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Preanalytical Errors that Occur During Specimen Collection

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Specimen collection errors cost the average 400-bed hospital \$200,000/year in recollections and medication errors.¹

If you consider all the errors that can affect the result of a blood test, most of them occur between the time the order is placed and when the specimen is actually tested.^{2,3} In the first article of this series, we focused on the errors that can occur from the time the patient is addressed up to the point where the phlebotomist is ready to insert the needle into the vein or perform the skin puncture. The focus of this article will be those errors that occur during the actual performance of the venipuncture or skin puncture until the specimen is obtained. This list summarizes the errors we will be exploring in this article:

1. Hemolysis
2. Order of draw
3. Discard tube/volumes
4. Inversion
5. Underfilling
6. Wrong tube

Hemolysis

Hemolysis occurs when the red blood cells are ruptured and release hemoglobin into the serum or plasma.

Plasma—The liquid portion of the blood after centrifugation of a specimen in which an anticoagulant has prevented clot formation.

Serum—The liquid portion of the blood after centrifugation of a specimen that is allowed to clot (e.g., red-top tubes).

Because hemolysis cannot be detected until and unless the specimen is centrifuged, it is important to know the causes of hemolysis and to recognize the signs that a specimen may be hemolyzing during the collection procedure. Taking measures to prevent hemolysis can avert the delays in testing and reporting results should the laboratory reject the specimen and request a recollection. Many factors can hemolyze a specimen during collection including:

1. Improper needle placement;
2. Excessive pulling pressure on the plunger of the syringe;
3. Vigorous mixing of the specimen;
4. Small needle size;
5. Inappropriate blood:anticoagulant ratio due to underfilling;
6. “Milking” the site of a capillary puncture;
7. Premature or excessive centrifugation.

As a result, the following analytes can be reported falsely higher or lower than their actual concentration in the patient:^{2,3}

Increased	Decreased*
Ammonia AST ALT LDH Magnesium Phosphorous Potassium	Hct RBC

*In addition to these analytes, there is also a dilutional effect of hemolysis. When a specimen is hemolyzed, the liquid contents of the red blood cells is released into the serum/plasma.

To prevent hemolysis, collectors can adhere to the following practices:

1. Avoid slow draws that come from improperly positioned needles;
2. Refrain from pulling too hard on the plunger of a syringe;
3. Fill tubes to their stated capacity;
4. Gently invert tubes instead of vigorously mixing;
5. Only use 23-gauge needles or larger;
6. Pre-warm infant heels or the fingers of older children and adults when performing a skin puncture;
7. Avoid draws from vascular access devices (VADs) such as arterial lines, PICC lines and central venous catheters.

Avoid slow draws that come from improperly positioned needles

If the sample enters the tube or syringe slowly, it may be an indication that the needle is not properly within the vein. Partial needle insertion can cause the bevel of the needle to adhere to the uppermost interior wall of the vein, leaving only a minute opening through which the blood must pass when extraction pressure is applied. If the needle was inserted too deeply, the bevel of the needle may be imbedded in the lowermost wall of the vein, partially occluding the bevel with the same effect. When the blood has only a fraction of the bevel opening through which to pass, the extraction force exerts a tremendous pressure on the blood cells causing them to rupture as they slowly pass through the needle into the syringe or tube.

If the blood flow is slow, move the needle slightly within the vein in order to remove the bevel from the vein wall based on your perception of needle placement. Needle relocation should never be attempted when trying to access the basilic vein due to the close proximity of nerves and the brachial artery.⁴

Refrain from pulling too hard on the plunger of a syringe. If drawing with a syringe, excessive pulling pressure on the plunger may adhere the bevel to the uppermost interior wall of the vein. If this is suspected, release the pulling pressure on the plunger of the syringe momentarily, then resume with only light pressure.

Fill tubes to their stated capacity

If tubes are underfilled, the blood:anticoagulant ratio may result in hemolysis. Avoid underfilling tubes by having an ample supply of tubes of various capacities. In the event that the collection yields a lesser amount of blood than anticipated, filling tubes of lesser volumes assures that all specimens will be filled to their stated capacity, minimizing the hemolytic affect of a decreased blood:anticoagulant ratio.

Gently invert tubes instead of vigorously mixing

If tubes are vigorously shaken after collection, hemolysis can occur. Gentle inversions are adequate to mix specimens with any additive that may be in the tube.

Use only 23-gauge needles or larger

There is a limit to how small of an opening that blood cells can pass without rupturing from the turbulence that is concentrated at the opening. Needles with an opening gauge of 25 or smaller exert tremendous pressure on cells as they pass through. To prevent hemolysis and the delays in testing, reporting and physician response to the results, limit needle use to 23-gauge or larger. Although hemolysis-free specimens are possible using smaller gauge needles, the probability that the specimen will be hemolyzed increases considerably, and may force delays in diagnosis or treatment due to specimen rejection. If tested, hemolyzed specimens can result in inaccurate results.

