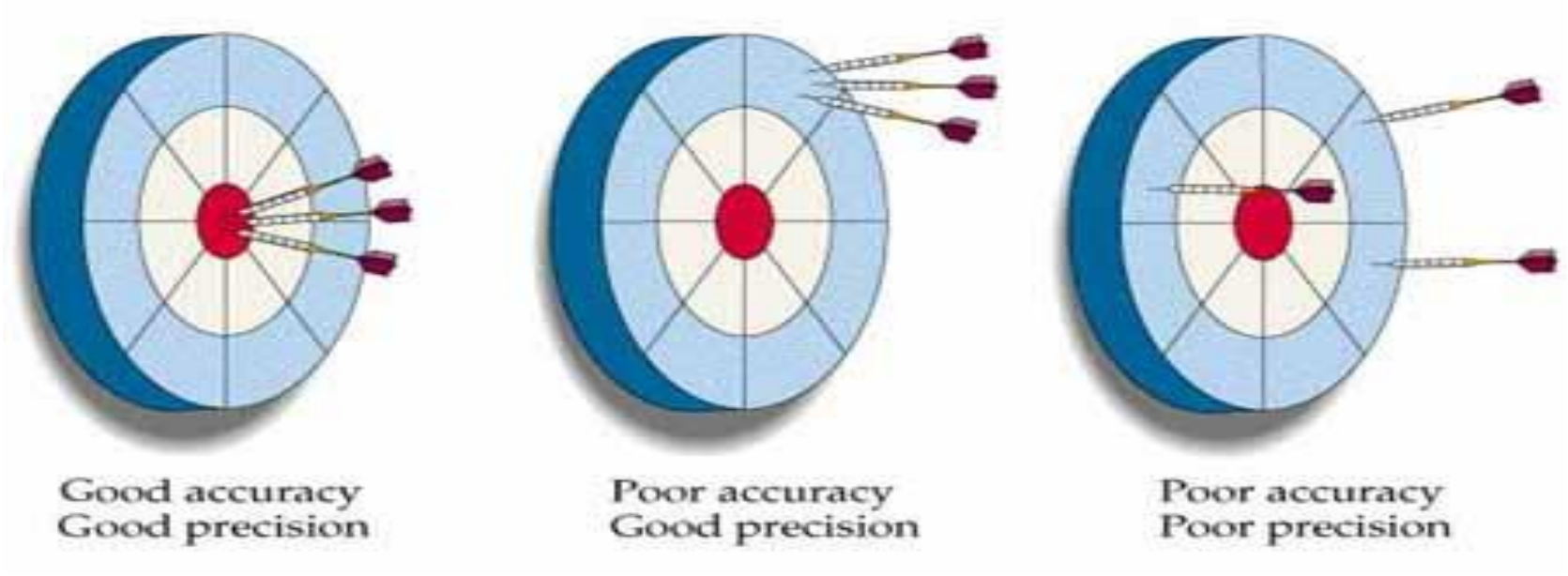


1.Precision and accuracy

Precision: is the reproducibility of an analytical method.

when the same sample is analysed on different occasions (replicate measurements) by the same person.

For instance, the precision is good, if the blood glucose level is 78, 80 and 82 mg/dl on replicates.



2.Analytical sensitivity and specificity

❖**The analytical sensitivity:** of an assay is a measure of **how little** of the analyte the method can detect. glucose can be specifically estimated by enzymatic glucose oxidase method.

❖ **Analytical specificity:** of an assay relates to how good the assay is at **discriminating** between the requested analyte and potentially interfering substances.

3.Quality assurance

1. ***Internal quality control*** refers to the analysis of the same stored sample on different days in a laboratory, the results should vary within a narrow range.

2. ***External quality control*** deals with the analysis of a sample received from outside, usually from a national or regional quality control centre. The results obained are then compared

4.Reference intervals

Biochemical test results are usually compared to a reference interval chosen arbitrarily to include 95% of the values found in healthy volunteers.

This means that, by definition, 5% of any population will have a result outside the reference interval.

Limit of Errors Allowable in Laboratory

In a laboratory, error may not be totally avoided; but should be kept at a minimum. The limits are denoted by the term, **percentage error.** The percentage of allowable error in anassay is given by the formula:

$$\frac{\text{Difference between maximum and minimum of normal range}}{\text{Mean of the normal range x 4}} \times 100$$

The percentage error, therefore, will vary from test to test. To take an example, in the case of blood glucose analysis, the normal range is 70 to 110 mg/dl. If these values are substituted in the formula:

$$\frac{(110 \text{ minus } 70)}{90x4} \times 100 = 10\%$$

Now, in the case of blood urea (normal range of 20-40 mg/dl), the percentage error will be

$$\frac{(40 \text{ minus } 20)}{30\text{-}x4} \times 100 = 16\%$$

Biological factors affecting the interpretation of results

- ***Sex.*** *Reference intervals for some* analytes such as serum creatinine are different for men and women.
- ***Age.*** *There may be different* reference intervals for neonates, children**,** adults and the elderly.
- ***Diet.*** *The sample may be* inappropriate if taken when the patient is fasting or after a meal.
- ***Timing.*** *There may be variations* during the day and night.
- ***Stress and anxiety.*** These may affect the analyte of interest.

