Hemolysis in Venipuncture

Course Contents:

- Quiz

Course Objectives

Upon completion of the course, the student is expected to have gained knowledge in the following:

1. The basic structure and components of red blood cells
2. The significance of hemolysis as an error in blood collection
3. Effects of hemolysis on blood specimens
4. Common causes of hemolysis in venipuncture
5. Differences between *in vivo* and *in vitro* hemolysis
6. Methods for detecting hemolysis in blood specimens

Hemolysis: A Significant but Preventable Error

Hemolysis is defined as the destruction of red blood cells (RBCs or erythrocytes), resulting in the release of their cellular contents. The term comes from Latin words *hemo* (blood) and *lysis* (loosening). In the body, hemolysis is a normal process and is part of a constant cycle wherein old cells are destroyed and replaced by new cells. This maintains the normal population of circulating erythrocytes in the blood.

However, hemolysis can also occur when blood is collected from the body, such as in venipuncture procedures. When erythrocytes in a specimen are destroyed, their contents are released into the serum or plasma. This alters the chemical composition of the specimen and causes problems in testing for specific analytes (chemicals in the blood which are being measured). Collection errors which produce hemolyzed specimens are both undesirable and preventable.

The prevalence of hemolysis in blood collection is almost five times higher than that of other collection missteps, making it the most common reason for specimen rejection and recollection. (wan Azman, et. al.) Furthermore, over 95% of hemolysis incidents occur during specimen collection and transport, (Cao, Branzell, Vink) meaning almost all hemolyzed specimens are due to the collector's mistakes. Phlebotomists and other healthcare professionals who obtain blood

samples should therefore be aware of the causes and effects of hemolysis and must adopt best practices to avoid this complication.

Components of Blood

Blood has two components: plasma and the formed elements.

Plasma is the liquid which makes up the majority (55%) of blood and is referred to as the *fluid portion* of the blood. Plasma itself consists of approximately 90% water. The remaining 10% are substances dissolved in the plasma (such as gases, nutrients, minerals, and hormones) which are transported by the bloodstream. When isolated from whole blood, plasma normally has a pale-yellow color.

The *formed (cellular) elements* are the different cell types (red blood cells, white blood cells and platelets) which float within the plasma and make up 45% of the blood. Unlike plasma, which is a liquid, the cells are solid elements of the blood. The majority of the formed elements are the red blood cells (also called RBCs or erythrocytes). Their primary function is to carry oxygen within the bloodstream. Oxygenated (oxygen-rich) blood is essential for the nourishment of cells within the various body organ systems.

Within the bloodstream, these components remain mixed. Although plasma by itself has a pale-yellow color, blood as a whole appears dark-red due to all the red blood cells flowing within the plasma. The fact that blood has two portions becomes more evident when blood is collected into a tube, after which plasma can be separated from the cells via centrifugation. (Insert picture of separated plasma and cells in collection tube)

Plasma versus Serum

Both plasma and serum are liquid portions of blood, and both have a pale-yellow appearance when separated from the cells, though they have different chemical properties. The main difference between the two is that plasma is the liquid portion of *non-clotted blood*, while serum is the liquid portion *after blood has clotted*.

The common requirement for obtaining both serum and 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.

Red Blood Cells

- RBC Structure - In adults, all RBCs are produced within the bone marrow, where they undergo several stages of development. Once fully formed, they live in the bloodstream for approximately 120 days. Unlike most other cells, mature RBCs contain no nucleus or organelles (cell organs); instead, they only contain the protein *hemoglobin* (see below). RBCs have a *biconcave* shape, meaning the cell has the form of a flattened disc with an indentation on both sides at the center. Both their shape and the presence of hemoglobin contribute to the cells' normal properties and function. (Insert picture of RBC structure)
- Cell Deformability - As they are carried within the bloodstream, RBCs have to navigate blood vessels of varying size and withstand different pressure levels. Particularly when passing through tiny capillaries, RBCs exhibit the ability to deform (temporarily change their shape then regain their original biconcave configuration), allowing them to squeeze through passageways less than half their size. (Univ of Bristol) This property is attributed to the RBC's biconcave shape, its contents, (Huisjes, et. al.) and the flexibility of the cell membrane. (Li and Lykotrafitis) (Insert picture of RBCs passing through a capillary)
- RBC Membrane - The cell membrane is the outer covering of the RBC. It maintains cellular integrity and mediates interactions between the extracellular (outside the cell) environment and the cell's interior. As previously mentioned, its flexible properties aid the cell in passing through the circulation. The membrane is composed of a double layer of lipid (fat) molecules, within which specialized protein molecules are interspersed. These proteins perform different functions, including preservation of membrane stability and pliability, transportation of substances across the membrane, and providing a negative

surface charge (which keeps RBCs from sticking to each other or to the blood vessel wall). (Goldman) (Insert diagram of RBC membrane)

