Cihan University/ Sulaymaniya

College of Health Science

Medical Laboratory Analysis

4th Stage- 1st Semester

Pr. Clinical Immunology

Lab- 1: Types of Samples

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Laboratories

Clinical laboratories are important in disease diagnosis, determination its severity and patient response to specific treatment.

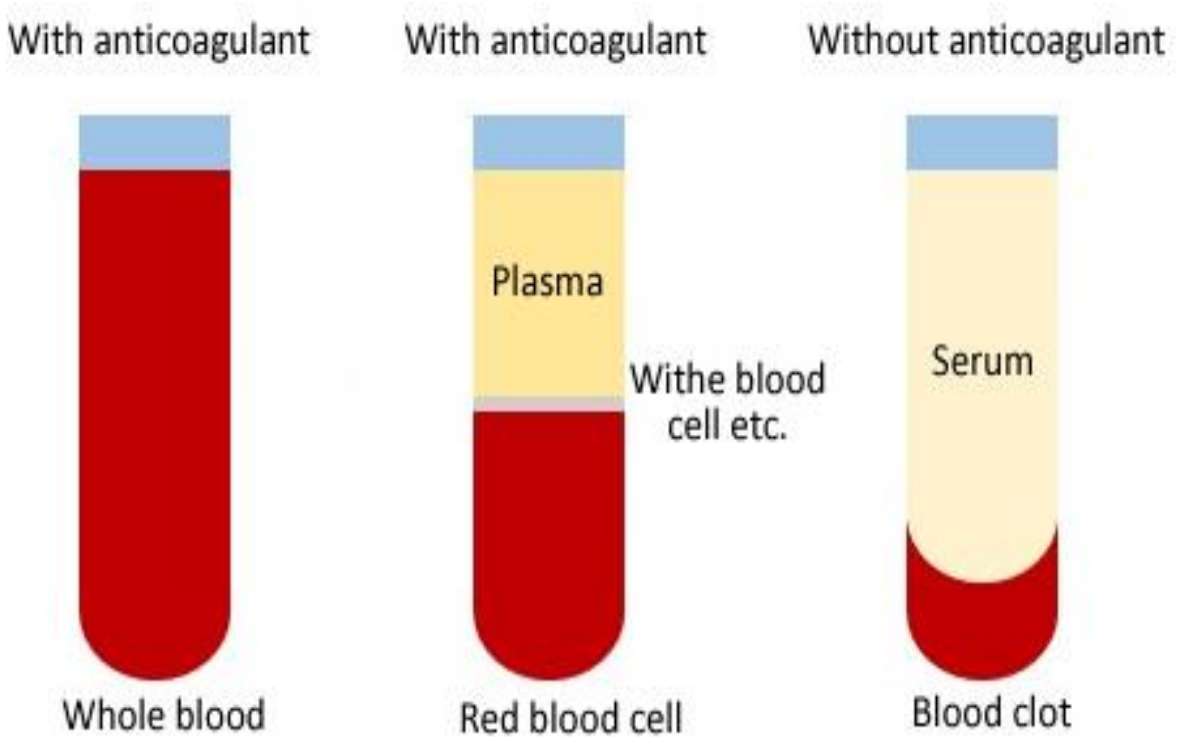
Diagnosis of any disease is first done by physical examination by physician and confirmed by laboratory diagnostic tests.

Lab values is very important in the determination disease severity, drug doses and in follow up.

Common Immunology Lab. samples

One of these samples may be used in immunology labs:

1. Whole Blood,
2. Plasma,
3. Serum,
4. CSF (Cerebrospinal fluid),
5. Urine,
6. Cells.



Phlebotomy or Blood Collection

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

The phlebotomy equipment:

For specimen collection the following materials will be required.

Phlebotomy or Blood Collection

Torniquet:

Venous blood sampling is usually performed using a torniquet to help locate and define peripheral veins to achieve safe and successful vein puncture.

