Blood Sampling

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Human Physiology Lab

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Blood sampling

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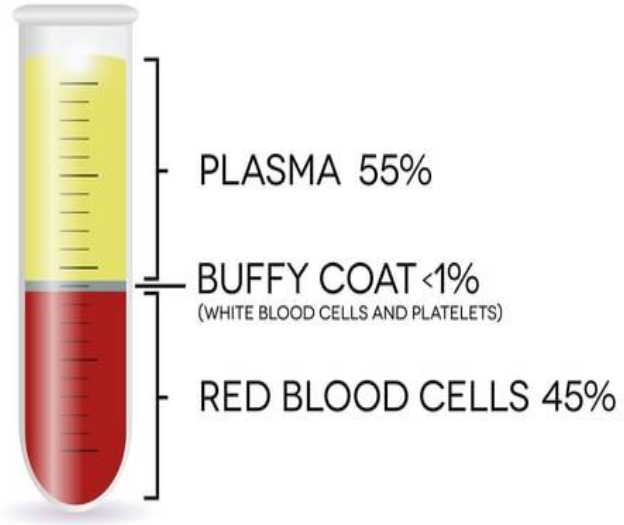
General precaution

- Hands should be washed thoroughly while entering or leaving the work area irrespective of the type of work undertaken
- Smoking, eating, and drinking should be prohibited in the laboratory.
- Infection :-Blood samples and urine samples are culture media for many organisms.
- Needle sticks and sharp injuries carry the risk of blood born infections, e. g AIDS, HCV, HBV. And others

Blood sampling

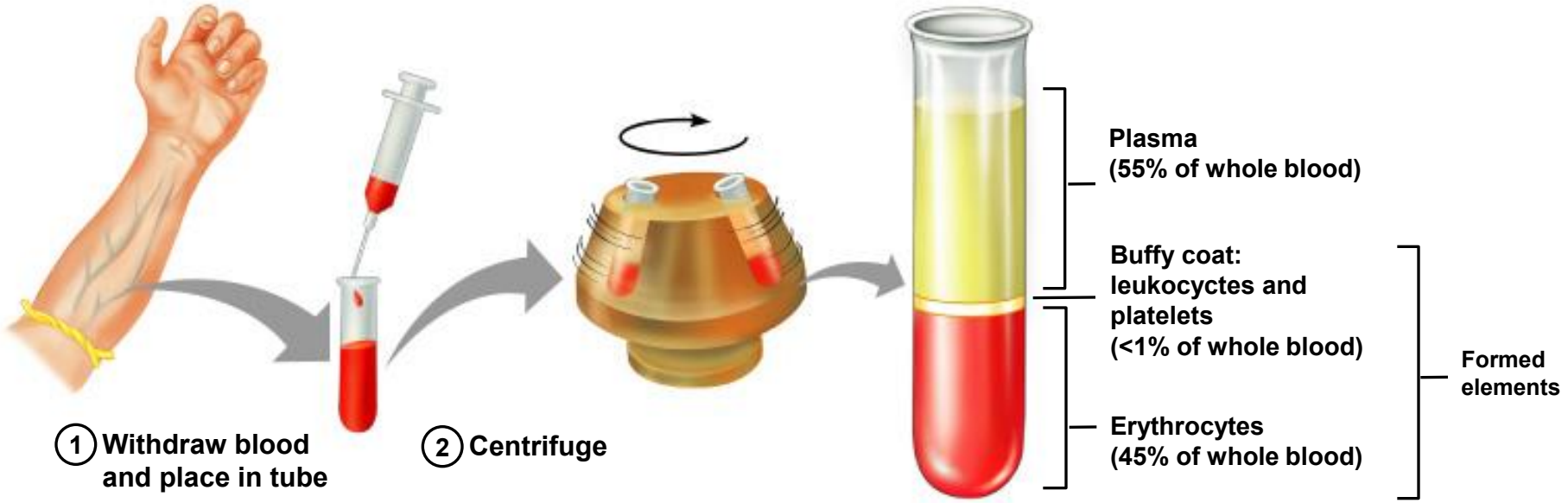
- **What's a blood**
- The body is just a huge bag of blood.
- An average" 70 Kgm human body contains only about 5 liters of blood, or 7% by volume
- Of the average 5 L of blood, only 2.25 L, or 45%, consists of cells. The rest is plasma.
- Plasma itself consists of 92% water (by weight) and 7% solids (mostly proteins, the greatest proportion of which is **albumin**).
- Of the 2.25 L of cells, only 0.037 L (1.6%) are leukocytes
- The total circulating platelet volume is even less -- about 0.0065 L (little over one teaspoonful)



Plasma consists of:

- Water (92%).
- Proteins (Albumin, Globulin, Fibrinogen).
- Glucose, lactate, pyruvate)
- Lipids(fat, lecithin, cholesterol)
- Amino acids, urea, uric acid, kreatine
- Inorganic substances(Na, K, Ca, Mg, Cl-, S,P,Fe, Mn, Co, Cu, Zn)
- Enzymes, hormones, vitamins, pigments.