Pre-warm infant heels or the fingers

Prewarming the sites of skin punctures increases the flow of blood through the capillary beds seven fold.⁵ Use a warm compress not to exceed 42°C for three to five minutes prior to performing a skin puncture. This decreases the time it takes to obtain a specimen and the necessity to excessively squeeze the site to force a blood flow. Since excessive squeezing of a puncture site hemolyzes specimens, pre-warming the site helps assure the specimen will be free of hemolysis, yielding a more accurate result and lessening the likelihood that the specimen will be rejected by the testing laboratory.

Avoid draws from VADs

Specimens drawn through vascular access devices by emergency room personnel are more frequently hemolyzed than specimens drawn by laboratory-based phlebotomists performing venipunctures.⁶⁻¹⁰ (A thorough discussion of the relationship between hemolysis and line draws is included in the article “Preanalytical Errors that Occur Before Collection,” available as a download from the Center for Phlebotomy Education’s web site, www.phlebotomy.com.)

Order of Draw

A great deal of material has been published, some incorrect, on the order in which tubes must be collected leading to substantial confusion. The order of draw is established to prevent the carryover of the blood:additive mixture from one tube to the next by the needle that pierces the stopper. The

following case study illustrates just one of the potential negative outcomes that can occur when tubes are filled in an incorrect order.

A physician orders pre-op blood work to make sure that the patient's potassium level is not too low for surgery, which could cause arrhythmia or cardiac arrest. Unbeknownst to the physician, the patient actually has a potassium level that disqualifies him as a candidate for surgery. The phlebotomist draws the tubes in the wrong order, filling the lavender-top tube before the serum tube. During the tube exchange, a minute amount of potassium-rich EDTA carries over from the lavender-top tube into the serum tube, falsely elevating the potassium in the serum tube. The laboratory tests the specimen, unaware that it has been cross-contaminated, and reports out a normal potassium level. The physician reviews the result and takes the patient to surgery. During the procedure, the patient goes into cardiac arrest and dies on the table.

This scenario is entirely possible. The contribution of potassium from one tube to the next can be significant.¹¹ The order of draw can also impact coagulation studies as well—for example, protime (PT) and partial thromboplastin time (PTT). If the needle transfers a minute amount of anticoagulant from a previous tube into a blue-top tube (used for coagulation studies), the introduction of the foreign anticoagulant may lengthen the protime result and make the patient appear to have a coagulation disorder or to be overmedicated.

It can also make an undermedicated patient appear well within therapeutic range. If a blue-top tube is drawn after a tube with a clot activator, the activator may affect the results of studies performed on the blue-top tube. Therefore, blue-top tubes should never be filled after a tube that contains an additive.

Besides cross-contamination of anticoagulants, violating the order of draw can contaminate blood culture collections. Because the tops of blood collection tubes are not sterile, needles that puncture them are capable of transporting any bacteria they collect from other stoppers into blood culture bottles. The result: the laboratory can report false-positive blood cultures on patients who are not septic. Such erroneous information can significantly lengthen a patient's stay and result in thousands of dollars of unnecessary tests and medication. Therefore, blood cultures must be collected before any other tubes are filled.

To prevent these and other scenarios that can affect how the physician treats and manages the patient, the order of draw as established by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) is the same for glass as for plastic tubes:⁴

Order of Draw

First	blood culture tubes or vials
Second	sodium citrate tubes (e.g., blue tops)
Third	serum tubes with or w/o gel or clot activator
Fourth	heparin tubes (e.g., green tops)
Fifth	EDTA tubes (e.g., lavender tops)
Sixth	oxalate/fluoride tubes (e.g., gray tops)

The order of draw is the same for vacuum-assisted draws as it is for filling tubes from a syringe. If only two or three tubes in the list above are to be collected, fill them as they fall within the recommended order.

Capillary Order of Draw

For skin punctures, a separate order of draw is required.⁵ Because skin punctures activate platelet adhesion and aggregation, the EDTA tube is the first tube filled during a fingerstick or heelstick. Other additive tubes follow the EDTA with the non-additive serum tube being last in the order.

	Capillary Order of Draw
First	EDTA (lavender)
Second	other additive tubes
Third	non-additive tubes

Discard tube

In the past, a discard tube was drawn prior to the filling of a citrate tube (blue-top) if the citrate tube was the first or only tube to be collected. The theory was that, during insertion, the needle may collect tissue thromboplastin and could contaminate a citrate tube with a non-circulating anticoagulant. However, tissue thromboplastin has never been shown to alter coagulation results. In fact, direct evidence that it has no effect on protime or aPTT results led NCCLS to rescind the recommendation in 1998 for those tests. Therefore, it is no longer necessary to draw a discard tube when the citrate tube for a prothrombin time or aPTT is the first or only tube being collected.^{12,13} However, no studies have been performed to prove or disprove the effect of tissue thromboplastin on special factor assays (e.g., Factor VII, Factor X, D-dimer, etc.) Therefore, CLSI recommends facilities establish their own policies and apply a discard tube as a precaution for special factor assays if preferred.

