Types of Blood Specimens

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Course Objectives

1. Review the basic concepts of blood composition, blood flow and coagulation
2. Discuss the differences between serum, plasma, and whole blood, including their composition, properties, and collection requirements
3. Describe the basics of collecting and processing the different types of blood specimens, and the specimen collection requirements for common types of blood tests
4. Emphasize the role of the phlebotomist in ensuring the accuracy of specimen collection, as part of the goals of patient care

Introduction

Physicians and other healthcare providers order a variety of blood tests for their patients, whether to diagnose conditions, assess organ function, or evaluate effectiveness of treatment. In the laboratory, diagnostic tests are typically performed on three types of blood samples: Serum, Whole Blood, and Plasma. Each of these is unique in its properties, and each varies in the method of collection. When carrying out orders for a blood draw, it is important for the phlebotomist to pay attention to the type of sample required for each test, and to be able to obtain all specimens properly. This includes knowledge of how to obtain clotted versus unclotted specimens, and the appropriate types of blood specimen tubes to be filled. Accuracy of specimen collection and processing allows the laboratory to perform testing in a timely manner, ensures the production of reliable results, andprovides laboratory and medical staffs with the vital information needed for making clinical decisions, ultimately benefitting patients.

This course reviews the basics of blood composition, blood flow, and coagulation and provides an understanding of the different types of blood specimens. It then discusses the methods for collecting serum, whole blood, and plasma, and explains the purpose for centrifugation and aliquoting. This course provides examples of medical tests involving each specimen type, including tests requiring special handling requirements.

Blood Composition

Blood is primarily comprised of two components: plasma and the formed (cellular) elements.

Plasma

Plasma makes up the majority (55%) of blood and is referred to as the *fluid portion* of the blood. Plasma itself is made up of 90% water. The remaining 10% are substances dissolved within the plasma (such as gases, nutrients, minerals, hormones, antibodies) that are being transported by the blood throughout the body. Plasma typically has a clear, pale-yellow appearance.

Formed Elements

Formed Elements are the variety of blood cells that float individually within the plasma. Unlike plasma, which is a liquid, these are solid cells which are carried by the bloodstream. Formed elements make up 45% of the blood, and are comprised of red blood cells, white blood cells and platelets.

The majority of the formed elements are the red blood cells (also known as RBCs, or *erythrocytes*). Although plasma by itself has a pale-yellow color, blood as a whole normally has a dark red appearance due to all the erythrocytes floating in the plasma. The major function of RBCs is to carry oxygen within the bloodstream. Oxygen-rich or *oxygenated* blood is essential for the nourishment of cells of the various body organ systems. The second type of blood cells are white blood cells (also called WBCs or *leukocytes*). WBCs play a role in the body's immune system, which primarily defends against invasion by foreign bodies such as bacteria and viruses. The third type of blood cells are platelets (also called thrombocytes) which are involved in the clotting process. In response to blood vessel injury, platelets form a plug that helps to seal up the vessel and prevent excessive blood loss. (Insert diagrams of Plasma and Formed Elements, RBCs, WBCs and Platelets)

Blood Flow

Blood relies on the pumping action of the heart in order to flow properly. With each contraction, the heart exerts pressure on the blood and causes it to run smoothly within the blood vessels (arteries, veins, and capillaries) that course throughout the body. Blood constantly travels in a circuitous route within the vessels to and from the heart, passing through the various organ systems. This is referred to as the circulation.

Arteries are blood vessels which carry blood away from the heart and deliver it to the various organs. The major arteries which exit the heart are the Aorta and the Pulmonary Trunk. The Aorta is the major artery of the *systemic* circulation. Upon leaving the heart, the aorta branches into the various arteries which transport oxygenated (oxygen-rich) blood necessary for nourishing the organs of the body. The Pulmonary Trunk is the major artery within the *pulmonary* circulation; it branches into the left and right pulmonary arteries and sends deoxygenated (oxygen-poor) blood exclusively to the lungs in order to pick up more oxygen.

