Cihan University/ Sulaymaniya

College of Health Science

Medical Laboratory Analysis

4th Stage- 1st Semester

Pr. Clinical Immunology

Lab- 1: Sample Collection

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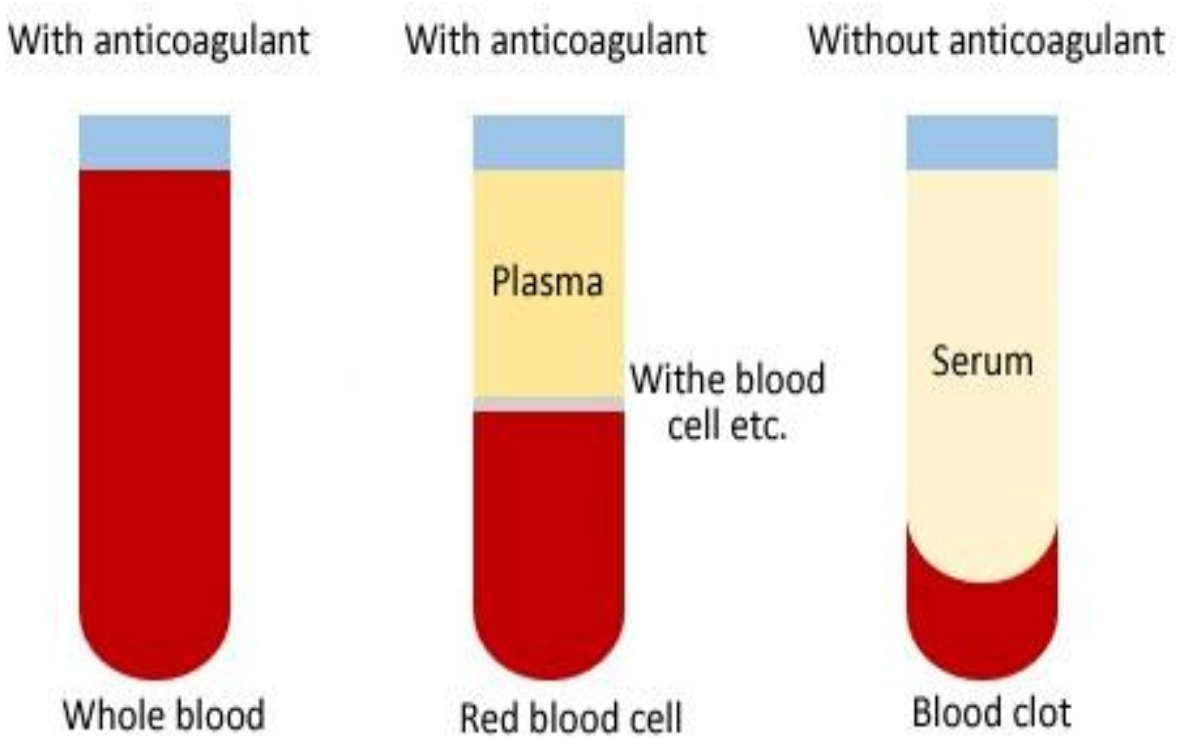
Laboratories

- 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.



Types of Specimens

- **Serum:** the supernatant of whole blood that has been allowed to clot. It is the most important specimen in serology because antibodies are found in it, the principle of serum preparation is whole blood are obtained by the vein puncture and allowed to clot, then the serum remove for testing.
- **Plasma:** is the fluid portion of the blood that has been prevented from clotting by the addition of anticoagulants (Oxalate, EDTA, heparin). It is rarely used in serotests because the fibrinogen precipitate and anticoagulants sometimes may alter the tests.
- **Cerebrospinal fluid (CSF):** Serotests on CSF are limited for syphilis.
- **Urine:** The only serotests done on urine is the pregnancy test, for the presence of HCG hormone in the urine sample during the pregnancy.