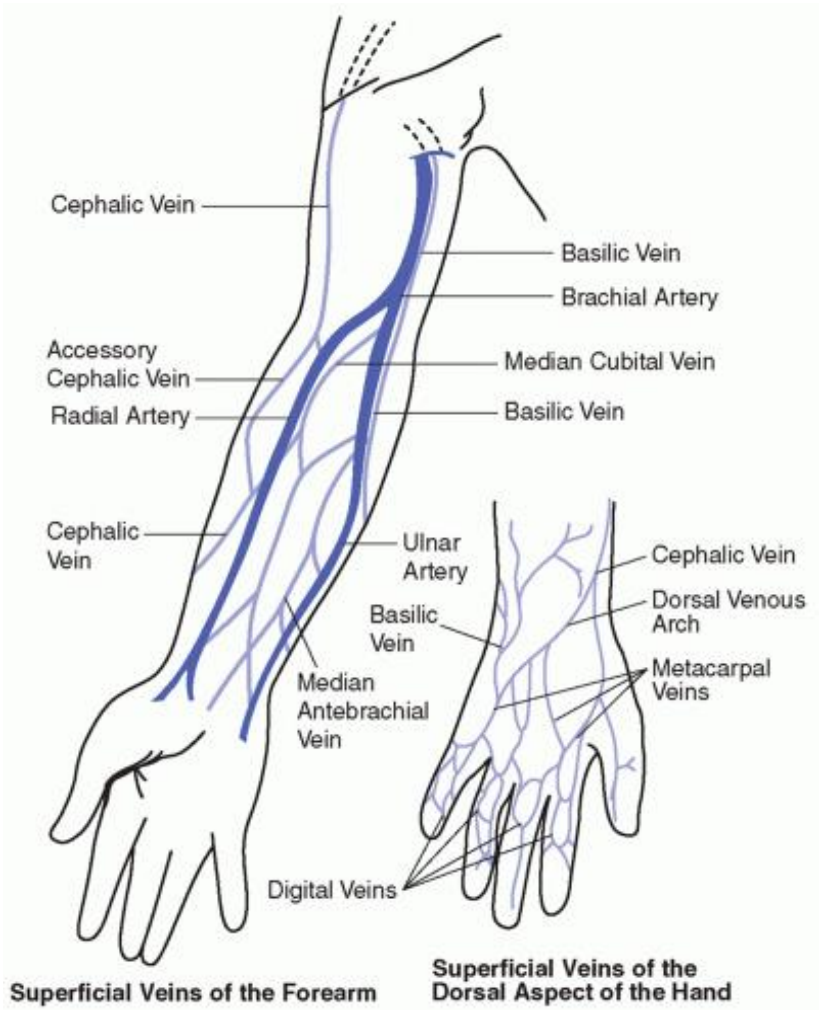
Selecting vein site

1- Whole blood Collection:

- Usually vein is used to collect blood by vein puncture procedure.
- In adult, most vein puncture procedure arm-vein is used.
- On arm, one of three arm vein is used: median cubital vein, located on the meddle, cephalic vein and basilic vein located on both sides.

Median cubital vein is the best choice, why?

- Because it has good blood flow than cephalic and basilic veins which has slower blood flow.
- However, if vein puncture procedure is unsuccessful in median cubital vein cephalic or basilic veins is used.
- **Artery blood** is rarely used, in special cases as when blood gases, pH, pCO_2, pO_2, and bicarbonate is requested. It is usally performed by physician.

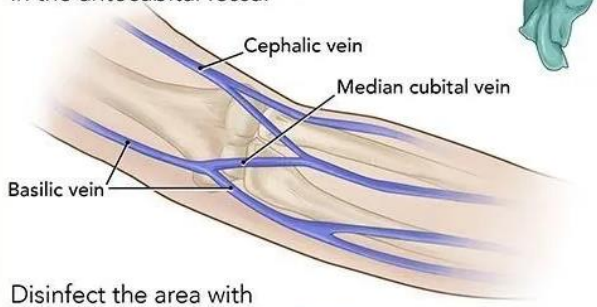


Venipuncture

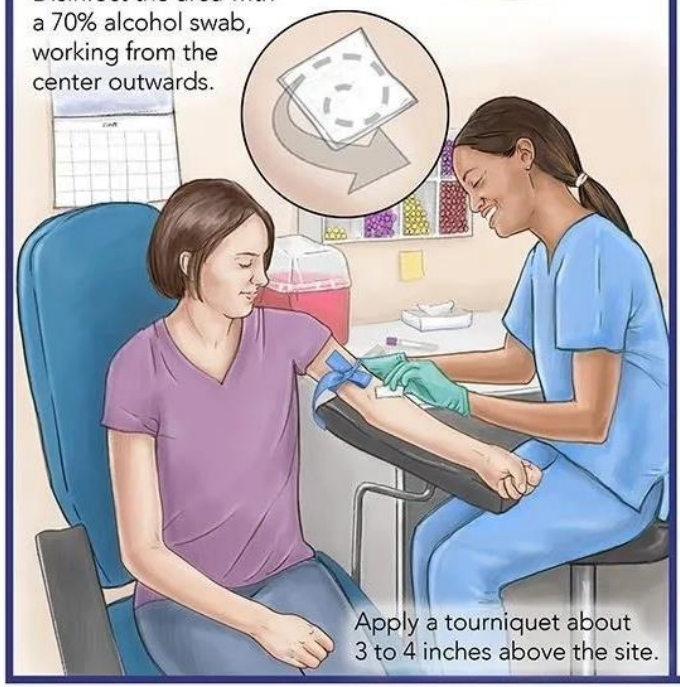
Blood collection procedure guide

Gather all equipment, wash hands, and put on sterile gloves.

Ask the patient to make a fist and select the venipuncture site in the antecubital fossa.

