**Specimen collection**

Biological specimens include :-

1-Whole blood

2- Serum (the clear liquid that separates from blood that is allowed to clot)

3- Plasma (the yellow liquid that remains after cellular components are removed by centrifugation)

4-Urine

5-Feces

6-Saliva

7-Others (**ascites** ,synovial, spinal, amniotic, pleural and pericardial) fluids

**Blood aspiration**

1-venipuncture (in adult) from vein

2- Arterial puncture (for blood gas analysis) from artery

3- Skin puncture( in young children) from capillary

**Venipuncture**

It is obtaining blood specimen from a patient s vein .

-Location

1- From median cubital vein is preferred because it is large and close to surface of skin

2-Cephalic and basilica v

3-Back of hand and ankle

The patient should be seated or supine and never performed on standing patient .

Preparation of site-

By gauze pad saturated with isopropanol

If specimen collected for ethanol determination you must use (zephiran chloride)

Povidone iodine should be avoided as cleansing agent because it interfere with several chemistry procedures

The skin should be allowed to dry in the air because traces may cause hemolysis

-Timing

Time at which specimen is obtained is important for those constituents that undergo marked diurnal variation like steroids, and specimens for alcohol or drug measurement .

-Venous occlusion

Tourniquet is applied 10-15 cm above the intended puncture site .This will obstruct the return of venous blood to the heart so will distend the veins .

Tourniquet leaved between 1-3 minutes because marked changes observed after 3 minute .

Pumping of fist before venipuncture should be avoided because it cause an increase in plasma potassium, phosphate, and lactate concentration

that lowering blood PH which will cause ionized calcium to increase .

**Skin puncture**

Skin is punctured with sharp lancet with depth less than 2.5 mm depth .

The first drop of blood is wiped off, and subsequent drops are transferred to collection tube by gentle contact, and avoid milking or squeezing of finger or foot.

This method is used for :-

1- Pediatric age group because we need limited sample

2- Sever venous damage like in repeated venipuncture

3-Unavailable veins like in burn or bandage

4-Point of care tests like glucose and hemoglobin

**Arterial puncture**

It is used primarily for blood gas analysis.

Preferred sites are:-

- Radial art. at wrist

-Brachial art. at the elbow

-Femoral art. at the groin

Factors affecting results because of blood collection

Technique use of anticoagulant, preservatives, site of

collection, hemolysis, and venous stasis

**Anticoagulant**

It is used to prevent clot formation when whole blood or plasma specimen are needed

- Heparin is the most widely used anticoagulant , it is available as sodium, potassium, lithium and ammonium salts .

It prevents fibrinogen change to fibrin by accelerating action of antithrombin 3 .

It inhibits acid phosphatase activity and inhibit binding of triiodothyronine and thyroxine to their carrier proteins thus producing higher free concentration of these hormones

It also unacceptable for most tests of PCR .

- EDTA (ethylene-diamine-tetra-acetic acid) is a chelating agent binding divalent cations such as Ca which is important for clotting

mechanism,so it is invalid for calcium estimation .

It is used as disodium,dipotassium, or tripotassium salts so it is unsuitable for potassium analysis .

- ACD (acid citrate dextrose) is used for samples for molecular diagnosis to preserve both the form and function of cellular component .

- Sodium fluoride is a weak anticoagulant added as preservative for glucose in blood

It inhibit enzyme involve in glycolysis, but it interfere with urea nitrogen through inhibition of urease enzyme .

- Sodium citrate is a chelating agent for calcium so it is unsuitable for measurement of Ca .

- Sodium iodoacetate used as substitute for sodium fluoride as anti-glycotic agent but it has no effect on urea test so it is used when

both glucose and urea tests are performed onthe same time .

**Site of collection**

There is no clinical significant difference between capillary blood and arterial blood in PH, PO2 and PCO2 .

Venous blood glucose is less than capillary glucose .So capillary blood have increased glucose and potassium and decrease bilirubin , calciumsodium, and total protein compared to venous blood .