- Na^+- K^+ pump - One of the cell membrane proteins is an enzyme which is crucial for RBC integrity and physiology. The Na^+- K^+ pump actively transports ions (charged particles) through the membrane, facilitating their entry or exit from the cell. The ions involved are Sodium (Na^+) and Potassium (K^+), both of which have a *positive* charge. To transport these ions, the pump utilizes ATP (adenosine triphosphate, an energy-providing compound for cells) as an energy source. For each unit of ATP spent, 3 Na^+ ions are pumped *out* of the cell, while 2 K^+ ions are pumped *in*. (Pirahanchi, et. al.) (Insert diagram of Na^+- K^+ pump)

  The pump needs to constantly perform this function to assure that there are always higher concentrations of Na^+ *outside* the cell, higher concentrations of K^+ *inside* the cell, and as a result, a net *negative* charge within the cell itself (in part because there are more positive ions being pumped out than are pumped in). These conditions make it ideal for important cellular physiological functions to take place, promoting the overall health of the cell.

  Maintaining stable ion concentrations is also crucial to keeping the cell intact. Without the action of the Na^+- K^+ pump, Sodium and other ions would build up inside the cell. These ions attract water, which would enter the cell and increase intracellular volume. Too much fluid within the RBC would cause cell swelling and eventual rupture, effectively destroying the cell. By preserving the right levels of intracellular ions, the pump also regulates the cell's fluid volume, thus maintaining cellular integrity.

- Hemoglobin - The cytoplasm of RBCs mainly contains hemoglobin, an iron-based protein, which as a pigment is responsible for giving these cells (and blood as a whole) their typical red color. Hemoglobin is crucial to oxygen transport, which is the primary function of erythrocytes.

  The hemoglobin molecule contains 4 *heme* groups, each of which contain an iron atom. As the bloodstream passes through the lungs, each iron atom attracts and binds to an oxygen molecule. This allows RBCs to transport oxygen, removing it from the lungs and carrying it throughout the body via the bloodstream. (Insert diagram of hemoglobin structure)

- Hemoglobin Recycling - Old and damaged RBCs are removed from the circulation by the liver and spleen, where the unwanted cells are lysed (destroyed) and their contents recycled or eliminated. The efficiency of hemolysis in these organs prevents most of the freed hemoglobin from being released directly to the bloodstream. (Thiagarajan, et. al.) Iron from the heme groups is sent to the bone marrow, where hemoglobin is reconstituted within newly formed RBCs. Hemoglobin is also repurposed by the liver to make *bilirubin*, a chemical with a yellow-brown color. Bilirubin from the liver is mainly used and eliminated by the digestive system; a small portion is excreted by the kidneys and gives urine its yellow color. Other proteins from RBC breakdown are also recycled or eliminated.

Risk Factors for Hemolysis in Venipuncture

Unlike hemolysis which occurs *in vivo* (in the body, either as a normal process or as the result of disease), hemolysis which takes place *in vitro* (outside the body, i.e., in a collection tube) is primarily the result of human error. Actions which expose the fragile RBCs to excessive shear forces (i.e., exceeding the tolerance of their cell membranes) lead to cell destruction. Examples include incorrect equipment selection, flawed venipuncture technique, and improper specimen handling, storage, transport, and processing.

