Course: Practical Clinical Chemistry, 5th stage
College of Pharmacy, University of Anbar
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HATEM HADEED

Lab: 1

Specimen collection and preservation

Introduction to Clinical Laboratories

When a person is ill, diagnosis begins with physical examination by a doctor. It may not possible to diagnose a disease only on the basis of physical examination. There are various diagnostic tests to confirm a suspected diagnosis. The clinical pathological laboratory tests are extremely useful to find out the causes of disease.

The functional components of the clinical laboratory are:
1) Clinical pathology
2) Hematology
3) Clinical biochemistry
4) Clinical microbiology
5) Serology
6) Blood bank
7) Histology and cytology

Functions: Clinical biochemistry deals with the biochemistry laboratory applications to find the cause of a disease as well as the severity of diseases of many organs such as liver, stomach, heart, kidneys, brain as well as the endocrine disorders and related status of acid-base balance of the body.

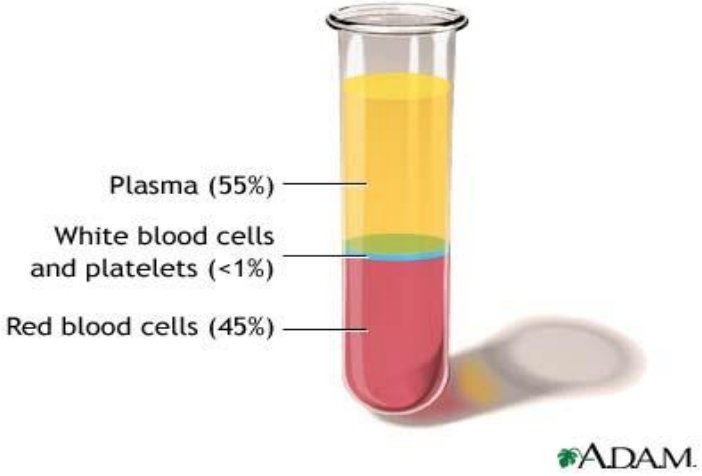
Specimen collection:

There are three types of blood specimens- serum, plasma and whole blood. Each different specimen is collected for various reasons. When blood is removed from the body, typically, it will coagulate or clot within 30 to 60 minutes. Serum can be separated from blood by centrifugation. Centrifugation is a process that spins the blood at high speeds in a machine called a centrifuge. This spinning separates the serum from the blood cells enmeshed in blood clot. Blood serum looks pale-yellow and has a similar composition to plasma. However, serum does not contain fibrinogen. Laboratory tests, like chemistry and immunology test are commonly performed on serum.

Coagulation tests cannot be performed on serum because the coagulation factors are separated out of the serum during the centrifuge process. Phlebotomy or blood collection: The term phlebotomy refers to blood draw from a vein, artery, or the capillary bed for lab analysis or blood transfusion

Blood:

Blood is a liquid tissue. Suspended in the watery plasma are seven types of cells and cell fragments. -Red blood cells (RBCs) -White blood cells (WBCs) -Platelets -Five kinds of Leukocytes (lymphocytes, monocytes, neutrophils, eosinophils, basophils) -After centrifugation of blood, the blood separate into three layers (see the figure)



Blood plasma: -

Plasma is the liquid component of blood. -It is mainly composed of water, blood proteins and inorganic electrolytes. -Roughly 92% water, mixed with organic and inorganic-substances. - The most abundant plasma solute is the plasma protein, of which there are three groups: albumin, globulins, and fibrinogen.

Blood clot:

-When a blood sample is left standing without anticoagulant, it forms a coagulum or blood clot. One of the normal components of plasma is a soluble plasma protein called fibrinogen. -On standing, this protein will be converted to insoluble substance called fibrin; this occurrence is referred to as blood coagulation or clotting. -The clot contains coagulation proteins, platelets, and entrapped red and white blood cells.

