



Biochemistry Laboratory

Lab.2



Specimen collection and processing

- **Many errors can occur during collection , processing , and transport of biological specimens.**
- **These errors have a major source of the preanalytical errors which represent 40% of the total laboratory errors.**
- **The accepted rate of errors in biochemical lab. is 0.4%.**
- **The percentage of each type of errors is :**
 - 1- preanalytical errors : 40%**
 - 2- analytical errors : 20%**
 - 3- postanalytical errors: 40%**

-Examples of biological specimen that are analyzed in clinical chemistry lab. Include :

1. whole blood.

2. serum.

3. plasma

4. Urine

5. feces

6. saliva

7. various types of fluids:

synovial >>>>>

spinal >>>>>

amniotic >>>>>

pleural >>>>>

pericardial >>>>>

ascitic >>>>>

COLLECTION OF BLOOD

-**Blood** for analysis may be obtained from: veins, arteries, or capillaries.

-Venous blood is usually the most widely used and *venipuncture* is the method for obtaining venous blood.

-**Capillary** blood is obtained using skin puncture which is the method of choice for young children and many *point of care tests*.

-**Arterial** puncture is used mainly for blood gas analyses.

-The phlebotomist should be properly dressed in protective equipment such as a gown and gloves.

-When the patient is in isolation in a hospital, the phlebotomist should put on a gown , gloves , a face mask , and goggles before entering the patient's room.

- At least three items of identification should be used: name, medical record, date of birth, room location if the patient is hospitalized or address if the patient is an outpatient.

Important tips to be considered

- 1. The** patient should be seated or supine for at least 20 minutes before collection of blood to minimize the variation in blood constituents caused by variation in blood volume (hemoconcentration and hemodilution).
- 2. Avoid** an arm with inserted intravenous line.
- 3. Avoid** an arm with extensive scarring or hematoma at the intended collection site.

An appropriate needle must be selected, the most widely used sizes are gauges 19 to 22; the larger the gauge size is, the smaller the bore.

When large volume of blood is needed to be collected (30ml-50ml), an 18 gauge needle is to be used while a 20 gauge needle is usually used in normal situation.

A needle is usually 3.7 cm long but 2.5 cm needles are also used.

B- Location

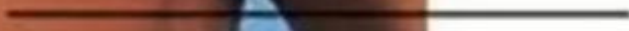
-The median cubital vein in antecubital fossa is the preferred site for collecting venous blood in adults because the vein is both large and close the surface of the skin.

-Other sites include the back of the hand and the ankle but are less desirable and should be avoided in diabetics and other individuals with poor circulation.