- Equipment Selection – Multi-sample needles or winged infusion 'butterfly' needles used in combination with evacuated tube systems are the common devices in standard venipuncture. Syringes are also utilized but much less frequently. Typically, a standard 21-gauge needle will suffice for most routine draws performed on a patient having easily accessible veins. Choosing a smaller needle (one with a higher gauge number) generally gives the collector easier venous access and causes less anxiety for the patient. However, a narrower needle opening exerts higher vacuum force on the RBCs during collection, increasing the risk of cell membrane rupture. Since 22-gauge needles and higher have been associated with a higher rate of hemolysis (Shah), butterfly needles should generally be reserved for pediatric draws, difficult veins, and special collections. While collectors should take patient's preference into consideration, they must also make a prudent decision on the

type of needle to use based on their assessment of the draw site and the collection requirements. (Insert pictures of ETS needle, and butterfly needle, vacutainer tubes, syringe)

- Site Selection – The preferable draw site for routine venipunctures is the *antecubital fossa*, the depression on the front of the elbow. It has superficial veins which provide easier access for blood draws and contains a layer of connective tissue that protects vital structures in the elbow. Drawing from a site other than the antecubital fossa has been associated with greater incidence of hemolysis. (Shah) Performing venipunctures on more difficult sites (such as the back of the hand) or access devices (such as peripheral IV catheters) should be reserved for those with appropriate training, supervision, and adequate experience. (Insert diagram of antecubital fossa)
- Suction Force - Evacuated tubes contain a vacuum which, upon tube activation, automatically suctions blood from the vein, with blood flow ceasing once the vacuum is used up. Larger tubes contain higher vacuum levels and exert greater suction force on the RBCs being collected, particularly when combined with a smaller needle. Hemolysis results when the cell membrane cannot tolerate the level of shear stress. Use of smaller tubes has been shown to decrease the incidence of hemolysis. (Phelan, et. al.)

  In syringe venipuncture, the collector has manual control over the amount of suction force being exerted. Pulling a syringe plunger too quickly creates excessive suction that could hemolyze cells.
- Tube Filling – Evacuated systems are designed to fill each tube with the adequate amount of blood once the vein is accessed. Collectors must ensure all tubes are filled completely to avoid causing hemolysis and other complications. As blood enters the tube, the initial portion of the specimen is suctioned into the tube at higher velocity, while the latter part is collected at a lower velocity. Higher suction speeds can cause RBCs to rupture. Thus, partially filled tubes result in a larger portion of the blood than normal being collected at higher velocity, thereby increasing the chances that the specimen will be hemolyzed (Neuwinger, et. al.).

  Collectors must also ensure all tubes are filled completely in order to satisfy minimum specimen volume testing requirements. Otherwise, collecting insufficient blood volume in a tube containing chemical additives can result in

an incorrect blood-to-additive ratio, possibly causing a specimen to be rejected. To avoid partially filled tubes when performing difficult blood draws, phlebotomists should consider using smaller tubes, provided that volume requirements are not compromised.

Maintaining good blood flow is crucial to filling tubes and depends largely on proper needle insertion and positioning, which are often reliant on the collector's skillfulness and experience level. If the patient has difficult veins and/or the phlebotomist lacks experience, the needle could either be inserted incorrectly or have excessive movement, leading to compromised blood flow. An improper technique whereby the needle is inserted too close to the inner vein wall causes slow tube filling. Hemolysis occurs in this situation due to blockage of the needle opening (aka lumen). (Shah) With proper training and experience, the collector is able to master correct needle insertion and stable needle positioning. (Insert diagram of needle lumen occluded by the vein's inner wall)

- Tube Inversion - Inversion facilitates the mixing of the blood with additive chemicals contained in the tube. For serum specimens, it *promotes* clotting. While for whole blood and plasma specimens, inversion *prevents* blood from clotting by adequately mixing the blood with anticoagulant chemicals. However, hemolysis occurs when specimens are mishandled (such as when tubes are excessively shaken). Proper inversion is accomplished by gently turning the tube upside down then right side up. The number of inversions required varies by tube type and by manufacturer requirements. (Insert diagram of proper tube inversion)
- Specimen Processing – Centrifugation separates the components of blood by spinning the specimen at a high rate of speed over a set time. This is done for both serum and plasma collection. When loading specimens, the operator must ensure that tubes are balanced in weight. During operation, if there is weight imbalance, the rotor may not spin properly, causing significant vibration, which in turn could damage the loaded specimens as well as the device itself. Relatedly, the operating parameters of speed, time, and temperature prescribed by laboratory protocol must be followed. Spinning a specimen at higher-than-prescribed speeds for a prolonged period can lead to

hemolysis. (Cadamuro, et. al.) (Insert diagram of balanced vs unbalanced centrifuge loading)