Components of Whole Blood



Fist test: Specimen collection

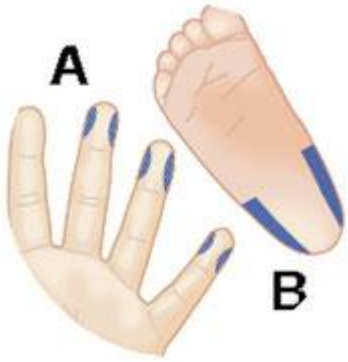
REAGENTS & EQUIPEMENTS

SYRINGES
NEEDLES
VACUTAINERS
GLOVES
MASKS
Tourniquet

Methods of Blood Collection

1. **Capillary blood collection**
2. **Vein Puncture**

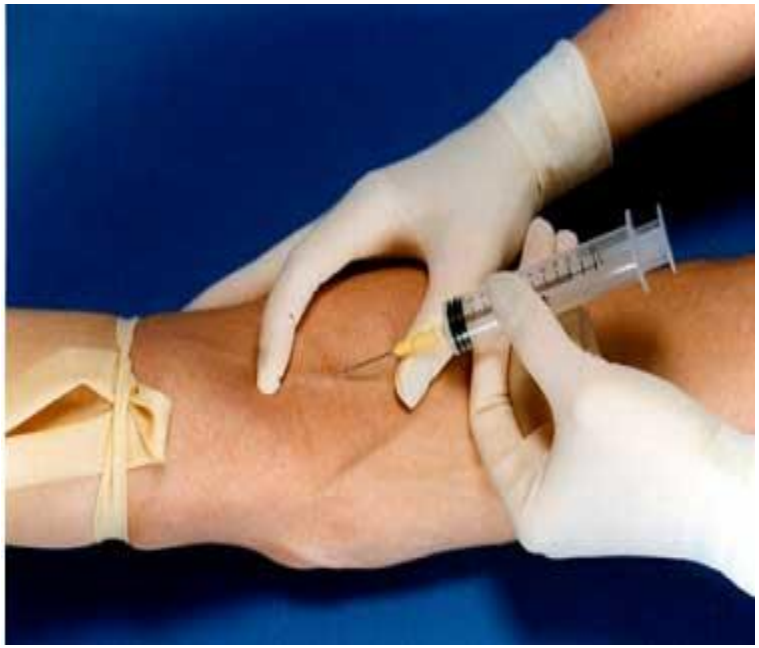
- **Capillary blood:**- Capillary blood is best taken from the radial side of middle fingertip. In case of infants, the blood can be taken from heel. The puncture site should be cleaned with ether or with 70% ethanol.