Veins are blood vessels which collect blood from the organs and carry it back to the heart. In the systemic circulation, the superior vena cava and inferior vena cava collect deoxygenated blood from the organs above and below the heart respectively. The heart will then send this blood to the lungs to become oxygenated once more. In the pulmonary circulation, the pulmonary veins collect oxygenated blood from each lung, returning the blood to the heart. The heart will then pump this oxygenated blood out to the body organs.

Capillaries are the smallest blood vessels. They are microscopic and serve as the connection between arteries and veins. Within the organ tissues of the systemic circulation, blood flows through small branches of arteries called *arterioles* which then connect with the capillaries. Due to their very thin walls (just one cell in thickness), capillaries allow the blood flowing within them to perform *gas exchange*, a process whereby oxygen is given to the cells and in turn carbon dioxide is removed. Since capillaries are connected to both arteries and veins, they contain mixed (both oxygenated and deoxygenated) blood. While passing through the capillary, the oxygen-rich blood is able to provide oxygen and nutrients to the cells. Conversely, the blood removes carbon dioxide and waste substances from the cells, thus becoming oxygen-poor. After passing through the capillaries, blood is collected by *venules*, small branches which combine to form the veins that return the blood to the heart.

While blood is transported within the circulation, it remains in a fluid state. In this form, the plasma flows like a river within the blood vessels, carrying along any dissolved substances, as well as all the blood cells. The plasma and formed elements remain mixed in the normal flow of the bloodstream. (Insert diagram of the circulation)

Coagulation

When blood vessels get damaged (such as by a deep cut in the skin), bleeding occurs because the vessel wall is no longer intact, and blood is able to leak out. Since losing too much blood is potentially harmful (or even deadly), a natural mechanism known as *hemostasis* is the body's way of repairing damaged blood vessels and stopping blood loss. An integral part of hemostasis is the process of blood clotting, by which blood basically turns from a fluid to a solid form. Simply put, when blood clots it becomes a solid mass that is meant to plug up the leak in the blood vessel, thereby preventing further bleeding.

Clotting or *coagulation* is a complex process involving many chemical and cellular components. In both the bloodstream and the body tissues, there are several protein-based chemicals (known as clotting factors) that are involved in precipitating the clotting process. One significant clotting factor is *fibrinogen*, which is found in plasma. While blood flows normally within the blood vessel, fibrinogen remains inactive. However, when there is vessel injury and subsequent bleeding, fibrinogen (through the action of other clotting factors in the blood) transforms into *fibrin*, which forms a type of meshwork that traps cells. This is the key step in the clotting process because fibrin acts as a binding agent, causing the freely floating cells in the bloodstream to become stuck together within its mesh-like structure. This solid mass of cells is what forms a solid *clot*, which essentially covers the damaged part of the blood vessel wall, plugging the leak and preventing further bleeding. Blood that has exited the body (such as blood that leaks from a cut onto the skin) is no longer flowing normally within the blood vessels, and also undergoes clotting, as evidenced by the solid scab that eventually forms over a bleeding cut. (Insert diagram of coagulation process)

When blood is drawn from a vein and collected into a specimen tube, the natural response of the blood in the tube will also be to clot, since blood undergoes clotting whenever it has been removed from the body or is no longer flowing properly. As previously mentioned, when clotting occurs the fibrinogen in the plasma is activated and transforms into fibrin. Fibrin then binds the individual blood cells into a solid mass. A blood sample within a collection tube that has been allowed to fully clot will no longer appear purely fluid in form; instead, a portion of it will have a solid appearance. When the tube is inverted, the clotted blood could be stuck to the inner tube wall, or it may detach and be viewed as a compact lump within the tube. This solid is comprised of RBCs, WBCs and platelets held together with fibrin. (Insert picture of clotted blood in a collection tube)

Components of Clotted Blood

Technically, the fluid portion of blood *must contain fibrinogen* in order to be called plasma. When blood is flowing properly (such as within the bloodstream), plasma retains its fibrinogen, and the blood remains unclotted. If blood is taken out of the body (either because of bleeding from injury or via blood draw procedure), the coagulation process is activated and fibrinogen turns into fibrin, which in turn binds the blood cells. Once the blood sample within the tube has completely clotted (typically after 30 to 60 minutes at room temperature), all the fibrinogen has been converted to fibrin. Therefore, in *fully clotted blood* that no longer contains any fibrinogen, the fluid portion is no longer referred to as plasma. The fluid portion of clotted blood is called *serum*. Clotted blood therefore is made of two components: serum and the clotted cells.