- Storage and Transport – Due to their fragility, RBCs are prone to rupture when they are not stored in ideal conditions or are mishandled during transport. Maintaining specimen tubes upright in racks or other appropriate holders facilitates clotting, minimizes agitation, and prevents dropping. Temperature also affects RBC integrity. When stored for up to 30 hours at 37°C (98.6°F) or lower, RBCs remain intact. Conversely, hemolysis occurs at increased storage temperatures, particularly beyond 45°C (113°F) where energy is sufficient to damage membrane proteins and enzymes. (Gershfeld and Murayama) When specimens are being transported, they must be handled gently to avoid hemolysis, as agitating or dropping a specimen tube could cause RBCs to rupture. This is true whether carrying specimens manually, transporting them in vehicles, or via automated systems. Although using rapid transport methods, like pneumatic tubes, facilitate quick delivery between departments (especially in large hospitals and laboratories), they have been shown to increase the frequency of hemolysis. (Kara, et. al.)

Detection of *In Vitro* Hemolysis

Visual Inspection

Typically, the first observable sign of hemolysis in a blood specimen is its appearance after being centrifuged. Once separated from the cells, the liquid portion of blood normally has a pale-yellow color. However, if the cells are lysed, hemoglobin is released and mixes with the serum or plasma. This changes the liquid's color to darker shades of yellow, pink, orange, or red depending on the severity of hemolysis (with a darker appearance implying the presence of more hemoglobin). A *hemolysis chart* is a visual reference to which the specimen's coloration can be compared. Images on the chart indicate the presence and severity of hemolysis as well as the hemoglobin content. (Insert picture of hemolysis chart)

Unfortunately, due to its dependence on the viewer's observational skill, experience and interpretation, gross examination is an inherently subjective method of detecting hemolysis. The lack of standardized measurement makes it prone to inaccuracy, particularly if the color change is very slight, if there are other

conditions affecting the specimen's appearance, or if the viewer fails to adequately inspect the specimen. Furthermore, hemolysis is particularly difficult to detect visually when the sample does not require centrifugation, such as in whole blood specimens. (Wilson, et. al.)

Chemical Analysis

While hemoglobin is the primary intracellular substance released by lysed RBCs, other cellular components leak into the serum or plasma when a specimen is hemolyzed. Quantitative measurement of these components in the laboratory is used to detect the presence and severity of hemolysis, as well as its effects on specific analytes.

- Hemoglobin – As previously discussed, hemoglobin is abundant in the RBC cytoplasm. It is responsible for blood's oxygen-carrying capacity and provides erythrocytes with their red color. The amount of hemoglobin released by damaged cells into the serum or plasma (called *Cell-Free Hemoglobin or CFH*) is a marker for hemolysis -- although the level of CFH measured in serum is less reliable (than that which is measured in plasma) since free hemoglobin increases during clotting. (Unger, et. al.) CFH levels are commonly determined via *spectrophotometry* (a tool for measuring chemical substances based on the absorption and reflection of light at different wavelengths). This method is viewed as the most accurate way of measuring hemolysis. (Van Buren, et. al.) Chemistry analyzers in the laboratory can perform an automated measure of hemolysis using the *Hemolysis Index* (HI), providing a standardized method for identifying hemolyzed specimens. (wan Azman, et. al.) HI results measure the degree of hemolysis (i.e., 1+, 2+, 3+). However, normal values vary among laboratories due to differences in the type of equipment, testing parameters and samples used. (Du, et. al.) (UCSF Health) The decision by laboratory staff on whether to reject a specimen or release appended reports of test results with hemolysis depends on hospital protocol. For example, a laboratory may routinely report 1+ hemolysis, whereas any result above 2+ renders the specimen unacceptable and requires the patient to be redrawn.
- Potassium - RBCs have higher intracellular concentrations of potassium ions (K^+) due to the Na^+- K^+ pump. When RBCs are hemolyzed, potassium is released

into serum or plasma, further increasing the overall amount of potassium in the specimen. When measured, the K^+ levels for these hemolyzed specimens are often found to be abnormally high (even though they are actually normal in the patient's blood) thereby producing an inaccurate result (i.e., false hyperkalemia). In cases where the patient has an abnormally low potassium level (hypokalemia), hemolysis during blood collection would increase K^+ in the specimen, producing a falsely normal result.