Phlebotomy or Blood Collection

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

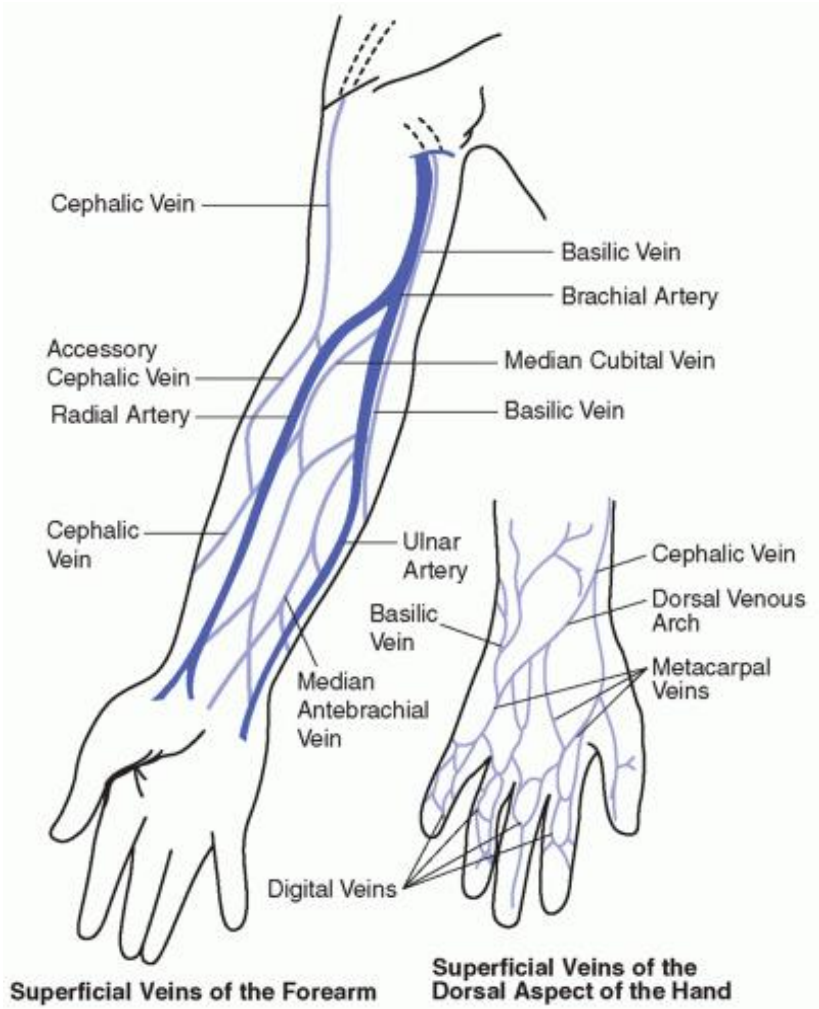
Selecting vein site

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.