Biological factors affecting the interpretation of results

- ***Posture of the patient***. *Redistribution* of fluid may affect the result.

- ***Effects of exercise***. *Strenuous exercise* can release enzymes from tissues.

- ***Medical history.*** *Infection and/or* tissue injury can affect biochemical values independently of the disease process being investigated.

- *Pregnancy*. *This alters some reference* intervals.

Biological factors affecting the interpretation of results

- ***Menstrual cycle.*** *Hormone* measurements will vary throughoutthe menstrual cycle.
- ***Drug history****. Drugs may have* specific effects on the plasma concentration of some analytes

4.Point of care testing

Outside the laboratory

The tests commonly performed **away** from the laboratory can be categorized as follows:

A. *Tests performed in medical or nursing settings. They clearly give* valuable information and allow the practitioner to reassure the patient or family or initiate further investigations or treatment.

B. *Tests performed in the home, or non-clinical setting. They can give* valuable information when properly and appropriately used.

C. *Alcohol tests. These are sometimes* used to assess fitness to drive. In clinical practice alcohol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Bayer
PRINT OUTPUT LIST SET
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Common tests on blood performed away from the laboratory Analyte Used when investigating

Analyte	Used when investigating	A
Blood gases	Acid-base status	
Glucose	Diabetes mellitus	
Urea	Renal disease	
Creatinine	Renal disease	
Bilirubin	Neonatal jaundise	
Therapeutic drugs	Compliance of toxicity	
Salicylate	Detection of poisoning	
Paracetamol	Detection of poisoning	
Glucose	Diabetic monitoring	B
Cholesterol	Coronary heart disease risk	
Alcohol	Fitness to drive/confusion, coma	C

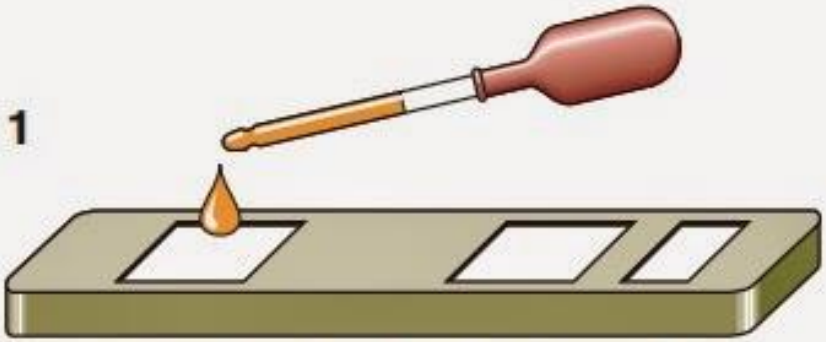
Tests on urine performed away from the laboratory

Analyte	Used when investigating	A
Ketones	Diabetic ketoacidosis	
Protein	Renal disease	
Red cells/haemoglobin	Renal disease	
Bilirubin	Liver disease and jaundise	
Urobilinogen	Jaundise/haemolysis	
pH	Renal tubular acidosis	
Glucose	Diabetes mellitus	B
hCG	Pregnancy test	

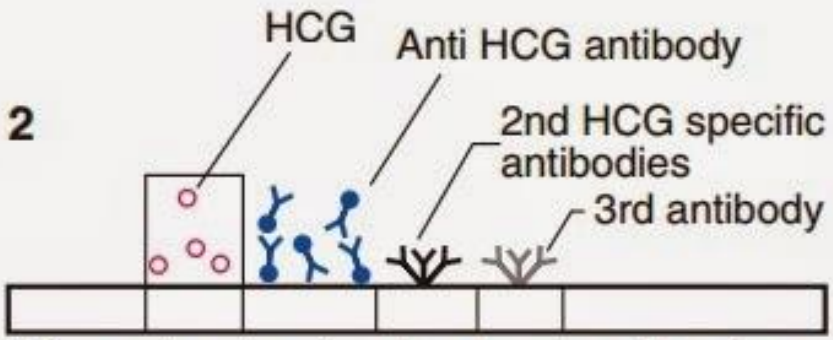
Methodology

It is a feature of many sideroom tests that their simplicity disguises the use of sophisticated methodology**. pregnancy test.**