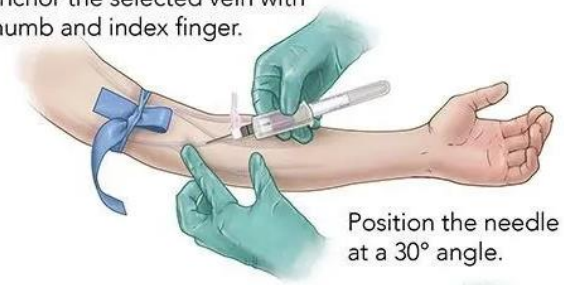


Disinfect the area with a 70% alcohol swab, working from the center outwards.



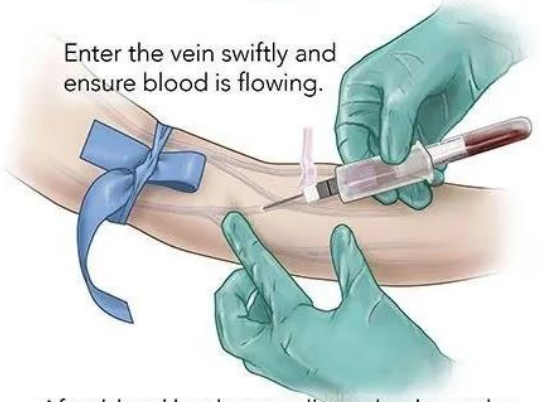
Apply a tourniquet about 3 to 4 inches above the site.

Anchor the selected vein with thumb and index finger.

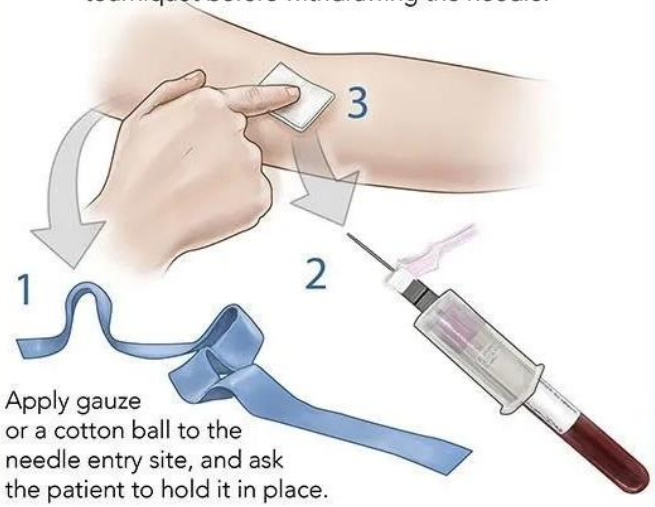


Position the needle at a 30° angle.

Enter the vein swiftly and ensure blood is flowing.

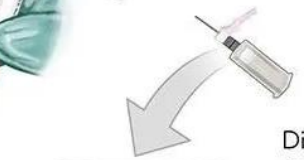


After blood has been collected, release the tourniquet *before* withdrawing the needle.



Apply gauze or a cotton ball to the needle entry site, and ask the patient to hold it in place.

Remove and immediately invert the tube 8 to 10 times to mix the sample with the tube additives.



Discard the used needle in the sharps container.

Remove gloves and wash hands with soap and water.

Label the tube for transport to the lab, indicating:

- Patient's full name
- Patient ID
- Birth date
- Date of sample.

For more information, visit:
World Health Organization Guidelines on Drawing Blood: Best Practices in Phlebotomy

ORDER OF DRAW FOR MULTIPLE TUBE COLLECTIONS

All specimens must be labeled with both the **patient's first and last name** as well as **a second identifier** such as **the patient's birth date** or medical record number matching the demographic information present on the accompanying requisition or other paperwork.

Blood collection tubes must be drawn in a specific order **to avoid cross-contamination of additives between tubes**.

When collecting multiple specimens, blood tubes should be drawn in the following order; mix all tubes by inversion 6 – 8 times:

ORDER OF DRAW FOR MULTIPLE TUBE COLLECTIONS

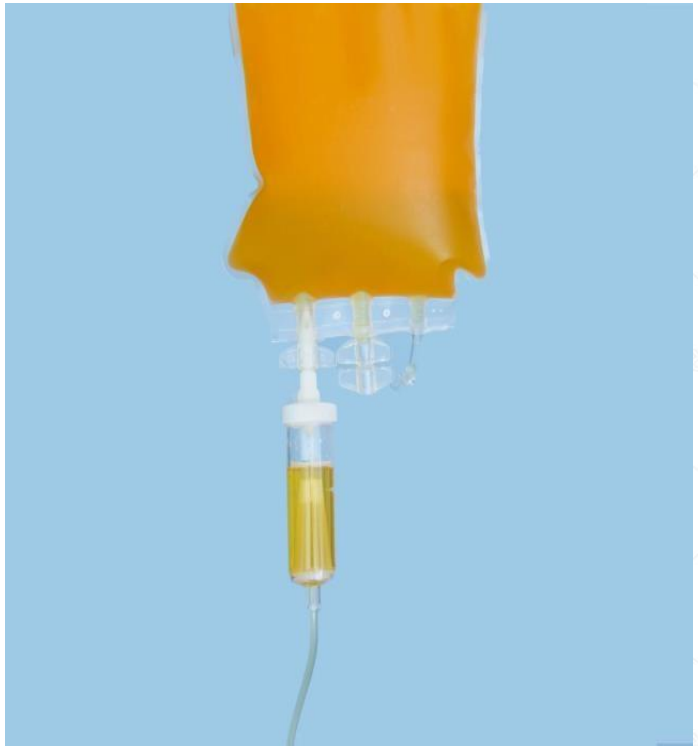
1. Blood Culture bottles,
2. Isolator tube,
3. Blue top (3.2% sodium citrate),
4. Red top (no preservative) and Gold top (SST),
5. Royal blue top (no preservative),
6. Green top (sodium heparin),
7. Lavender top (EDTA), Pink top (EDTA), and Royal blue top (EDTA),
8. Gray top (sodium fluoride),
9. Yellow top (ACD) Solution A or B,
10. TB Gold QuantiFeron: Nil (gray top), TB antigen (red top), and Mitogen (purple top).