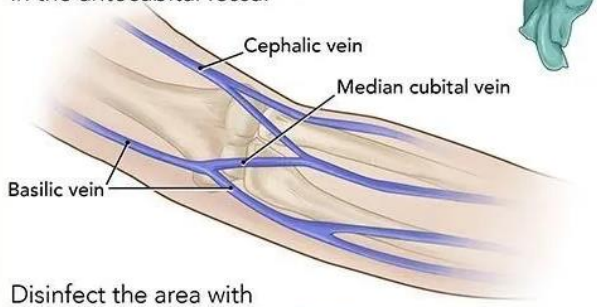


Venipuncture

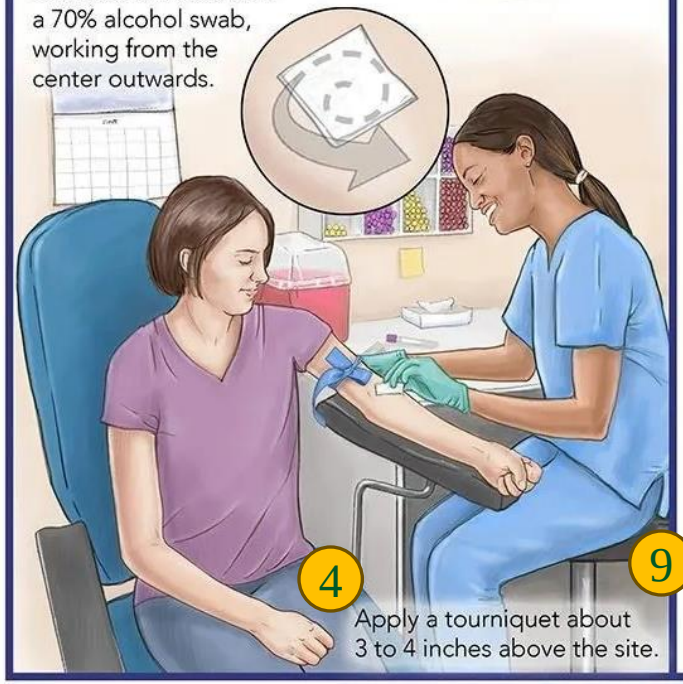
Blood collection procedure guide

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

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

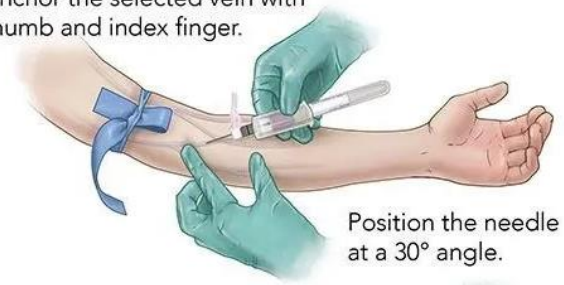


3. Disinfect the area with a 70% alcohol swab, working from the center outwards.



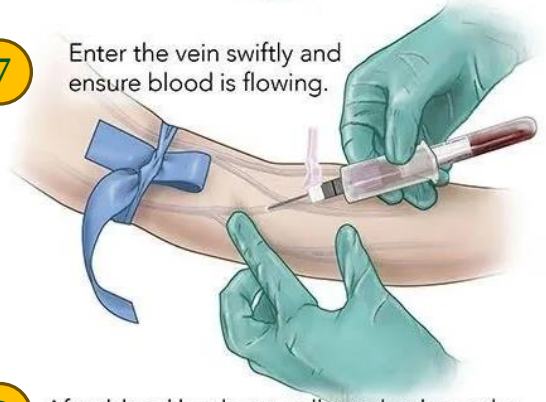
4. Apply a tourniquet about 3 to 4 inches above the site.

6. Anchor the selected vein with thumb and index finger.

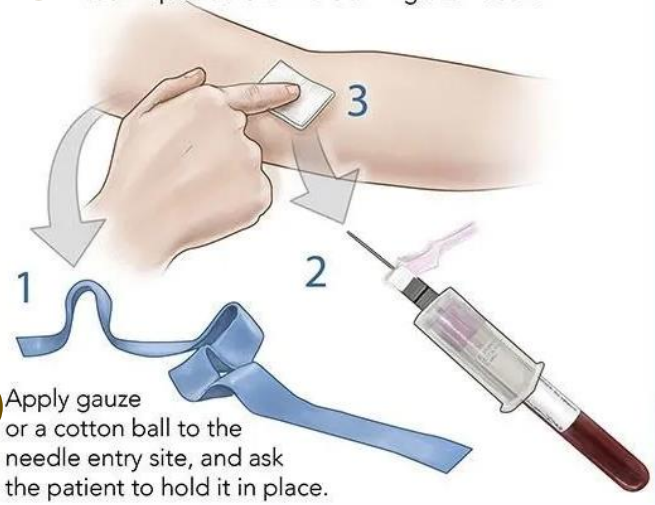


Position the needle at a 30° angle.