The first component, serum, which is the liquid portion of clotted blood, has a pale-yellow appearance similar to plasma, but unlike plasma, serum *contains no fibrinogen*. Serum is formed once all the fibrinogen in plasma has been converted to fibrin. Although serum and plasma basically have the same composition, a major difference is that serum does not contain any of the clotting factors (including fibrinogen), since these chemicals were all used up during the process of coagulation. *(Phlebotomy Essentials p.170)*

The second component of clotted blood is the clot itself, composed of red blood cells, white blood cells and platelets that have all been bound together by the fibrin. This is the solid portion of the clotted blood. (Insert picture of clotted specimen with visible serum)

Although the serum and the clotted cells are two distinct components of clotted blood, they will not be immediately distinguishable in the specimen tube. Initially, the coagulated blood will just have the appearance of a solid lump within the tube. After the tube is left standing for a few minutes, the two components will naturally begin to separate. *(Guyton and Hall, 2021)* In a specimen tube which is kept upright and undisturbed, the clot, being composed of solid cells and heavier, can be seen at the bottom of the tube. The serum, being a liquid and weighing less than the clot, will be seen floating in the upper portion of the tube. At this point the serum and clot will become easier to differentiate.

Obtaining a Serum Specimen

To collect a serum specimen, the phlebotomist first draws blood from the vein. Initially this is blood in its original unclotted form, composed of the liquid plasma (which contains fibrinogen) mixed with all the blood cells. The blood specimen is then allowed to *clot completely*, after which *the fluid portion of the blood has changed from plasma to serum*.

A blood sample within a collection tube that has been allowed to fully clot will have a solid appearance. At this point, the serum and the clotted cells are still not separately identifiable within the tube. After the clotted specimen has been left upright for a few minutes, the components will naturally begin to separate into two layers. The upper layer will contain liquid serum, and the bottom layer will have the solid clot.

In the laboratory, the serum within the tube can be fully separated by placing a clotted blood sample in a *centrifuge*. This instrument is able to spin the blood at a high rate of speed over a set amount of time, during which the centrifugal force pushes the heavier component of the blood (the clotted cells) to the bottom of the tube while the lighter component (serum) remains floating at the top. The operating procedure for centrifuge machines depends on the specific model type being used by the laboratory; a phlebotomist must be trained in centrifuge operation, maintenance, and safety protocols. Once centrifugation is complete, the serum becomes fully separated from the clot, seen as a pale-yellow liquid in the upper layer, while the clotted cells are seen as a dark-red, solid mass at the bottom of the tube. (Insert picture of centrifuged serum specimen)

When a serum specimen is ordered, it is crucial to wait for the clotting process to be completed before centrifuging is done. Typically, it takes a blood sample 30 to 60 minutes to clot fully at room temperature (22 to 25°C). Centrifuging a specimen before it has fully clotted can lead to the presence of residual fibrin clots in the serum, which is undesirable because these can cause delays in testing or affect testing results. (*Phlebotomy Essentials p. 393*) (Insert picture of fibrin clots)

After clotting is complete, it is then necessary to fully separate the serum from the clot, as the laboratory only requires *serum-only to be submitted* for testing. When retrieving and handling the specimen tube after centrifugation, it is very important to *keep the tube upright* as much as possible. Although the serum and clot appear as distinct layers, any excessive motion or tilting of the sample can lead to the clot re-mixing with the serum. This must be avoided, since it will require the sample to be re-centrifuged, thus prolonging the processing period. Furthermore, centrifuging a sample more than once carries the risk of altering the specimen in certain ways (i.e., hemolysis), which could lead to testing issues in the lab and the possibility of inaccurate results.