Blood serum:

Serum is the same as plasma except that clotting factors (such as fibrin) have been removed. -For many biochemical laboratory tests, plasma and blood serum can be used interchangeably. Serum resembles plasma in composition but lacks the

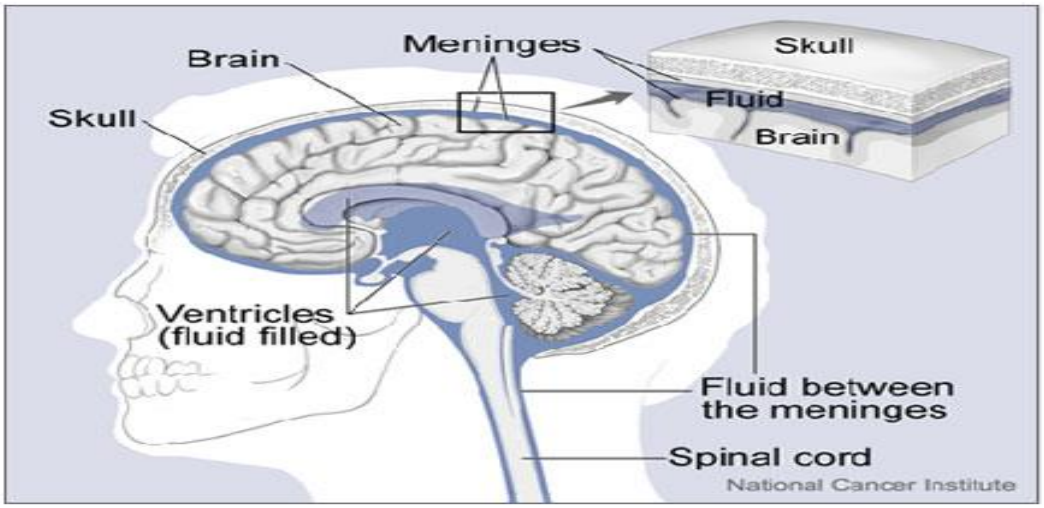
coagulation factors. - It is obtained by letting a blood specimen clot prior to centrifugation.

1- Whole blood specimens are usually required for hematology tests. These types of tests require the blood to remain in the same form as it is in the bloodstream. It is important that the blood specimen does not clot or separate. An anticoagulant must be added and the specimen should be mixed for at least 2 minutes immediately before performing the test. It must be analyzed within limited time (why?)

2- Urine has a long, rich history as a source for measuring health and well-being and remains an important tool for clinical diagnosis. The clinical information obtained from a urine specimen is influenced by the collection method, timing and handling.

3- Saliva testing is a diagnostic technique that involves laboratory analysis of saliva to identify markers of endocrine, immunologic, inflammatory, infectious, and other types of conditions. Saliva is a useful biological fluid for assaying steroid hormones such as cortisol, genetic material like RNA, proteins such as enzymes and antibodies, and a variety of other substances. Saliva testing is used to screen for or diagnose numerous conditions and disease states, including Cushing's disease, anovulation, HIV, cancer, parasites, hypogonadism, and allergies.

4- Cerebrospinal fluid (CSF) is a clear watery liquid that is formed and secreted by the choroid plexus, a special tissue that has many blood vessels and that lines the small cavities or chambers (ventricles) in the brain. About 17 ounces (500 mL) are produced each day. This rate of production means that all of the CSF is replaced every few hours. A CSF analysis is a group of tests that evaluate substances present in CSF in order to diagnose conditions affecting the central nervous system.



Phlebotomy:

The term phlebotomy refers to blood draw from a vein, artery, or the capillary bed for lab analysis or blood transfusion. Usually vein is used to collect blood by vein puncture procedure. In adults: most venipuncture procedure use arm vein.

On arm, one of three arm veins is used: **median cubital** vein "located on the middle", **cephalic** vein or **basilic** vein "located on both sides".

Median cubital vein is the best choice (why?).

However if venipuncture procedure is unsuccessful in median capital; cephalic or basilica is used.

Artery blood is rarely used in special cases as when blood gases, pH, PCO2, PO2 and bicarbonate is requested. It is usually performed by physicians.