7. Enter the vein swiftly and ensure blood is flowing.



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



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

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

11. Discard the used needle in the sharps container.

12. 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

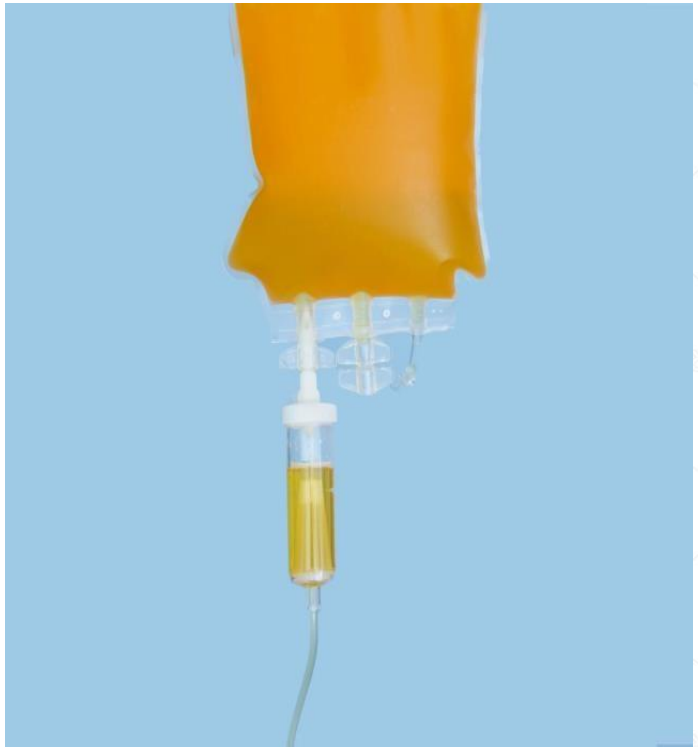
- All specimens must be labeled with both the **patient's first and last name** as well as **a second identifier** such as **the patient's birth date** or medical record number matching the demographic information present on the accompanying requisition or other paperwork.
- Blood collection tubes must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

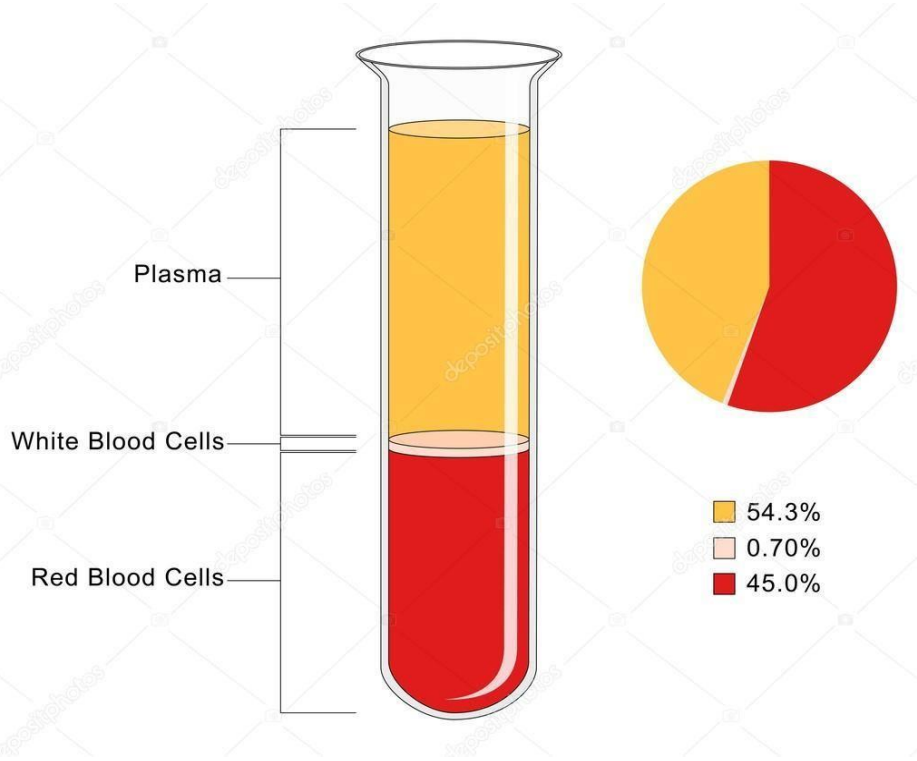
Catalogue of blood vacuum tube

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).