Blood from site of intravenous infusion give false result of hyperglycemia .

**Hemolysis**

It is disruption of red cell membrane resulting in release of hemoglobin and other cellular components.it lead to increament of lactate

dehydrogenase, potassium, magnesium, AST and PO4,

Hemoglobin may interfere with some chemical reactions so falsely increase plasma bilirubin and lowering alkaline phosphatase

activity .

**Delayed separation of blood**

In vitro the erythrocytes use up the available glucose in it so glucose will pass from plasma to RBC to continue using glucose so blood glucose level will below .

**Refrigeration**

Refrigeration of whole blood cause reduce activity of adenosine triphosphatase pump so this not suitable for urea and elecrolytes measurement . Freezing results in hemolysis, therefore blood specimens must be centrifuged and plasma separated from cells before storing .

**Venous stasis**

if occlusion is maintained for more than short time the combined effect of raised intracapillary pressure and hypoxia of vessel wall increase rate of passage of water and small molecules from lumen to surrounding interstitial fluid but large molecules not pass like proteins and protein bound calcium will be increased.also potassium leakage causing falsely

high plasma concentration .

**Specimen handling**

\* Specimen identification or labeling: every specimen container must be adequately labeled with minimum information that include :

-Patient name, identify number, date and time of collection.

-Use request form wrapped around the container is not acceptable as specimen label.

\* Test request: that must contains

-Patient name and address

-Patient gender

-Patient age

-Date and time specimen obtained

-Test required

-Type of specimen

-Name of physician ordering test

\* Specimen preservation:

\* Separation and storage: plasma or serum should be separated from cells as soon as possible , but early separation before half hour may permit continued formation of fibrin and lead to obstruction of sample probe in testing equipment. if it is impossible to centrifuge a blood specimen within 2 hours ,the specimen should be held at room temperature rather than at 4c to decrease hemolysis. Avoid freeze-thaw cycle bec. It may degrade analyte of interest.

\* Specimen transport : in sealed plastic biohazard bags designed for specimen transport .

**Other specimens**

\* **Urine specimen**

Random early morning specimen is preferred bec. It is most concentrated ,used for microscopical examination , detection of abnormal amounts of constituents such as proteins, HCG in urine.

Bacterial examination of first 10 ml of urine voided is most appropriate to detect urethritis, whereas midstream specimen is best for bladder disorders.

Double voided specimen is urine excreted during timed period after complete emptying of bladder used in glucose tolelance test.

**Urine preservatives**

It is added to reduce bacterial action or chemical decomposition or to solubilize constituents that might precipitate out of solution.

Most satisfactory forms of preservation of urine is refrigeration after collection.

Refrigeration is even more successful when combined with chemical preservatives like acidification to below PH 3 to preserve 24 h. specimen , and specimen for calcium, steroid, and vanillmandelic acid (VMA).

Weak base such as sodium bicarbonate or small amount of sodium hydroxide is used to preserve specimens for porphyrins, urobilinogen, and uric acid testing.

\* **Feces specimen**

Feces are most commonly tested for microorganism as the cause of diarrhea , and tested for heme or occult blood as an indicator of bleeding ulcer or malignant disease in gastrointestinal tract. Feces from children may be screened for trypsin activity to detect cystic fibrosis.

In adult , fecal excretion of nitrogen and fat is used to assess the severity of malabsorption and the measurement of fecal porphyrins is required to characterize type of porphyria.

Usually , no preservative is added to feces , but container should be kept refrigerated throughout collection period and care should be taken to prevent contamination of urine.

\* **Cerebrospinal fluid specimen**

Spinal fluid is normally obtained from lumber region , it is examined for

1. Cerebrovascular accident

2. Meningitis

3. Demyelinating disease

4. Malignant disease

The initial specimen may be contaminated by tissue debris or skin bacteria so the first tube should be used for chemical or serological tests, the second for microbiological tests, and the third for microscopic and cytological tests.