Cephalic vein



Biceps brachii



Basilic vein



Median cubital vein



Pronator teres



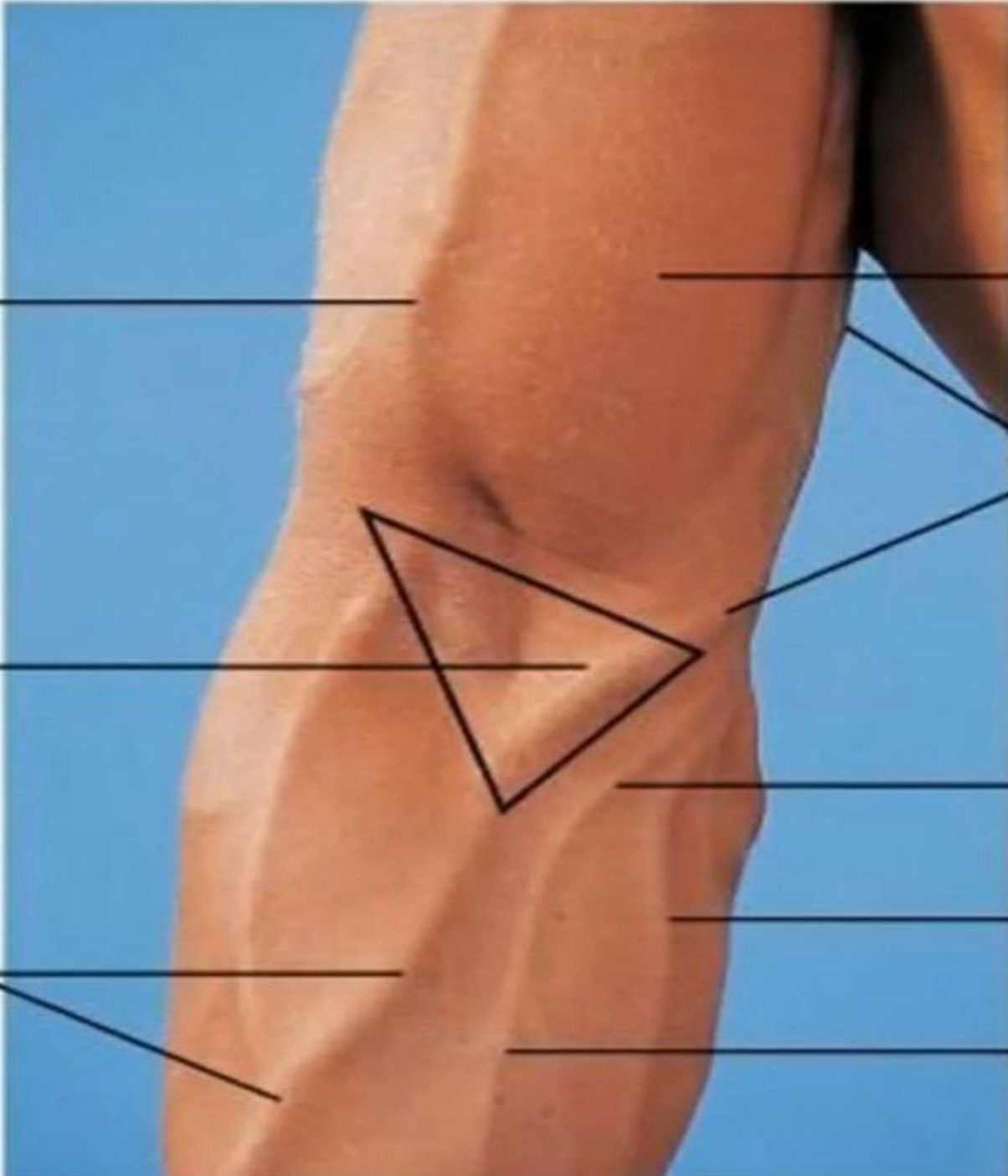
Basilic vein



Cephalic vein



Median vein of forearm



Important tips

1. **Collection** of blood through cannula is allowed only at the first time of insertion.
2. **An** arm containing a cannula or arteriovenous fistula should not be used.
3. **If** there is an infusion fluid at the arm, it should be shut off at least 3 minutes before collecting blood from that arm, but we can use the other arm or below the infusion site of the same arm for all tests except those found in the infusion solution (e.g. glucose, Na, K).

C- Preparation of the site

-The area around the puncture site should be cleaned with a gauze pad saturated with 70% isopropanol. Cleaning should be in a circular motion from the site outward.

Important tips

1. Allow alcohol to dry before aspiration of blood because traces of alcohol may cause hemolysis.
2. The skin should be cleaned with benzalkonium chloride if the collected sample will be used for ethanol determination. %%

- Venous occlusion

Tourniquet is applied 10-15 cm above the puncture site. It is rarely needed to leave tourniquet more than 1 minute

In all circumstances changes in blood composition will occur but marked changes have been observed after 3 minutes.

Important tips

1. Pumping of the fist before venipuncture should be avoided because it will increase serum potassium, phosphate, lactate, and ionized calcium while pH is decreased.
2. Ionized calcium returns to normal 10 minutes after releasing of tourniquet.