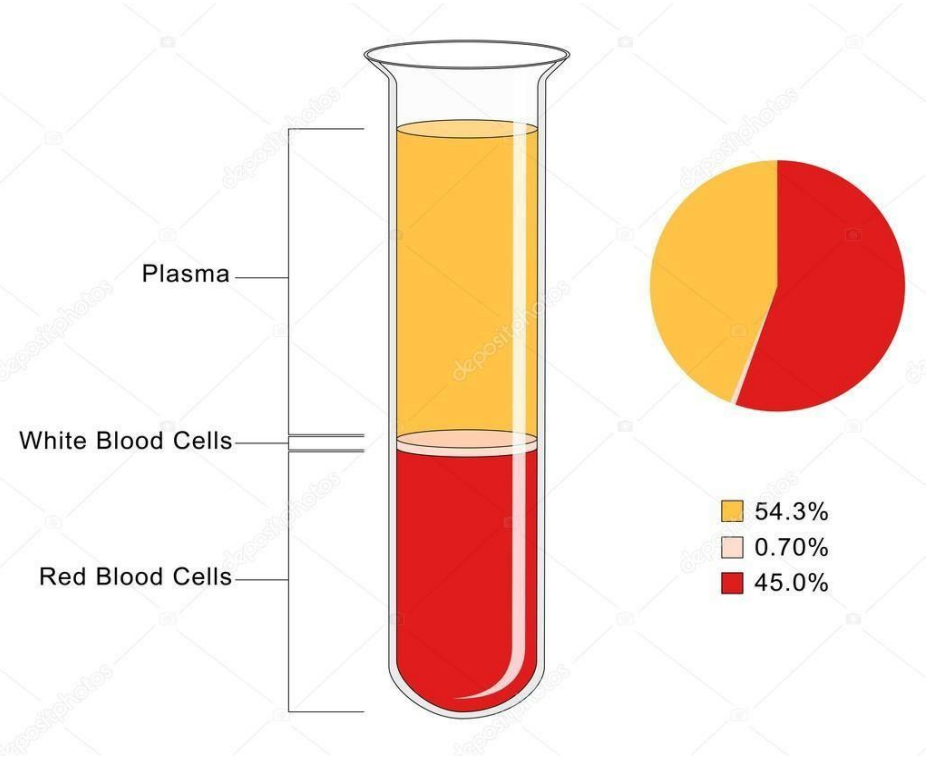
Catalogue of blood vacuum tube

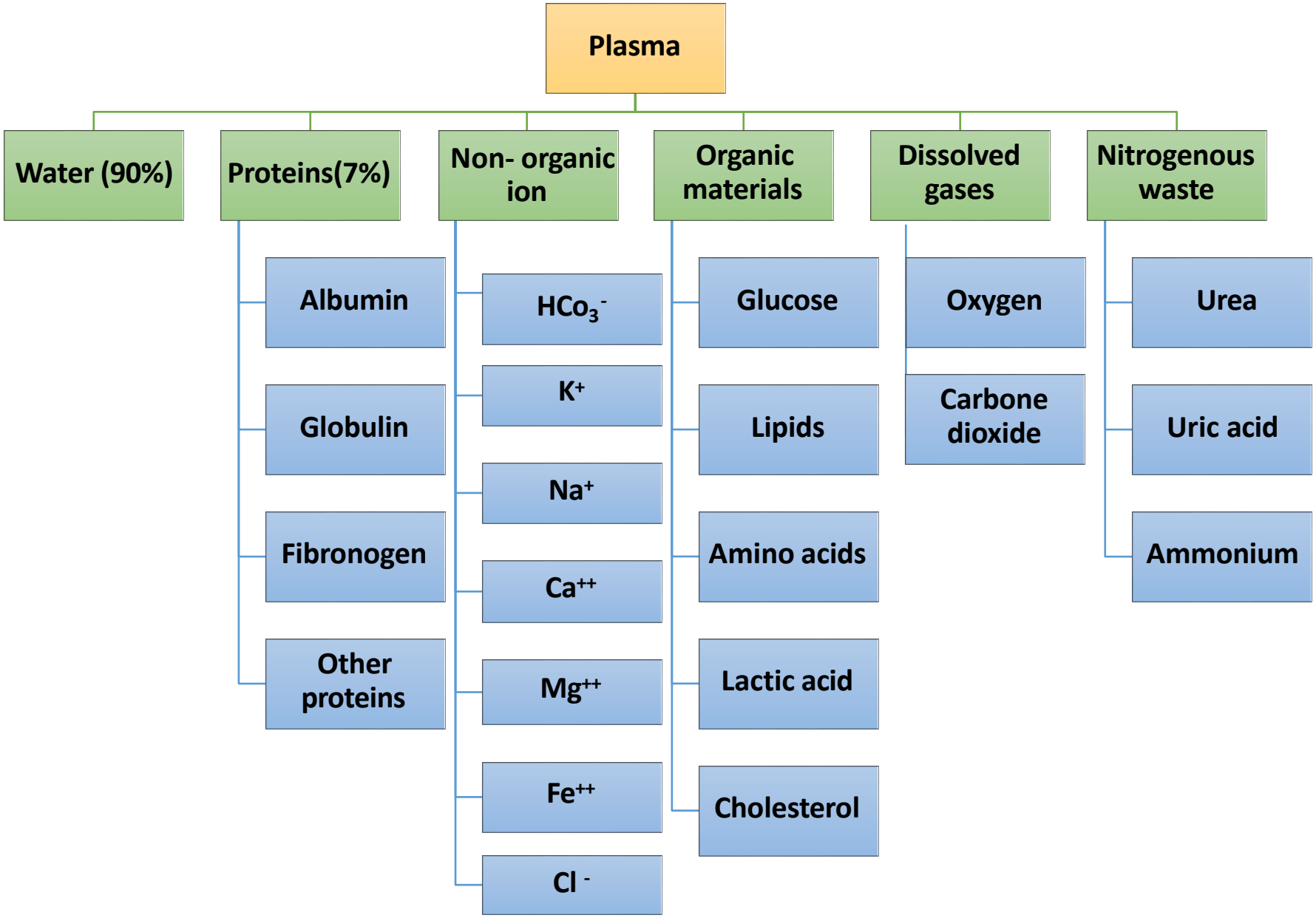
Type	Name	Cap	Additive	Spec. of Body (diameter*Length)	Material of body	Quantity/ tray	Quantity/ carton	Dimension of carton
Serum Blood Collection Tube	Plain tube		No additive	13mm×75mm 13mm×100mm 16mm×100mm	Glass/ PET/ PP.	100pcs	1200pcs	13mm×75mm: 510×360×210mm 13mm×100mm: 510×360×250mm 16mm×100mm: 580×390×250mm 8mm×120mm: 460×360×285mm
	Pro-coagulation tube		Clot activator					
	Gel & Clot Activator tube		Gel & Clot activator					
Plasma Blood Collection Tube	Glucose tube		Sodium Fluoride/ Potassium Oxalate					
	PT tube		0.109M Sodium Citrate(1: 9)					
	Heparin tube		Sodium Heparin/ Lithium Heparin/ Lithium Heparin & Gel					
Whole Blood Collection Tube	EDTA tube		EDTA-K2/ EDTA-K3/ EDTA-K2 & Gel					
	ESR tube		0.129M Sodium Citrate(1:4)	13mm×75mm 13mm×100mm 8mm×120mm				

Types of Specimens In Immunology Lab.

2. Plasma: is the fluid portion of the blood that has been prevented from clotting by the addition of anticoagulants (Oxalate, EDTA, and heparin).