MiniCollect+ Complete
MiniCollect+ Complete

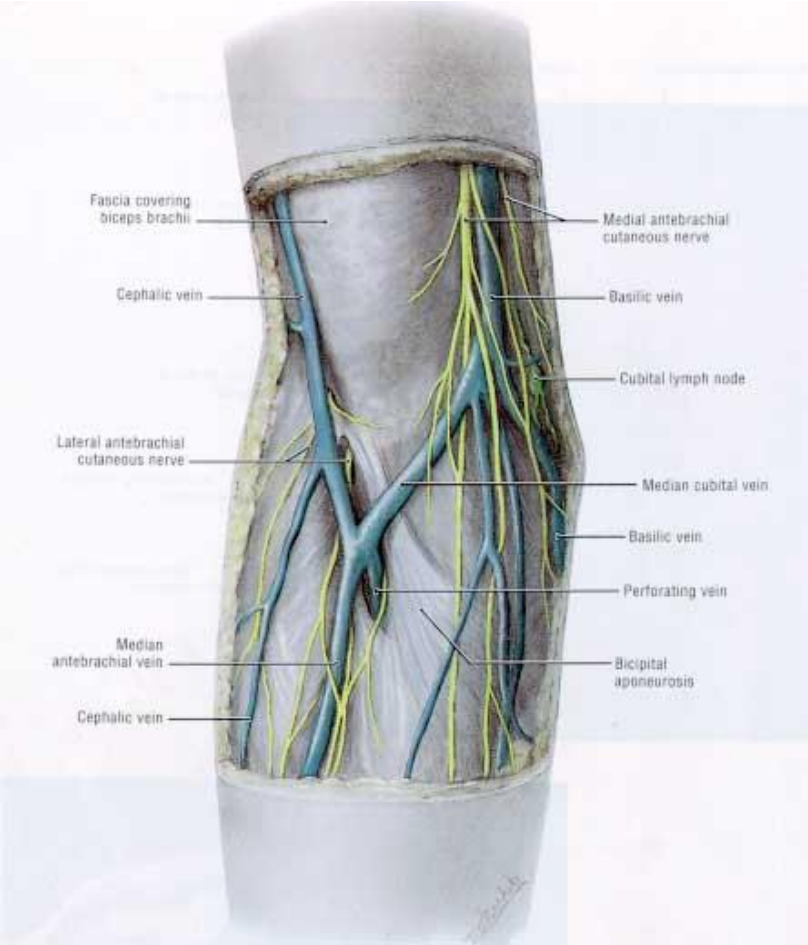
Veinepuncture :-

- For preparation of serum, blood should be collected in plain vial for plasma blood should be collected into vial with suitable anticoagulant.
- Blood is best drawn from the arm veins at the bend of the elbow
- The arm is compressed for 30 seconds at a pressure slightly exceeding diastolic pressure - a uniform pressure of 100 mm Hg may be used for 30 seconds.
- Blood should be drawn with a wide-bore needle into a cannula and dropped into the vial
- A syringe should be used only if the veins are poor. Blood should never be pushed out fast from the syringe.



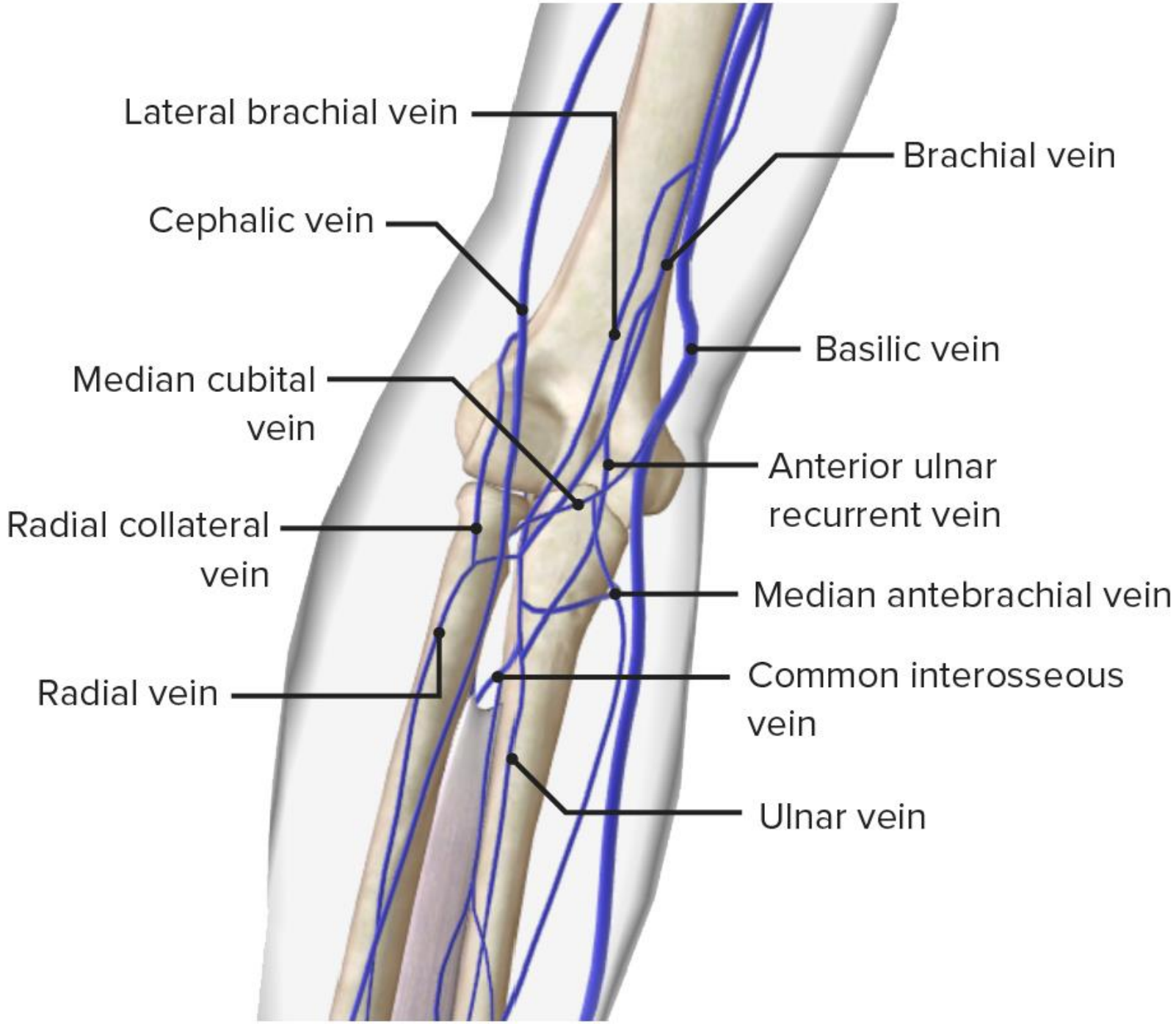
Blood also can obtained from, wrist vein, ankle vein, femoral vein, jugular vein in infants and vein of the forearm.