- Lactate Dehydrogenase (LDH) - LDH is an enzyme involved in cellular metabolism and is found in the cells of various organs, particularly the muscles, liver, and kidneys. Red blood cells contain moderate amounts of LDH (Farhana and Lappin), but upon hemolyzing they release the enzyme into the serum or plasma. Elevated LDH activity is associated with *in vitro* hemolysis (wan Azman, et. al.); however, it may also be caused by tissue damage due to injury, infection, or other disease processes, as well as by drugs and medication.
- Aspartate aminotransferase (AST) – AST is an enzyme found mainly in the liver, but also in muscles and other body organs. Erythrocytes use AST to synthesize glutathione, an antioxidant which repairs damage caused by toxic substances. (Ellinger, et. al.) Hemolysis causes AST levels to become mildly elevated. (Murakami and Shimizu)

An increase in the levels of Potassium, LDH, and AST in serum or plasma correlates with the amount of hemoglobin released from hemolysis. (wan Azman, et. al.) However, the levels of individual markers such as these can become abnormal due to other disease conditions. The laboratory can more accurately detect the presence, cause, and extent of hemolysis when there is correlation of several test results, ruling out of other causes, and a review of the patient's symptoms and medical history.

Detection of *In Vivo* Hemolysis

Abnormal hemolysis can occur within the liver and spleen as a result of various stressors and diseases, including splenic disorders, genetic defects, and immune hyperactivity. When hemolysis is excessive, it causes more RBCs to be destroyed than are being produced, leading to a decrease in RBC population. RBCs in the bloodstream can also be damaged and destroyed prematurely (*intravascular*

hemolysis) if they are exposed to forces which exceed cell membrane tolerance, or if disease processes cause the cell membrane to stop functioning properly. As a result, the membrane loses its integrity and hemolysis occurs. Severe injury, infection, genetic defects, immune hyperactivity, blood transfusion reactions, drugs, toxins, or foreign objects in the circulation can cause hemolysis of circulating RBCs. Hemoglobin and other intracellular contents are spilled directly into the plasma instead of being processed and recycled by the liver or spleen.

Although *in vivo* hemolysis is not a common cause of hemolyzed specimens, it is still important to detect so that any underlying disease processes can be diagnosed and treated accordingly. A variety of lab tests (including those discussed below) are available for this purpose.

- Cell-Free Hemoglobin - The higher concentration of hemoglobin released from hemolyzed cells in serum or plasma is viewed as a reliable marker for RBC injury. Assessment of CFH using the hemolysis index has become readily available, and has potential as an accurate, rapid, and inexpensive screening tool for intravascular hemolysis. (Lippi, et. al.)
- Haptoglobin - Haptoglobin is a protein made by the liver which binds to free hemoglobin in the bloodstream, such as that produced by hemolysis in the blood vessels. The liver clears haptoglobin-hemoglobin complexes from the circulation and eliminates them from the body. As a result, plasma haptoglobin levels decrease in the presence of *in vivo* hemolysis but remain unaffected in *in vitro* hemolysis. Haptoglobin is therefore useful in differentiating hemolysis caused by disease processes within the body from hemolysis caused by collection errors. (wan Azman, et. al.)
- Indirect Bilirubin - As mentioned previously, bilirubin is made from the hemoglobin released by RBCs which have been hemolyzed in the liver. Liver cells then secrete enzymes which convert bilirubin into a form (called *direct* bilirubin) which can be excreted from the body. However, when RBCs are hemolyzed prematurely in the blood vessels, hemoglobin is released directly into (and breaks down in) the plasma. The bilirubin produced in this case becomes bound to *albumin*, a plasma protein which performs regulatory and transport functions. Bilirubin in this form is called *indirect* bilirubin. Levels of indirect bilirubin become elevated due to *in vivo* hemolysis.