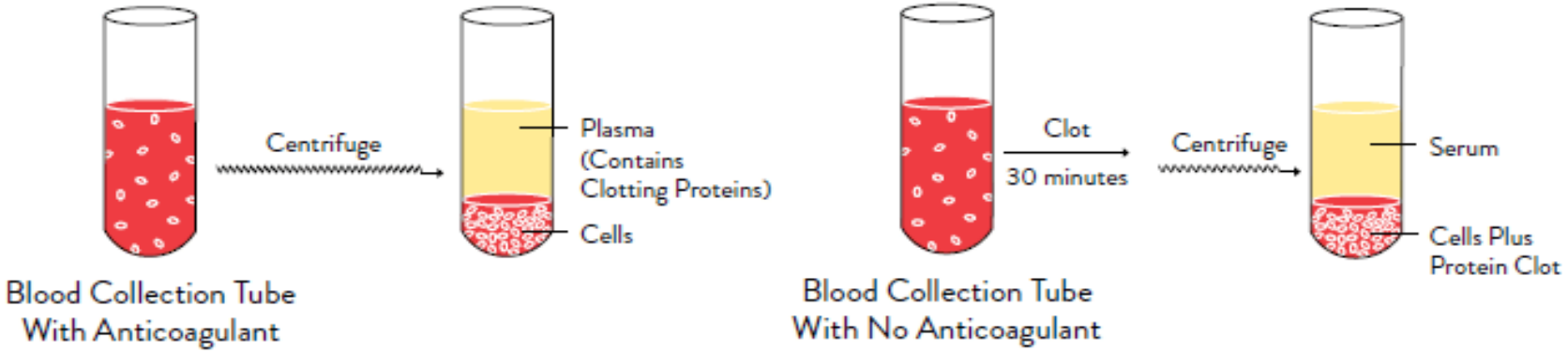




3. Serum

Procedure for Serum Preparation:

1. We obtain the blood in the vein, should be discard the needle and put in the centrifuge tube.
2. Leave the blood to clot at room temperature for 30 min. – 1 hr. for blood clotting (conversion of fibrinogen to fibrin).
3. Centrifuge the blood for 5 minutes (2500-3500 rpm).
4. By using he Pasteur pipette remove the serum in to the another tube.
5. Testing immediately or preserve the serum.



Serum Preservation

- **Physical preservation:**

1. Refrigeration: This is short term preservation, the serum keep in refrigerator at (4-8 ºC).
2. Freezing: For one week place it in freezer at -20 ºC.
3. Deep freezing: for 6-12 months place it in deep freezer at (-70 ºC to -80 ºC).
4. Lyophilization: Removing the water from serum by freeze drying (dehydration), can be kept in very long time at (-70 ºC to -80 ºC).