Changes of serum composition after 3 minutes of venous occlusion

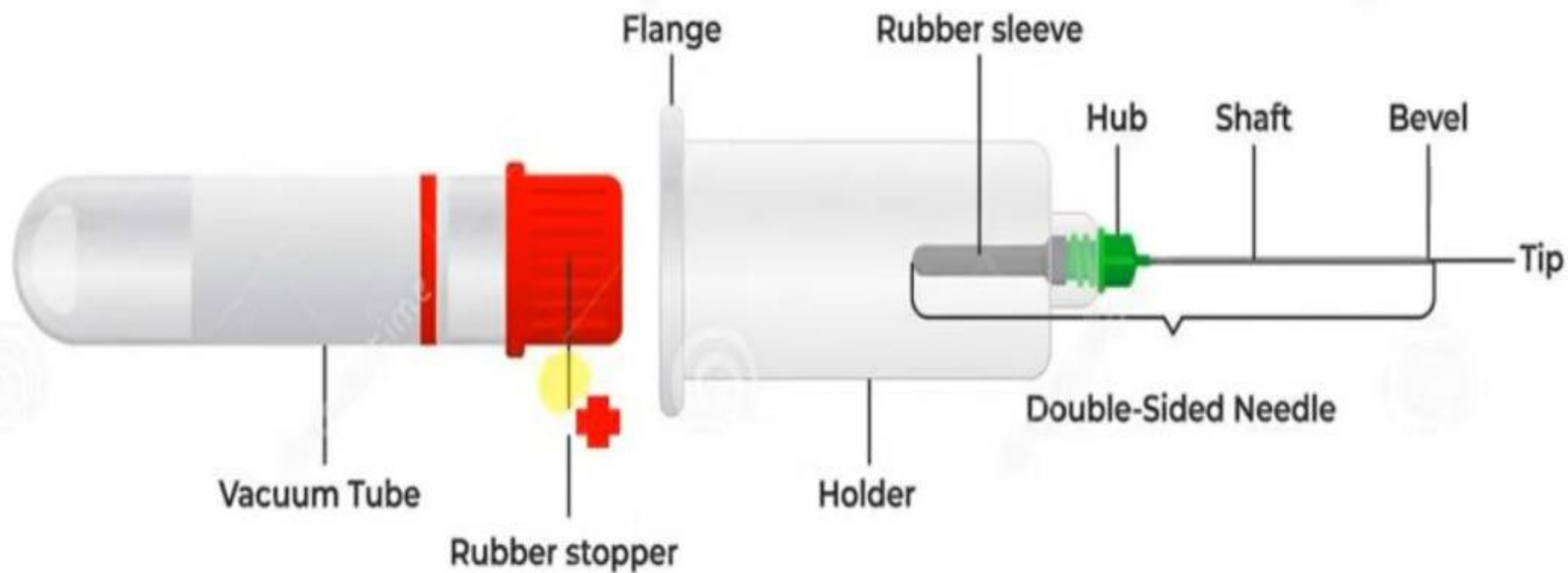
Increase	%	Decrease	%
Total protein	4.9	Potassium	6.2
Iron	6.7		
Cholesterol	4.7		
Total lipids	5.1		
AST	9.3		
Bilirubin	8.4		

Evacuated blood tubes

- **Using** these tubes is considered less expensive and more easier than using syringes.
- **There** are several types of evacuated tubes
- **Each** type is identified by the color of the stopper used.
- Some** glass tubes are siliconized to reduce adhesion of clots to the wall and decrease risk of hemolysis.
- other tubes contain thrombin to accelerate clotting but silicone coated wall may also accelerate clotting.
- **To** be used, each tube is connected to a holder which is connected to a needle on the other side, once the needle is inside the vein, the blood will flow spontaneously into the tube due to negative pressure inside it.