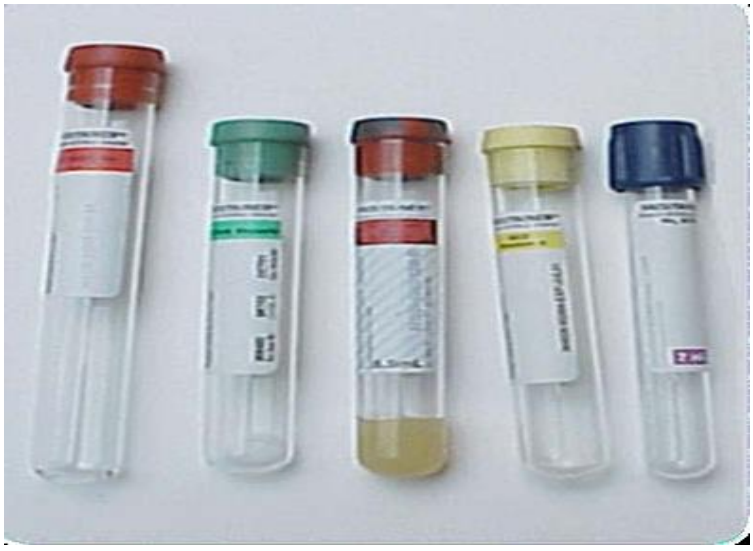
Hemolysis :

- It means liberation of hemoglobin due to rupture of RBCs.
- Due to hemolysis plasma or serum appears pink to red color.
- It causes elevation in: K^+, Ca^{2+}, phosphate, SGOT, SLDH and acid phosphatase.
- Hemolysis is occurred due to sampling, transporting and storage (too hot or too cold).

Changes in the serum color indicate one of the following:

- **Hemolyzed**: serum appears **pink** to red due to rupture of RBCs
- **Icteric**: serum appears **yellow** due to high bilirubin.
- **Lipemic**: serum appears **milky** or turbid due to high lipid.

Blood collection tubes:



The tubes are covered with a color-coded plastic cap. They often include additives that mix with the blood when collected, and the color of the tube's plastic cap indicates which additives that tube contains. The tubes may contain additional substances that preserve the blood for processing in clinical laboratory. Using the wrong tube may therefore make the blood unusable.

Top Color	Additives	Principle	Uses
Lavender	**EDTA**	**-The strongest anti-coagulant** **- Ca^{+2} chelating agent** **- To preserve blood cells components**	**- Hematology** **- Blood bank (ABO)** **- HbA1C (Glycosylated Hb)**
Light Blue	**Sodium Citrate**	**Ca^{+2} chelating agent**	**- PT: Prothrombin Time** **- PTT: Partial Thromboplastin Time** **(in case of unexplained bleeding and liver disease)**
Green	**Sodium Heparin or Lithium Heparin**	**Heparin binds to Thrombin and inhibits the second step in the coagulation cascade** **(Prothrombin → Thrombin)** **Fibrinogen ↛ Fibrin**	**Enzymes** **Hormones** **Electrolytes (Na^+, K^+, Mg^+, Cl^-**

Top Color	Additives	Principle	Uses
Black	**Sodium Citrate**	**Ca^{+2} chelating agent**	**ESR (Erythrocyte Sedimentation Rate) to test how much inflammation in the patient, unexplained fever, Arthritis, Autoimmune Disorder**
Gray	**-Sodium Fluoride** **-Potassium Oxalate**	**Glycolysis inhibitor** **Anti-Coagulant**	**Glucose tests**
Royal Blue	**Heparin** **Na-EDTA**	**Anti-Coagulant** **Tube should not be contaminated with metals**	**Toxicology** **Trace Elements and metals**
Yellow	**ACD (Acid-Citrate Dextrose)**	**Anti-Coagulant**	**DNA Studies** **Paternity Test** **HLA Tissue Typing (Human Leukocyte Antigen)** **The body used this protein to differentiate the self-cells from non-self-cells**

Anticoagulant

An **anticoagulant** is a substance that prevents coagulation (clotting) of blood. A group of pharmaceuticals called anticoagulants can be used in vivo as a medication for thrombotic disorders. Some anticoagulants are used in medical equipment, such as test tubes, blood transfusion bags, and renal dialysis equipment.

Heparin is a biological substance, usually made from pig intestines. It works by activating antithrombin III, which blocks thrombin from clotting blood. Heparin can be used in vivo (by injection), and also in vitro to prevent blood or plasma clotting in or on medical devices.

Anticoagulants outside the body

Laboratory instruments, blood transfusion bags, and medical and surgical equipment will get clogged up and become nonoperational if blood is allowed to clot. In addition, test tubes used for laboratory blood tests will have chemicals added to stop blood

clotting. Apart from heparin, most of these chemicals work by binding calcium ions, preventing the coagulation proteins from using them.