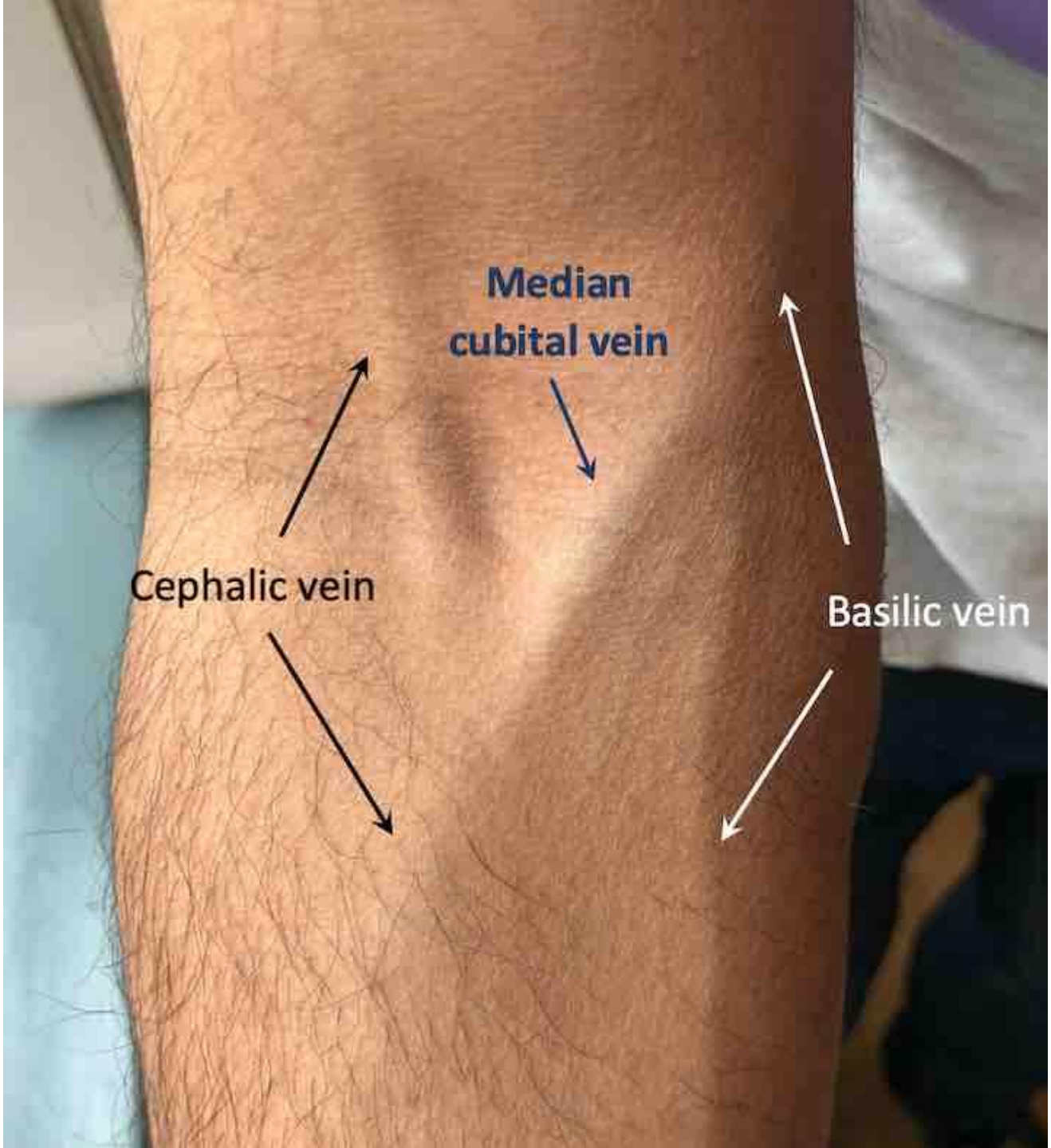
Selection of Vein



Veins used for drawing blood

1. Median cubital **vein** - first choice, well supported, least apt to roll
2. Cephalic **vein** - second choice
3. **Basilic vein** - third choice, often the most prominent **vein**, but it tends to roll easily and makes venipuncture difficult


Lateral brachial vein
Brachial vein
Cephalic vein
Basilic vein
Median cubital vein
Anterior ulnar recurrent vein
Radial collateral vein
Median antebrachial vein
Radial vein
Common interosseous vein
Ulnar vein


Median
cubital vein
Cephalic vein
Basilic vein

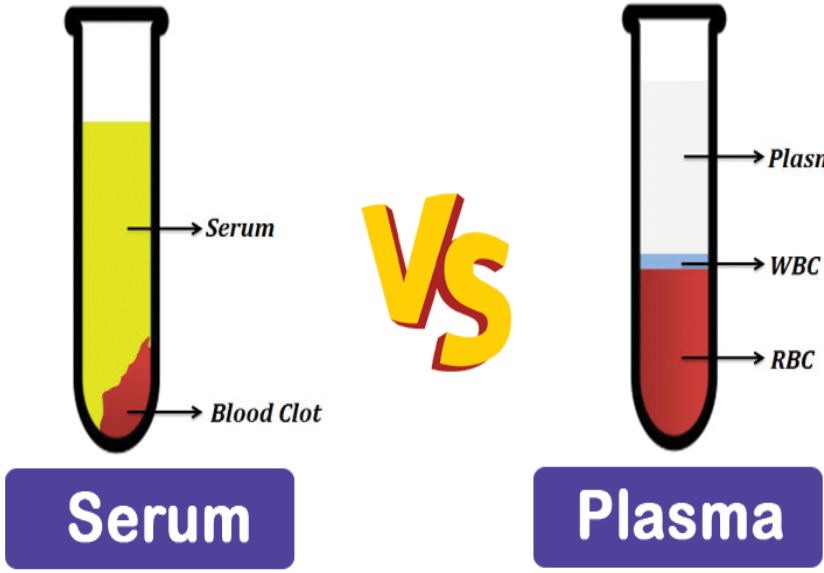
Plasma and serum

- What is plasma ?

The virtually cell-free supernatant of blood containing anticoagulant obtained after centrifugation.

- What is serum?

The clear yellowish fluid obtained upon separating whole blood into its solid and liquid components after it has been allowed to clot.

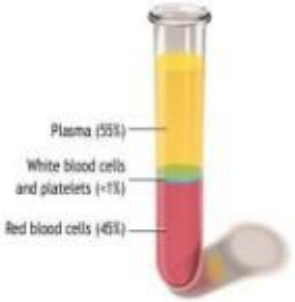


2. Plasma vs. serum

Plasma is the liquid, cell-free part of blood, that has been treated with anti-coagulants.

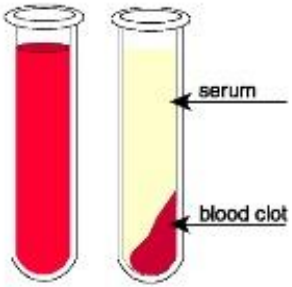
Serum is the liquid part of blood AFTER coagulation, therfore devoid of clotting factors as fibrinogen.

Anticoagulated



serum= plasma - fibrinogen

Clotted



Anticoagulants

- Are Additives that inhibit blood and/or plasma from clotting ensuring that the constituent to be measured is non-significantly changed prior to the analytical process.