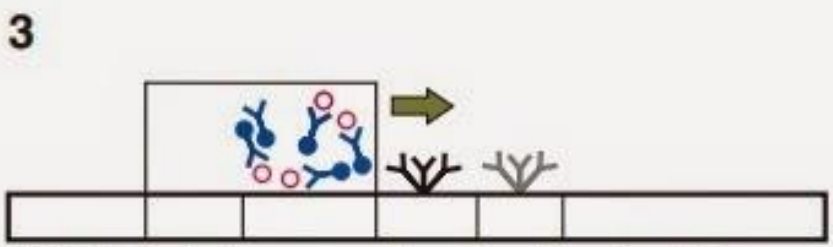
Human Chorionic Gonadotropin (hCG)



A urine sample is applied to the test strip.



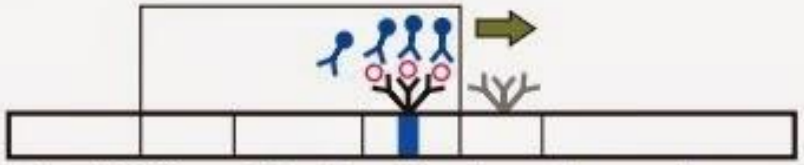
Urine saturates absorbent pad and begins to move along test strip.



HCG binds to monoclonal antibody–blue bead complex, which then moves along the plate as the urine diffuses.

How a pregnancy test kit works

4



The HCG–antibody–blue bead complex binds to a 2nd HCG specific antibody fixed to the plate along a straight line. This produces a blue line on the plate.

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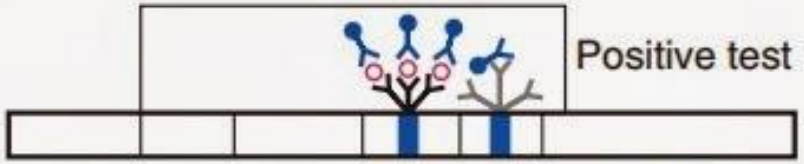

Excess of the monoclonal antibody–blue bead complex in the urine binds to a third antibody forming another blue line. This signals that the test is complete.

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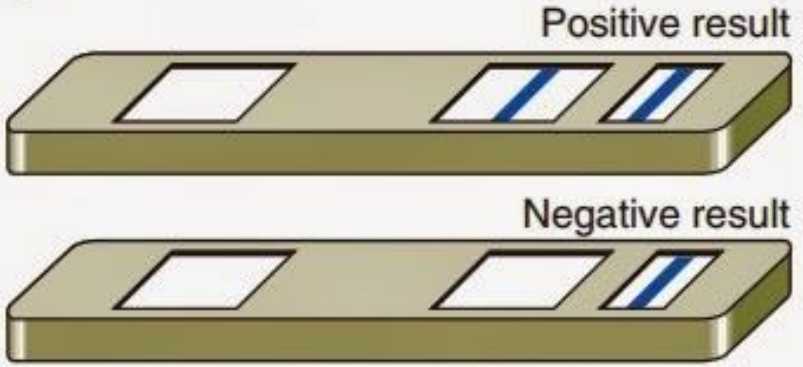

A positive result is shown by 2 blue lines; a negative result is shown by 1 blue line.

How a pregnancy test kit works

General problems

1.Cost.

2. Responsibility

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Analytical problems

The most commonly encountered analytical errors arise because of failure to:

1. calibrate an instrument
2. clean an instrument
3. use quality control materials
4. store reagents or strips in appropriate conditions.

The clinical biochemistry laboratory

- Biochemical tests are used in diagnosis, monitoring treatment, screening and for prognosis.
- Core biochemical tests are carried out in every biochemistry laboratory. Specialized tests
- may be referred to larger departments. All hospitals provide for urgent tests in the
- ‘emergency laboratory’.
- Laboratory personnel will readily give advice, based on their knowledge and experience, on
- the use of the biochemistry laboratory, on the appropriate selection of tests, and about the
- interpretation of results.

The interpretation of results

❖**The interpretation of results**

❖Biochemistry results are often reported as concentrations.

❖ Concentrations change if the amount of the analyte changes or if the volume of solvent changes.

❖ Variability of results is caused by both **analytical factors and biological factors.**

❖The reference range supplied with the test result is only a guide to the probability of the

❖results being statistically ‘normal’ or ‘abnormal’.

❖ Different reference intervals may apply depending on the age or sex of the patient.

❖ Sequential changes observed in cumulative reports when placed in clinical context are as

Point of care testing

❖**Point of care testing**

❖Many biochemical tests are performed **outside the normal laboratory** setting, for the convenience of patient and clinician.

❖Although apparently simple, such tests may yield erroneous results because of operator errors.

❖It is important that advice be readily available to interpret each result in the clinical context