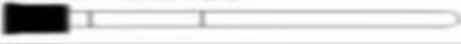





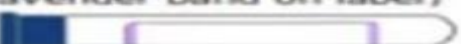



Vacuum blood collection system

INFOGRAPHIC ELEMENTS



VENEPUNCTURE



Order of Draw	Color of Stopper	Invert	Additive	Comments/Common Tests
1	Clear 	Not required	No additive	Tube used ONLY as a discard tube.
2	Blood Culture Bottle 	Invert gently to mix	Bacterial growth medium and activated charcoal	When a culture is ordered along with any other blood work, the Blood Cultures MUST be drawn first.
3	Yellow with clear label 	8 to 10 times	Sodium polyanethol sulfonate (SPS)	Tube used for Mycobacteria (AFB) blood culture.
4	Royal Blue (with red band on label) 	Not required	Glass tube with no additive	Tube used for serum trace element tests. Available from CLS Referrals, the red band on label is applied by CLS Referrals before sending tubes to collection site.
5	Red GLASS 	Not required	Glass tube with no additive	Tube used for serum tests, which CANNOT be collected in SST tubes. NOTE: red PLASTIC tubes are preferable for lab tests.
6	Light Blue 	3 to 4 times	3.2% buffered Sodium citrate anticoagulant	Tube used mainly for PT (INR), PTT, and other coagulation studies.
7	Black GLASS 	3 to 4 times	3.2% Sodium citrate anticoagulant	Tube used for ESR ONLY.
8	Red 	5 times	Clot activator, and no anticoagulant	Tube used for serum tests, which CANNOT be collected in SST tubes.
9	Gold 	5 times	Gel separator and clot activator	Usually referred to as "SST" (serum separator tube). After centrifugation, the gel forms a barrier between the clot and the serum.
10	Dark Green 	8 to 10 times	Sodium heparin anticoagulant	Tube used mainly for Amino Acids and Cytogenetics tests.
11	Light Green (mint) 	8 to 10 times	Lithium heparin anticoagulant and gel separator	Usually referred to as "PST" (plasma separator tube). After centrifugation, the gel forms a barrier between the blood cells and the plasma. Tube used mainly for Chemistry tests.
12	Royal Blue (with blue band on label) 	8 to 10 times	K ₂ EDTA anticoagulant	Tube used for Trace Elements.
13	Royal Blue (with lavender band on label) 	8 to 10 times	Na ₂ EDTA anticoagulant	Tube used for whole blood trace element tests. Available from CLS Referrals, the lavender band on label is applied by CLS Referrals before sending tubes to collection site.
14	Lavender 	8 to 10 times	K ₂ EDTA anticoagulant	Tube used mainly for CBC, pre-transfusion testing, Hemoglobin A1C, and anti-rejection drugs. Note: EDTA tubes specifically for Catecholamines also include Sodium metabisulfite.
15	Yellow with yellow banded white label 	8 to 10 times	Acid citrate dextrose solution "A" (ACDA)	Tube used for Tissue Typing and some Flow Cytometry testing.
16	Grey 	8 to 10 times	Sodium fluoride and Potassium oxalate anticoagulant	Tube used for Lactate.

Collection of blood should follow the following order :

1. **blood** cultures (yellow)
2. **Non** additive tubes (red stoppers)
3. **coagulation** or citrate – containing tube (blue stopper)
4. **serum** separator tube containing gel
5. **heparin** containing tube (green stopper)
6. **EDTA** –containing (lavender stopper) and oxalate – fluoride containing tube (green stopper)

Skin puncture

-**This** is the second method of blood collection in which the skin is punctured with a lancet and a small volume of blood is collected into a capillary tube.

-**The** first drop should be wiped off and subsequent drops are collected.

-**The** depth of the incision should be less than 2.5 mm to avoid contact with bone.

In practice, this method is used in situation where:

1. **Pediatric** age group (sample volume is limited).
 2. **Severe** veins damage due to repeated venipuncture.
 3. **Unavailable** veins because they have been burned or bandaged.
 4. **Point** of care tests (e.g. blood glucose or hemoglobin).
- Many sites can be used for skin puncture including tip of fingers, earlobe, heel or big toe of infants.

Arterial puncture

-This is usually performed only by physicians or specially trained technicians.

-The preferred sites of arterial puncture are , in order ,

1- radial artery at the wrist ,

2- brachial artery at the elbow ,

3- femoral artery at the groin.

-Heparinized capillary tubes are used to collect blood , which should be sealed and sent immediately to the lab

ANTICOAGULANTS AND PRESERVATIVES FOR BLOOD

-Various types of anticoagulants can be used including:

Heparin,

EDTA,

Sodium fluoride,

Sodium citrate

HEPARIN

- It causes the least interference with tests, except for polymerase chain reaction (PCR).
- It prevents the formation of fibrin from fibrinogen by accelerating the action of antithrombin III.