- Reticulocytes - In adults, all RBCs are formed in the bone marrow. Within this tissue are *stem cells* from which all mature blood cells originate. They undergo several stages of development until mature cells eventually emerge and are released into the bloodstream. Part of this process involves each cell losing its nucleus and organelles (cellular organs) as it matures. Towards the end of cell development, reticulocytes are immature RBCs which still have remnants of their nucleus present. Upon entering the bloodstream, they completely lose all their nuclear material and organelles, becoming mature erythrocytes within 1 to 2 days thereafter. Extensive hemolysis leads to decreased RBC numbers. This causes the bone marrow to compensate by increasing RBC production, resulting in higher numbers or reticulocytes released into the bloodstream. All modern hematology analyzers are capable of measuring reticulocyte levels. An elevated reticulocyte count is seen in *in vivo* hemolysis, whereas in *in vitro* hemolysis the reticulocyte count remains normal. (wan Azman, et. al.) (Insert diagram of RBC production in bone marrow)

As discussed previously, individual tests cannot exclusively be relied upon as markers for hemolysis. The determination of hemolysis and its cause are better made by correlating several tests, ruling out other causes, and a review of the patient's symptoms and medical history.

Conclusion

Accuracy in laboratory testing depends on a properly collected specimen. Hemolysis causes analytical interference due to the questionable reliability of test results as well as difficulty of interpretation. The false indication of hyperkalemia as a consequence of hemolysis is a prime example. However, hemolysis has also been found to cause interference in the chemical analysis of other tests as well, including phosphorus, creatine kinase, magnesium, iron, total protein, and albumin. (Yang, et. al.)

More importantly, hemolysis negatively impacts patient care. Upon determining that a specimen has been hemolyzed, the lab typically deems the specimen to be unreliable and requests that the specimen be recollected, resulting in the patient being inconvenienced by repeated blood draws and delayed test results. Proper

diagnosis, clinical decision-making, patient monitoring and treatment are all hampered.

The American Society for Clinical Pathology sets a hemolysis frequency of less than 2% as the benchmark of best practice. (Cao, Branzell, Vink) To meet this standard, phlebotomists and other professionals must continuously strive for improvement of their techniques. The abundance of hemolysis risk factors associated with venipuncture makes it imperative for proper methods and precautions to be emphasized in training and clinical practice.

Quiz

Choose the best answer:

1. Which of the following statements is true?

    a. New RBCs are formed in the liver and kidneys

    b. The average lifespan of RBCs is 60 days

    c. RBCs have a biconcave shape

    d. Mature RBCs have a nucleus and organelles

2. Once blood has clotted completely, the fluid portion is called:

    a. Plasma

    b. Serum

    c. Formed elements

    d. None of the above

3. Which of the following statements is false?

    a. Hemolysis is the most common reason for blood specimen rejection

b. Hemolysis mostly occurs during specimen collection and transport

c. Hemolysis only occurs during blood collection procedures

d. Hemolysis is the most common reason for blood specimen recollection

4. In the presence of *in vitro* hemolysis, all of the following substances in the specimen can become abnormally elevated, except:

a. LDH

b. Potassium

c. AST

d. Haptoglobin

5. Which of the following is less likely to cause hemolysis in a blood specimen?

a. Venipuncture using a gauge 23 needle

b. Performing gentle tube inversions

c. Quickly pulling back on a syringe plunger

d. Drawing blood from a peripheral IV catheter

6. Which of the following statements about the RBC membrane is true?

a. The RBC membrane contains fat and proteins

b. The RBC membrane maintains cell integrity

c. The RBC membrane contributes to the cell's deformability

d. All of the above

7. Which of the following procedures is more likely to cause hemolysis?

a. Storing blood specimen tubes upright at 37°C

b. Ensuring the weights of specimen tubes are balanced in a centrifuge

c. Using pneumatic tubes to transport specimen tubes

d. Filling smaller specimen tubes for difficult blood draws

8. Which of the following findings is *not* associated with *in vivo* hemolysis?

a. A normal reticulocyte count

b. Elevated levels of indirect bilirubin

c. Decreased levels of haptoglobin

d. Elevated levels of cell-free hemoglobin

9. A plasma specimen is hemolyzed due to mishandling. Which of the following can happen as a consequence?

a. The plasma has a reddish color

b. The level of potassium in the plasma is elevated

c. The specimen is rejected for testing

d. All of the above

10. Which of the following is the most likely cause of *in vitro* hemolysis?

a. Errors in blood collection

b. Infections

c. Drugs and toxins

d. Genetic defects

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