- **EDTA** is denoted by mauve or purple caps on Vacationer (A **vacutainer** blood collection tube is a sterile glass or plastic tube with a closure that is evacuated to create a vacuum inside the tube facilitating the draw of a predetermined volume of liquid. Most commonly used to draw a blood sample directly from the vein, these also are used to collect urine samples) brand test tubes. This chemical strongly and irreversibly binds calcium. It is in a powdered form.
- **Citrate** is usually in blue Vacutainer tube. It is in liquid form in the tube and is used for coagulation tests, as well as in blood transfusion bags. It binds the calcium, but not as strongly as EDTA. Correct proportion of this anticoagulant to blood is crucial because of the dilution. It can be in the form of sodium citrate or ACD.
- **Oxalate** has a mechanism similar to that of citrate. It is the anticoagulant used in fluoride (grey top) tubes.

Spectrophotometry

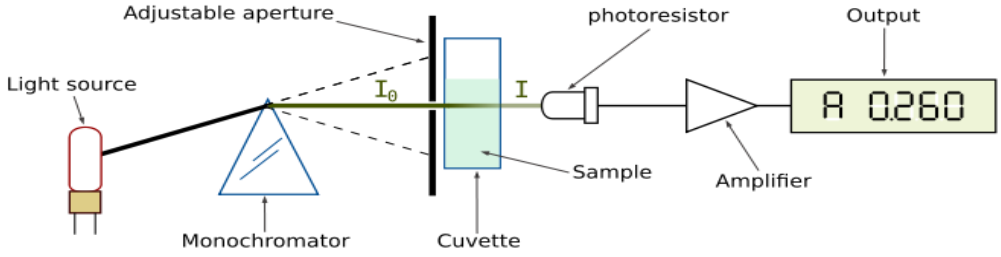
In chemistry, **spectrophotometry** is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. It is more specific than the general term electromagnetic spectroscopy in that spectrophotometry deals with visible light, near-ultraviolet, and near-infrared.

Spectrophotometry involves the use of a spectrophotometer. A spectrophotometer is a photometer that can measure intensity as a function of the light source wavelength. Important features of spectrophotometers are spectral bandwidth and linear range absorption or reflectance measurement

In short, the sequence of events in a modern spectrophotometer is as follows:

1. The light source is imaged upon the sample
2. A fraction of the light is transmitted or reflected from the sample
3. The light from the sample is imaged upon the entrance slit of the monochromator

The monochromator separates the wavelengths of light and focuses each of them onto the photodetector sequentially.



Beer-Lambert Law

Introduction

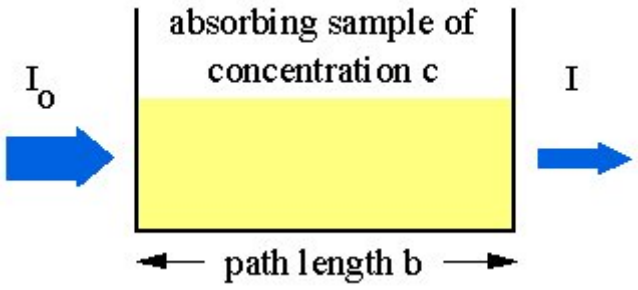
The Beer-Lambert law (or Beer's law) is the linear relationship between absorbance and concentration of an absorbing species. The general Beer-Lambert law is usually written as:
$A = a(\lambda) * b * c$

Where A is the measured absorbance, $a(\lambda)$ is a wavelength-dependent absorptivity coefficient, b is the path length, and c is the analyte concentration. When working in concentration units of molarity, the Beer-Lambert law is written as:
$A = \varepsilon * b * c$
where ε is the wavelength-dependent molar absorptivity coefficient with units of M^{-1} cm^{-1}.

Instrumentation

Experimental measurements are usually made in terms of transmittance (T), which is defined as:
$T = I / I_o$
where I is the light intensity after it passes through the sample and I_o is the initial light intensity. The relation between A and T is:
$A = -\log T = -\log (I / I_o)$.

Absorption of light by a sample



Modern absorption instruments can usually display the data as either transmittance, %-transmittance, or absorbance. An unknown concentration of an analyte can be determined by measuring the amount of light that a sample absorbs and applying Beer's law. If the absorptivity coefficient is not known, the unknown concentration can be determined using a working curve of absorbance versus concentration derived from standards.