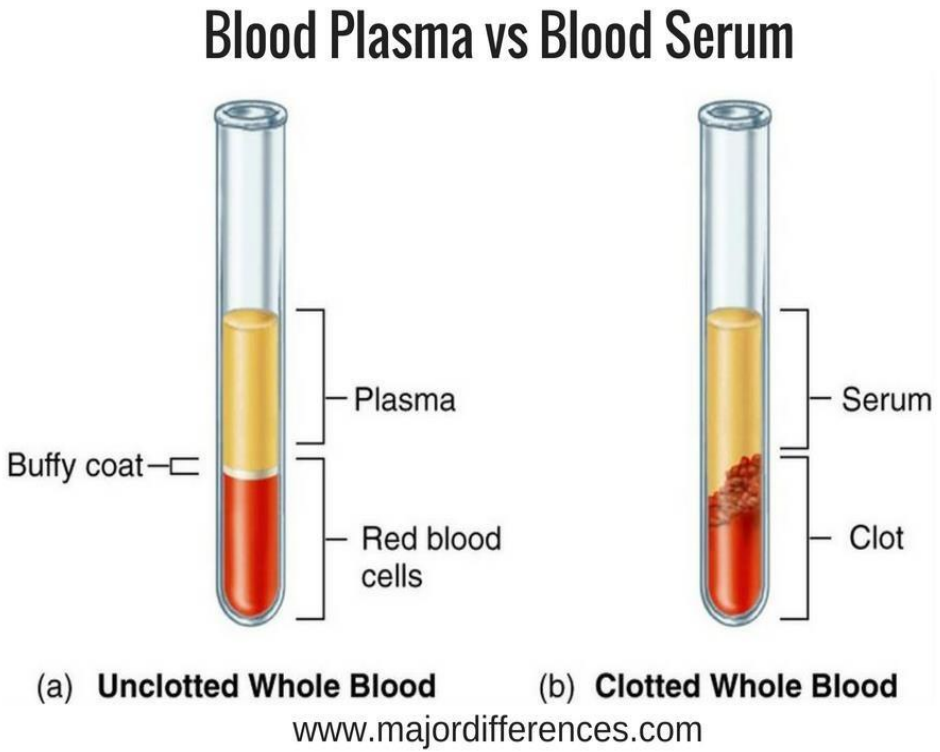



Plasma
Water (90%)
Proteins(7%)
Non- organic ion
Organic materials
Dissolved gases
Nitrogenous waste
Albumin
Globulin
Fibronogen
Other proteins
HCo_3^-
K^+
Na^+
Ca^{++}
Mg^{++}
Fe^{++}
Cl^-
Glucose
Lipids
Amino acids
Lactic acid
Cholesterol
Oxygen
Carbone dioxide
Urea
Uric acid
Ammonium

3. Serum: is the liquid part of the blood after coagulation (clotting). It is the most important specimen in serology because it contains antibodies.

The principle of serum preparation is whole blood obtained by the vein puncture and allowed to clot, then the serum is moved for testing.

In short: Serum= Plasma- Fibrinogen (coagulant factor).



	Definition	How It's Obtained	Appearance	Density	Composition
Serum	Liquid that remains after the blood has clotted	Centrifuging clotted blood	Light yellow, clear	1.024 g/ml	Water, albumin, globulins, amino acids, hormones, enzymes, nitrogenous waste, nutrients, gases. Higher in TGFbeta, VEGF and IL-8.
Plasma	Liquid that remains when clotting is prevented	Centrifuging whole blood with anticoagulant	Light yellow, clear	1.025 g/ml	Water, albumin, globulins, amino acids, hormones, enzymes, nitrogenous waste, nutrients, gases, fibrinogen. Dependent on anticoagulant used. Low Ca++, Mg++ in EDTA and citrate plasma. Lower levels of inflammatory mediators.

Procedure of serum preparation:

- We obtain venous blood specimen, and then put them in (serum separator) tubes.
- Leave the blood to clot at room temperature for 15- 30 min. for blood clotting (conversion of fibrinogen to fibrin).
- Centrifuge the blood for 5 minutes (2500-3500 rpm).
- Serum should be separated from the red cells (prevent RBCs destruction).
- Testing immediately or preserve the serum.

Common Serum Preparation Errors:

1. Failure to separate serum from red cells within 30 to 45 minutes of vein puncture.
2. Hemolysis: Red blood cells damaged and intracellular components spilled into serum. It causes elevation in $K^{+,}$ $Ca^{2+,}$ Phosphate, SGOT, SLDH, and Acid phosphatase.
3. Hemolysis is occurred due to sampling, transporting and storage (too cold or too hot).
4. 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.
5. mixation of serum and cells after centrifuging.

Notes:-

Sero -tests must be done with the fresh serum prepared (as soon as possible).

Sometimes the serum may be preserved for short or long time before doing the tests.