Certain tubes used to collect serum contain a *separator gel* at the bottom, which makes processing the blood more convenient: during centrifugation, the gel moves up within the tube and forms a barrier between the serum and the clot. These *serum separator tubes* make serum collection easier by preventing the clot from re-mixing with the serum after the specimen is centrifuged. (Insert picture of serum separator tube before and after centrifugation)

Serum can be extracted from the sample tube via *aliquoting*, a process where a portion of the specimen is siphoned out and transferred into a secondary tube(s) using a device such as a pipette. A phlebotomist must always wear appropriate protective equipment and follow laboratory safety protocols when aliquoting specimens, in order to avoid any biohazard exposure incident. Aliquoting should be done in a timely manner in order to isolate the serum from the clotted cellular portion, though in tubes with a separator gel that has properly formed a barrier, aliquoting may not need to be done immediately. (Insert picture of aliquoting with pipette)

While aliquoting, it is important that only the serum (and none of the clot) is collected. Also, when serum separator tubes are used, the tip of the pipette must not be allowed to touch the gel barrier. Once aliquoted, the serum in the secondary tube must be stored according to laboratory protocols and subsequently submitted to the lab for testing.

Whole Blood

A whole blood sample is basically the same type of blood *as what is normally flowing within the blood vessels* of the body. When a whole blood sample is requested, it means that the blood specimen collected needs to remain in its original fluid form and contains all its components. Therefore, in a whole blood specimen the blood must remain *unclotted*, and *both the plasma and the cells* are submitted together in the specimen tube.

Blood that has just been drawn from the vein is initially in its original form. Since this blood has just recently been collected from the body, it has not yet begun to clot and is still in a fluid state. Fibrinogen has not yet been activated, and the blood cells continue to float freely within the plasma.

However, because the blood is no longer flowing within the body, its natural tendency will be to start clotting. Since a *whole blood sample is required to remain unclotted*, in this type of specimen it is critical to prevent the coagulation process from taking place.

Obtaining a Whole Blood Specimen

To collect a whole blood specimen, the phlebotomist must draw blood *using tubes that contain anticoagulants*. An *anticoagulant* is a chemical which prevents blood from clotting. Examples of anticoagulants used in blood collection include SPS, Citrate, Heparin, EDTA, and Oxalate. When adequately mixed with blood in the collection tube, these chemicals inhibit the clotting process, thereby preventing fibrinogen from converting to fibrin. As a result, the individual blood cells remain separate within the sample and plasma is not converted to serum.

To ensure that a blood specimen does not clot, the anticoagulated tube must be *inverted* immediately after it has been filled with blood. To properly invert a tube, it is gently turned upside down, then right side up. The method and number of tube inversions should be done in accordance with laboratory protocol. Proper inversion ensures that the entire blood specimen is adequately mixed with the anticoagulant in the tube. In this way, the clotting process is suspended, and the blood remains in its fluid form. (Insert diagram of tube inversion)

Once tube inversion is completed and the blood is prevented from clotting, there is typically no further processing necessary for a whole blood sample. Since the laboratory requires both plasma and cells to be submitted for testing, *centrifuging and aliquoting do not need to be performed for whole blood collection*. However, in the laboratory a tube left standing on a rack for a sufficient amount of time will cause the cells to begin to separate from the plasma. Since whole blood testing requires these components to remain mixed, if the plasma and cells have begun to separate, the specimen is re-mixed for at least 2 minutes before testing is done. (*Phlebotomy Essentials p. 171*)

Plasma

As mentioned previously, plasma is the fluid portion of blood within the bloodstream. Plasma is composed of 90% water and 10% dissolved substances. For certain tests, a plasma specimen may be preferred. When blood is collected into a specimen tube, the fluid portion remains as plasma so long as the specimen is not allowed to clot. Similar to how whole blood is obtained, collecting a plasma specimen requires the use of tubes which contain anticoagulant.