Disadvantage

- high cost.
- temporary action
- inhibits acid phosphatase activity
- false increase in free T3 and free T4

EDTA

- It is a chelating agent useful for hematological examination because it preserves the the cellular components of blood.
- It is usually used as dipotassium or tripotassium salt at a concentration of 1-2 g/L of blood as higher concentration shrinks RBCs.
- It prevents coagulation by binding calcium which is essential for clotting mechanism.
- It inhibits ALP and CPK activity, and unsuitable for specimen for calcium and iron analyses using photometric techniques, it has little effect on other clinical tests.

SODIUM FLUORIDE

-This a weak anticoagulant often used as a preservative for blood glucose at a concentration of 2 g/L when used with another anticoagulant such as potassium oxalate.

-It acts by inhibiting the enzyme systems involved in glycolysis.

-The specimen can be preserved for 24 hours at 25 C and 48 hours at 4 C.

-Important tips

1. without antiglycolytic agent agent, blood glucose decreases by 10 mg/dl per hour at 25 C.
2. the rate of decrease is higher in newborns and in leukemic patients.
3. when used alone, 3-5 times higher concentration of the usual 2 g/l of sodium fluoride is required.

EFFECT OF HEMOLYSIS ON BLOOD TEST

-In case of hemolysis: the main elevation occurs in constituents that are present in higher concentration inside RBC than plasma, these includes:

Potassium, magnesium, phosphate, LDH, AST.

An additional band will be seen on serum protein electrophoresis.

-Regarding constituents that present in lower concentration within the RBC, hemolysis will lead to slight dilutional effect (decrease in their concentration ex. Na).

Urine samples

It is indicated in:

The best timing is:

Timed collection urine sample:

Double voided sample:

Urine preservative

Refrigeration is one of the most acceptable way of urine preservation especially when combined with chemical preservation.

Some tablets used as preservative contain a mixture of chemicals which act mainly by lowering pH and releasing formaldehyde.

Acidification to below pH 3 is used to preserve 24-hour urine specimen for calcium, steroids, and VMA determinations.

This is done by adding 10 ml HCl, 6mol/L, per 24-hour excretion.

However, this acidification will precipitate urate and thus the specimen becomes unsuitable for uric acid measurement.

FECES

No preservative is required to fecal specimen Uses:

-Occult blood in stool is a very important clue to the presence of bleeding ulcer or a malignant disease of the gastrointestinal tract.

-Trypsin activity test is applied to children feces as a screening test of cystic fibrosis.

Fecal porphyrins can be used to determine the type of porphyria.

- What else????

SPINAL FLUID

- **Because** the initial specimen may be contaminated by tissue debris or skin bacteria:

the first tube should be used for chemical or serological tests
the second for microbiological tests

the third for microscopic and cytological examination.

- **Up** to 20 ml of spinal fluid can be safely removed from an adult but such amount is not usually required.

For proper interpretation of spinal fluid glucose values, a simultaneous blood specimen should be obtained.

OTHER COLLECTION PROCEDURES

Amniocentesis : aspiration of amniotic fluid.

Paracentesis : aspiration of fluid from pleural , pericardial , or peritoneal cavities

Thoracentesis : aspiration of fluid from pleural cavity.

Pericardiocentesis : aspiration of fluid from pericardial cavity.

Separation and Storage of Specimens

- **Plasma** or serum should be separated from cells as soon as possible and optimally within 2 hours.
- **Premature** separation of serum, however, may permit continued formation of fibrin and lead to obstruction of sample probes in testing equipment.
- **Specimen** tubes should be centrifuged with stoppers in place
 - (1) reduce evaporation particularly of volatiles, such as ethanol,
 - (2) prevent aerosolization of infectious particles, and
 - (3) maintain anaerobic conditions, which is important in the measurement of carbon dioxide and ionized calcium.

THANKS

The image features a solid blue background. In the bottom right corner, there are several white, parallel diagonal lines that create a sense of motion or a modern design element.