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:-

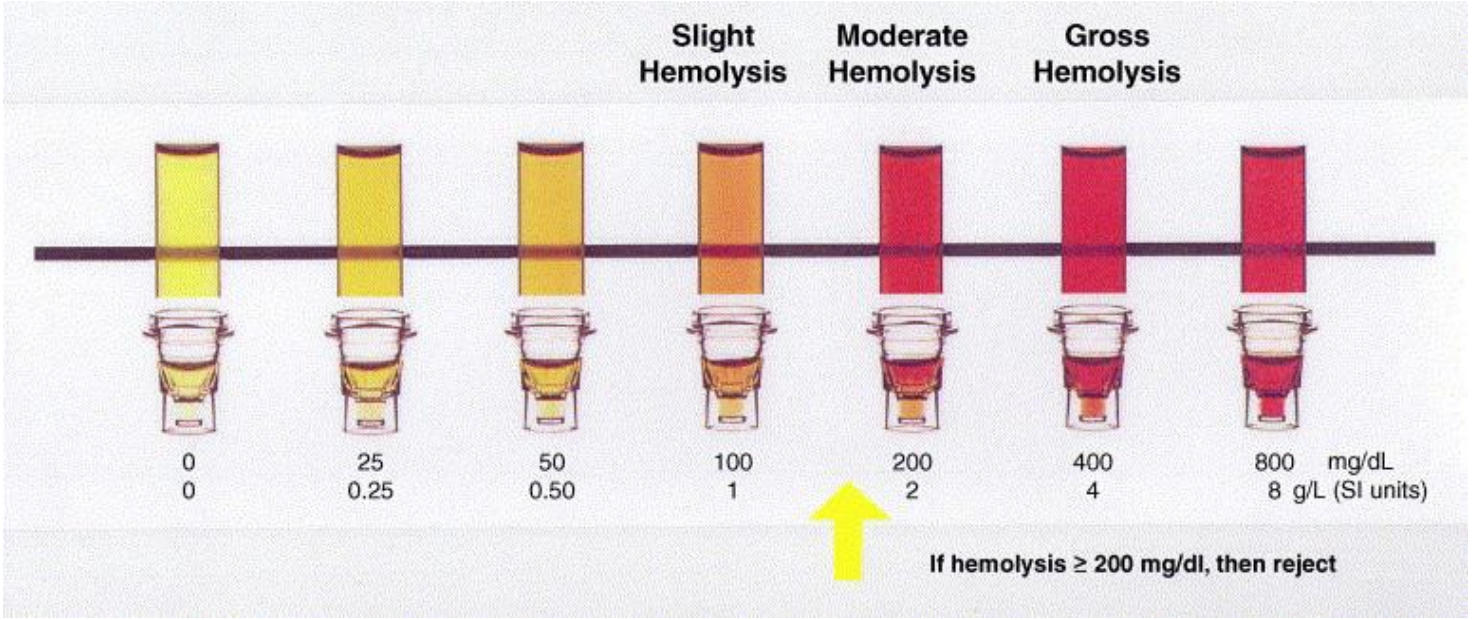
Sero -tests must be done with the fresh serum prepared (as soon as possible).

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.



Chemical preservation

1. Normally chemical preservation should not be added to serum sample in serotests, but in rare conditions some chemicals can be used as preservatives e.g. for syphilis tests.
2. The preservative used is Merthiolate, However in some situations it causes lysis of organisms such as Vibrio cholerae.
3. Sodium salicylate is used in the activation of serum complement.

Note// The complements in patients serum interferes with several serotests, 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. The activity is returned to the complements after several hours, mostly after 4 hours. So, we must do second inactivation (reinactivation) by heating the serum at 56 ºC for 10 minutes.

How to send Samples

A laboratory request form is the authorization that enables the laboratory to do specified procedures. It must accompany each specimen. The following information must be provided:

1. Patient name or other unique identifier
2. Date of specimen collection
3. Type and/or source of specimen
4. Name and location of submitter
5. Examination requested
6. Where there are multiple samples, taken at different times, the times should be recorded on the request.

Additionally, age and sex of patient, disease suspected, symptoms, patient address, etc., may be required for some specified tests.

Unacceptable or rejected specimens

Specimens may be rejected for the following reasons

1. There is a patient name or file number discrepancy between specimen label and request form.
2. There is no patient name or other unique identifier on specimen
3. Specimen is too old when received
4. There is apparently no specimen in container
5. Are over- or under-filled specimens in container
6. The expiration date of the transport medium has been exceeded
7. Previously frozen samples that have thawed in transit
8. Sample is broken or has leaked in transit,

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).