Obtaining a Plasma Specimen

To collect a plasma specimen, the phlebotomist must *first draw a whole blood specimen*. This requires filling an *anticoagulated tube* then immediately and adequately inverting it to ensure that blood does not clot. After inversion is completed, the tube is then placed in the *centrifuge to separate the plasma from the cells*. Centrifuging spins the sample at a high speed for a set amount of time. This process pushes the heavier component of the blood (the blood cells) to the bottom of the tube while the lighter plasma remains floating at the top. Once centrifugation is complete, the plasma becomes fully separated from the blood cells, and is seen as a pale-yellow liquid in the upper layer. Since the blood is not clotted and the cells are not bound together, during centrifugation the cells are pushed to the bottom of the tube individually based on weight. Given that the red blood cells are the heaviest, they end up at the very bottom, appearing as a dark-red RBC layer. The white blood cells and platelets also get pushed downward, but since they weigh less than the RBCs, they end up in a separate layer (a thin film called the *buffy coat*) on top of the red blood cells.

Therefore, after whole blood has been centrifuged, *three layers* are produced within the tube: the bottom layer contains the RBCs, the middle layer or buffy coat contains white blood cells and platelets, while the top layer contains the liquid plasma. (Insert picture of centrifuged plasma specimen)

It is necessary to completely separate the plasma from the cells, since the laboratory *requires that plasma-only be submitted* for testing. When retrieving and handling the specimen tube after centrifugation, it is very important to *keep the tube upright* as much as possible. Otherwise, since the blood is not clotted, excessive motion or tilting of the sample will cause the cells to re-mix with the plasma, which in-turn will require the sample to be re-centrifuged and thus prolong the processing time. Furthermore, centrifuging a sample more than once carries the risk of altering the specimen in certain ways (i.e., hemolysis), which could lead to testing issues in the lab and the possibility of inaccurate results.

Certain tubes used to collect plasma contain a *separator gel* at the bottom which makes processing the blood more convenient: during centrifugation, the gel forms a barrier between the plasma and the cells. These *plasma separator tubes* make collection easier by preventing the plasma from re-mixing with the cells after the specimen has been centrifuged. (Insert picture of plasma separator tube before and after centrifuge)

Plasma can be extracted from the sample tube via aliquoting, a process where a portion of the specimen is siphoned out and transferred into a secondary tube(s) using a device such as a pipette. A phlebotomist must always wear appropriate protective equipment and follow laboratory safety protocols when aliquoting specimens, in order to avoid any biohazard exposure incident. Aliquoting should be done in a timely manner in order to isolate the plasma from the unclotted cellular portion, though in tubes with a separator gel that has properly formed a barrier, aliquoting may not need to be done immediately. When aliquoting, it is important to collect only the plasma and none of the cells. The tip of the pipette must not touch the buffy coat or RBC layer. When the specimen has been collected using a plasma separator tube, it is also important that the tip of the pipette does not touch the gel barrier. Once aliquoted, the plasma in the secondary tube must be stored according to laboratory protocols and subsequently submitted to the lab for testing.

Serum versus Plasma

Both plasma and serum are liquid portions of blood, and both have a pale-yellow appearance when separated from the cells. However, plasma differs from serum as follows:

> Plasma is the liquid portion of non-clotted blood whereas serum is the remaining liquid portion after blood has clotted; AND
>
> Plasma contains fibrinogen *whereas serum does not*.

Note, although blood will still have a liquid portion even after it has clotted, the terminology used is different for technical reasons.

The common requirement for collecting either serum or plasma specimens is that *the blood sample needs to be centrifuged*. This process quickly and completely separates the components of the blood, so that either the serum or the plasma can be aliquoted and submitted to the lab. Only *whole blood samples do not require centrifugation or aliquoting*, since the lab requires both the liquid and solid components of the blood sample to be submitted for testing.

Specimen requirements of some common blood tests

Depending on laboratory protocols, blood tests may be performed specifically on whole blood, serum, or plasma. Whether the specimen collected needs to be clotted or unclotted is a major factor in collection requirements. Another factor is whether the test is requested as routine or STAT.