- Anticoagulation occurs by binding calcium ions (EDTA, citrate) or by inhibiting thrombin activity (heparinates, hirudin)

Common types of anticoagulants

1. **salt EDTA**
2. **Sodium citrate**. usually in the form of trisodium citrate,
3. ***Heparin***
4. ***Oxalate***. 2-3 mg per ml blood is required.

- **Oxalate or EDTA may be mixed with sodium fluoride to prevent glycolysis in blood sugar estimation.**

ESR TUBE (EST)

EDTA TUBE (EDT)

GLUCOSE TUBE(GLL)

PT TUBE

HEPARIN TUBE (HET)

NO ADDITIVE TUBE (NO)

PRO-COAGULATION TUBE (PRC)

GEL & CLOT ACTIVATOR TUBE (GEL)

Important Notes	Tube Colour	Tube Type	Information
The sample should be inverted about six times to ensure the blood and gel mixes to activate the clotting process. **The samples must not be shaken to mix the blood**	 **Gold**	Serum Separating Tube (SST)	This tube type is used for the vast majority of Biochemistry, Immunology and Serology tests. Please check the website if unsure of the correct sample type.
The tubes should be filled up to the line indicated on the tube to ensure the correct ratio of blood to anticoagulant is achieved for analysis. The sample should be inverted about six times to ensure the blood and anticoagulant mixes. **The samples must not be shaken to mix the blood**	 **Green**	Lithium Heparin/Plasma Separating Tube	A few of the tests that use this tube type require the plasma to be frozen within half an hour of the sample being take. Please check the test information on the website.
The tubes should be filled up to the line indicated on the tube to ensure the correct ratio of blood to anticoagulant is achieved for analysis. The sample should be inverted about six times to ensure the blood and anticoagulant mixes. **The samples must not be shaken to mix the blood**	 **Royal Blue**	Sodium Heparin Tube	These sample are used to check the levels of trace metals, lead and mercury in the blood.
These tubes should not be shaken	 **Red**	Plain Tube	These tubes are very rarely used and should only be used for a specimen under instruction from the laboratory.
The tubes should be filled up to the line indicated on the tube to ensure the correct ratio of blood to anticoagulant is achieved for analysis. The sample should be inverted about six times to ensure the blood and anticoagulant mixes. **The samples must not be shaken to mix the blood**	 **Grey**	Fluoride Oxalate Tube	Anticoagulant and fluoride salt act as a glycolysis inhibitor to stabilise the glucose concentration in the sample. Main tests analysed on the sample: Glucose, Lactate and Alcohol.
The tubes should be filled up to the line indicated on the tube to ensure the correct ratio of blood to anticoagulant is achieved for analysis. The sample should be inverted about six times to ensure the blood and anticoagulant mixes. **The samples must not be shaken to mix the blood**	 **Lavender**	Potassium EDTA Tube	Potassium EDTA serves as the anticoagulant for the performance of almost all haematological tests. Larger EDTA bottles are used for transfusion samples only.
The tubes should be filled up to the line indicated on the tube to ensure the correct ratio of blood to anticoagulant is achieved for analysis. The sample should be inverted about six times to ensure the blood and anticoagulant mixes. **The samples must not be shaken to mix the blood**	 **Light Blue**	Sodium Citrate Tube	The ratio between anticoagulant and blood for physiological examinations is 1:9

- **Hemolysis**
- Hemolysis is the breakage of the red blood cells (RBC's) membrane, causing the release of the hemoglobin and other internal components into the surrounding fluid. Hemolysis is visually detected by showing a pink to red tinge in serum or plasma

The following steps may prevent haemolysis

1. Moisture should be avoided in needle, cannula, syringe and the vial.
2. The arm should not be over-compressed. The sphygmomanometer cuff is the most suitable appliance for regulated pressure.
3. Blood should not be pushed out fast from the syringe. lt should not be shaken vigorously with the anticoagulant.
4. Only moderate speed should be employed to centrifuge the sample.
5. Serum should be separated within 45 minutes.
6. Without touching, allow the venipuncture site to air dry

- **Storage**

- In room temperature, whole blood plasma and serum **Up to 6–8 hours** (best) in room temperature

- In Refrigerator (2–8°C) whole blood **Up to 24 hours** , serum up to 72 h

Serum

- **In Freezer**

- **–20°C:** stable for **weeks to months** depending on the test.

- **–70°C or below:** stable for **years** (used for research or viral studies).