\* **Synovial fluid specimen**

The technique of obtaining synovial fluid for examination is called arthrocentesis which is used to characterize type of arthritis and to differentate non-inflammatory effusion from inflammatory infusion.

Normally, only a very small amount of fluid is present in any joint but this volume is usually very much increased in inflammatory conditions.

Sterile plain tube is used for culture and for glucose and protein measurement. EDTA tube is necessary for total leukocyte, differential and erythrocyte count.

\* **Amniotic fluid**

The collection of amniotic fluid is called (amniocentesis), which is used for:

1. Assessment of prenatal diagnosis of congenital disorders

2. To assess fetal maturity

3. To look for Rh isoimmunization

If specimen is for determination of fetal lung maturity using lecithin-sphingomyelin ratio or albumin to surfactant ratio, the container is immediately placed in ice.

If for spectrophotometric analysis , the specimen should be transferred to brown tube to prevent photo-degradation of bilirubin.

\* **Pleural, pericardial, and ascetic fluid**

these cavities normally contain a small amount of serous fluid that lubricate the opposing parietal and visceral membrane surfaces. The collection procedure is called paracentesis which is used to know that fluid accumulate is an effusion or an exudateby presence of protein .

\* **Saliva**

Its use is very limited except measurement of blood group substances to determine secretor status and genotype , and most recently to detect the presence of anti-HIV antibodies.

**Requesting lab. Results**

They are used for:

1. To help diagnosis and confirming a clinical suspicion.

2. Screening for metabolic diseases

3. To monitor treatment and prognosis

4. For researches

**Repetition of the same test Or how often I should investigate the patient?**

1. How quickly numerically significant changes occur like concentration of main plasma protein fractions do not changed in less than 1 week also TSH do not changed in less than 6 weeks.

2. Whether significant numerical changes will alter treatment like potassium concentration alter rapidly in patient taking large doses of diuretics which need to change treatment.

**Result interpretation to find sources of error**

\* **Pre-analytical errors:** which represent 40% of lab. Errors

**A.technical factors:**

**1.** Accurate labeling for identification

**2.** Collection into correct container

**3.** Appropriate preparation of patient like fasting

**4.** Rapid and safe transport to lab. Like arterial blood gases samples should be sealed and cooled , and samples of porphyrin should be protected from light.

**B. biological factors**

**-Endogenous**

1. Age related differences like bilirubin in neonate and plasma alkaline phosphatase is higher in children and elderly.

2. Sex related differences like plasma urate is higher in males, and HDL is higher in premenopausal women than in men.

3. Ethinic differences like creatine kinase is higher in black than in white people.

4. Regular i.e. changes occur throughout the 24-h period called circadian or diurnal rhythms like plasma cortisol which is higher in the morning and liw at night.

Also plasma iron concentration may fall by 50% between morning and evening .also there is monthly variation especially in women during menstrual cycle like estradiol.

5. Body mass: obese individuals tend to have higher insulin and TG **--Exogenous**

**1)** Stress: pituitary and adrenal hormones are affected by stress (ACTH,cortisol,prolactin,catecholamines)

**2)** Posture: that affect renin and aldosterone which are higher in sanding than recombinant .also protein and protein bound molecules are 10% higher in upright than when recumbent.

**3)** Fasting: food intake affect many analytes like glucose, TG, and insulin because they increase after a meal, also protein rich meal result in increase blood urea.

**4)** Medications: statin affect creatin kinase and liver enzymes, also glucocorticoid suppress ACTH and decrease cortisol, also thiazide diuretics decrease plasma K , also increase total thyroxine.

**5)** Exercise: can cause increment in plasma potassium and creatine kinase.

**\*Analytical errors:** this occur in lab during analysis, and test methods should be checked for accuracy, precission, specificity, sensitivity, and linearity.

**\*Post-analytical errors:** can occur during calculation and recording results.