Chemistry

Chemistry tests involve the study of various chemical substances in the blood and include both individual and group (or panel) tests. Routine tests for chemistry can be done using serum specimens obtained from clotted blood. However, certain chemicals such as ammonia and potassium cannot be tested using clotted blood since coagulation causes the cells to release these chemicals into the serum, leading to falsely elevated results. (*Phlebotomy Essentials p.170*) Therefore to ensure accuracy, testing using a plasma sample is preferred in these cases.

When a STAT chemistry test is ordered, time is a critical factor. As such, serum specimens are generally less desirable for use in STAT testing since the sample has to first be allowed to completely clot before it can be centrifuged and aliquoted. Whole blood and plasma require less processing time since the specimen is not required to clot in either of these. As soon as an adequate amount of blood has been obtained and properly mixed with anticoagulant, a whole blood specimen can be tested. If plasma is required, the tube containing whole blood can immediately be centrifuged then aliquoting of plasma can be done.

Coagulation

Tubes containing citrate as the anticoagulant are used for collecting tests for coagulation, such as Prothrombin Time (PT) and Partial Thromboplastin Time (PTT). These tests require plasma collection to evaluate the proper function of the clotting process, especially in cases where patients have bleeding disorders or are routinely taking blood thinner medications. For coagulation studies to be done, clotting factors need to be present for testing in the blood, so the specimen needs to remain in unclotted form.

Hematology

Hematology involves the study of blood and its cellular components, as well as the blood-forming tissues. Hematology tests such as a complete blood count (CBC) assist in the diagnosis and assessment of various blood-related diseases, such as anemia and leukemia. Testing for hematology generally requires whole blood specimens collected using tubes which contain EDTA as the anticoagulant.

Summary

In clinical laboratory testing, obtaining accurate results is of paramount importance as health care practitioners rely upon them to make diagnostic and therapeutic decisions. Ultimately, the health and well-being of patients hinges on the laboratory producing precise and reliable testing outcomes. As part of the health care team, phlebotomists play a critical role in ensuring accuracy of testing by collecting the correct type of blood sample, as well as by appropriately handling and processing all specimens.

Quiz

1. The fluid portion of blood after the blood sample has completely clotted is called:

   a. Serum

   b. Plasma

   c. Formed elements

   d. RBC layer

2. Approximately 90% of Plasma is composed of:

   a. Oxygen

   b. Carbon Dioxide

   c. Water

   d. Hormones

3. The type of blood specimen which is basically the same form as the blood flowing

within the blood vessels is:

a. Plasma
b. Whole Blood
c. Serum
d. Both A and C

4. In an unclotted blood specimen that has been centrifuged, the middle layer which contains the WBCs and platelets is called the:

a. Clot
b. Buffy Coat
c. RBC layer
d. Plasma

5. In the systemic circulation, the type of blood that flows through the arteries is:

a. Oxygenated blood

b. Deoxygenated blood

c. Mixed blood

d. None of the above

6. The process of clotting is also known as:

a. Oxygenation

b. Centrifugation

c. Gas exchange

d. Coagulation

7. Which of the following statements is true?

a. Plasma is the fluid portion of a clotted blood sample

b. To obtain serum, blood must be collected in tubes with anticoagulant

c. Plasma is obtained from unclotted blood and contains fibrinogen

d. Serum contains fibrinogen and is obtained from a clotted blood sample

8. Which of the following types of blood specimen does not need to be centrifuged?

a. Plasma

b. Serum

c. Whole Blood

d. All of the above

9. Anticoagulants are chemicals which:

a. prevent clotting of blood

b. prevent oxygenation

c. promote clotting of blood

d. keep the blood sample cool

10. Which of the following types of blood specimen should be collected using a tube without anticoagulants?

a. Plasma

b. Serum

c. Whole Blood

d. All of the above

11. Which of the following specimen types requires a non-clotted blood specimen

to be collected?

a. Whole blood
b. Serum
c. Plasma
d. Both A and C

12. Hematology tests are typically collected using:

a. Plasma in a Citrate tube

b. Serum in a Gel Separator tube

c. Whole Blood in an EDTA tube

d. Plasma in a Heparin tube

References

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