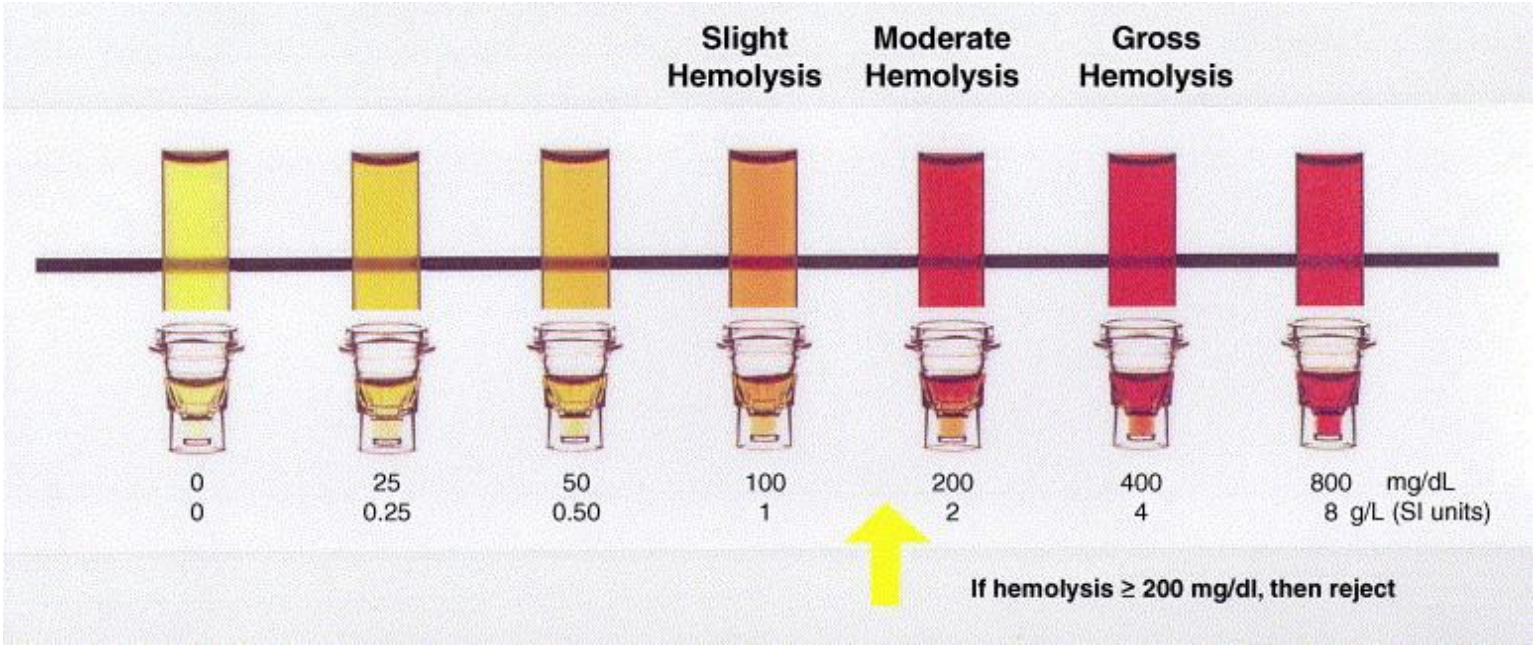
Specimen Integrity Chart for Hemolysis

Changes in the serum or plasma color indicate one of the following:

Hemolyzed Plasma/ Serum : appears pink to red due to the rupture of RBCs and not the normal clear straw color.

Icteric Plasma/ Serum: is caused by the presence of excess bilirubin in the blood stream.

Lipemic Plasma/ Serum: appears milky or turbid due to high lipid concentrations of triglyceride.



Serum preservation

A- Physical preservation:

- Refrigeration: This is short time preservation, the serum is kept in a refrigerator at (4-10 °C).
- Freezing: For a week. Place in freezer at -20 ºC.
- Deep freezing: for 6-12 months. Place it in a deep freezer at (-70 ºC to -80 ºC).
- Lyophilization: Removing the water from serum by freeze drying (dehydration), can be kept to very long time at (-70 ºC to -80 ºC).

B- Chemical preservation:

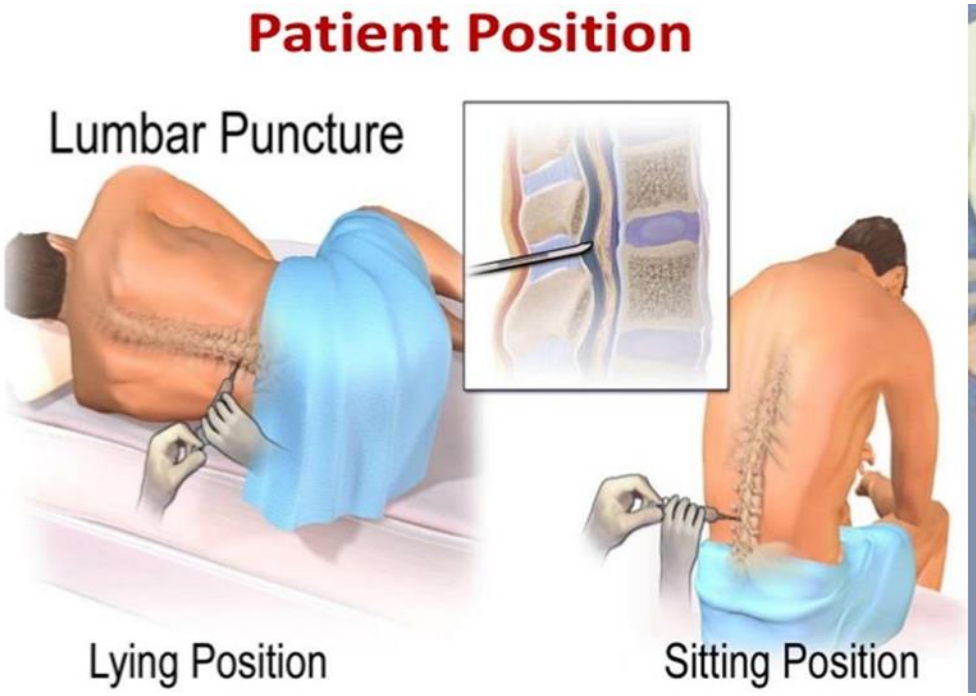
Normally chemical preservatives should not be added to serum samples in sero-tests, except in rare conditions:

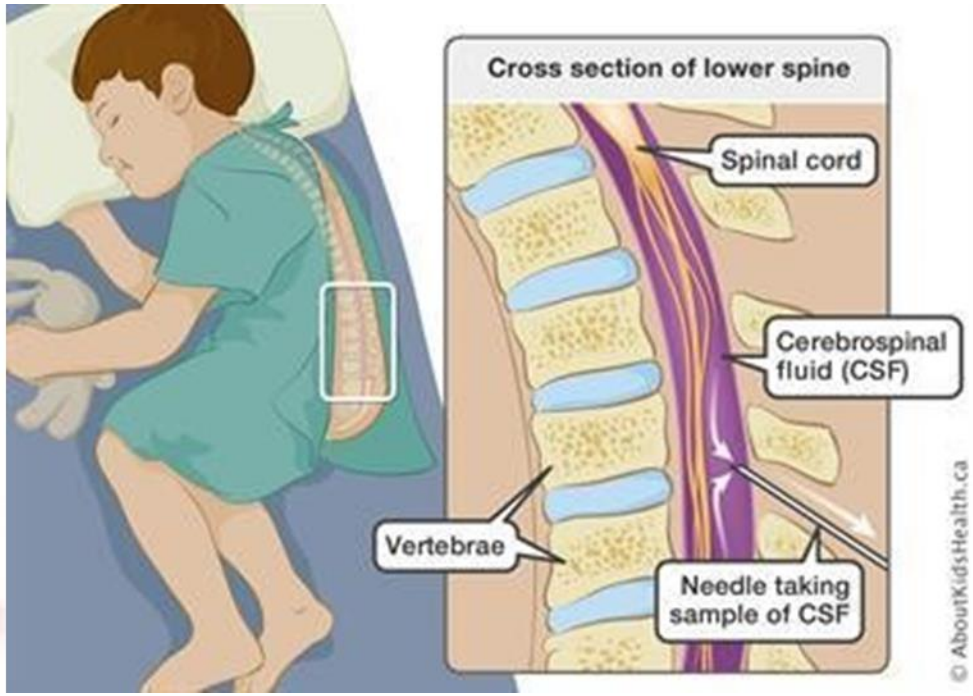
- The preservative used is Merthiolate in activation of serum complement.
- The complements in patients serum interferes with several sero-tests, so it must be inactivated before the tests.
- The inactivation is done by heating the serum sample in water bath at 56 ºC for 30 min. or at 63 ºC for 3 min.

4. CSF (Cerebrospinal fluid):

- Cerebrospinal fluid (CSF) analysis is a set of laboratory tests that examine a sample of the fluid surrounding the brain and spinal cord.
- This fluid is an ultra filtrate of plasma.
- It is clear and colorless.
- It contains glucose, electrolytes, amino acids, and other small molecules found in plasma, but has very little protein and few cells.
- CSF protects the central nervous system from injury.

- Provides it with nutrients, and removes waste products by returning them to the blood.
- CSF is withdrawn from the subarachnoid space through a needle by a procedure called a lumbar puncture or spinal tap.
- CSF analysis includes tests in clinical chemistry, hematology, immunology, and microbiology to diagnose meningitis and encephalitis, which may be viral, bacterial, fungal, or parasitic infections.





5. Urine:

- The only sero tests done in our lab on urine is the pregnancy test, for the presence of HCG hormone in the Urine sample during the pregnancy.

References

- Chapel, H., Haeney, M., Misbah, S. A., & Snowden, N. (2014). Essentials of clinical immunology. John Wiley & Sons.
- Bazzano G, Galazzi A, Giusti GD, Panigada M, Laquintana D. The Order of Draw during Blood Collection: A Systematic Literature Review. Int J Environ Res Public Health. 2021 Feb 7;18(4):1568. doi: 10.3390/ijerph18041568. PMID: 33562241; PMCID: PMC7915193.
- https://www.ncbi.nlm.nih.gov/books/NBK138659/
- Find a Test | MLabs (umich.edu).