Discard tubes are required when drawing through a winged-collection set when the citrate tube is the first or only tube drawn to prevent underfilling. The empty tubing can contain over 0.5 mL of dead space. Therefore, if the blue-top tube is the first tube drawn, the vacuum of the tube will pull a volume of air equal to its dead space volume into the tube before any blood enters the tube. This results in the tube being underfilled. To prevent an erroneous results and a patient medication error based on it, a discard tube should be applied before the blue-top tube to “prime” the line. The discard tube---a plain, non-additive tube or another citrate tube---need not be filled, but applied only long enough to remove the air from the line of the winged blood collection set.

Inversion

Failure to invert tubes immediately after collection is a preanalytical error that can lead to specimen rejection and erroneous results due to the formation of micro clots. Giving specimens 5-10 inversions immediately after filling can prevent a recollection and the delays they bring to patient care.⁴ Particularly vulnerable are the small capillary tubes used for CBCs. If not mixed periodically during collection, they are almost certain to form clots and prompt recollection, or form undetectable clots that alter results and affect patient care.

Under-filled volume

The amount of anticoagulant manufacturers place in their tubes is calculated to provide the proper blood: anticoagulant ratio when completely filled. Short draws disrupt the physiology of the specimen and may affect results. Therefore, all tubes should be filled to their stated volume.

If not possible because of a difficult draw or because only a limited volume of blood has been obtained by syringe, smaller volume tubes that require less volume to achieve optimum filling should be on hand. Collectors who submit a tube that does not reach the stated fill volume put the patient at risk of being diagnosed, medicated and/or treated according to erroneous results.

The tube most sensitive to underfilling is the sodium citrate tube (blue top) used for coagulation studies. Any citrate tube filled less than 90 percent of its stated volume will yield falsely lengthened aPTT results and can result in the physician adjusting anticoagulant dosage to a degree that risks serious complications.¹⁴ Phlebotomists should carefully monitor the fill volumes of all tubes, but especially citrate tubes, to avoid the delays of recollection and/or the potential for patients to be medicated based on erroneous results. The following chart reports the effect on results of an underfilled EDTA tube.

	A <u>Proper Fill</u>	B <u>Underfilled (1 mL/5 mL tube)</u>	C <u>% change</u>
WBC	3.2	2.4	25
RBC	2.8	2.4	14
Hgb	10.1	8.6	15

Column A represents the results obtained in completely filled liquid EDTA tube. Column B represents the results of a 5 mL EDTA tube that contained less than 1 mL of blood. Note that, although the difference in the WBC, RBC, and Hgb may appear miniscule because of the low numbers, they constitute a significant change in percentage of the total (Column C).

It would be a mistake to attribute this affect on the dilution of the liquid EDTA. Underfilling a lyophilized (dry) EDTA tube results in erroneous results due to excessive anticoagulation. When the ratio of EDTA to blood is too high, the red cells tend to shrink. As a result, hematocrit, mean cell volume (MCV), and the mean corpuscular hemoglobin concentration (MCHC) will be affected. It is prudent, therefore, to submit for testing only tubes that have been filled with volumes that comply with the minimum fill volumes recommended by the tube manufacturer.

What is the minimum fill volumes on EDTA and heparinized tubes? Manufacturers make no recommendations. That's because tubes are manufactured with a carefully calibrated amount of additive in each to in order to prepare it for testing when completely filled. When tubes with additives are underfilled, the blood:additive ratio is not as the manufacturer has intended and accurate results cannot be guaranteed. Therefore, every tube should be filled to its stated volume. In order for a laboratory to accept underfilled tubes, rejection criteria must be established based on internal studies involving a wide variety of patients and ranges of results. It's easier to simply stock all collection trays with a wide variety of tube sizes. When prepared for draws that yield unexpectedly low volumes, smaller-volume tubes can be completely filled and submitted for testing.

Underfilled blood cultures

Organisms that cause septicemia can be in concentrations as low as one organism per milliliter of blood. Therefore, the more blood that is collected for a blood culture, the better the chances are of harvesting the causative organism of bacteremia. The optimum volume for adult patients is 20 mL of

blood evenly distributed between two bottles, not to exceed 10 mL each. Collecting 1 mL of blood for every year of life is the recommended blood culture volume for pediatrics.¹⁵

Collectors should be careful not to exceed the manufacturer's recommended fill of the culture vials since overfilling can cause some detection instrumentation to identify negative cultures as positive due to the interference of excessive white blood cells.

If a collection yields less than 20 mL of blood on an adult patient, evacuate up to the maximum recommended volume into the aerobic vial instead of dividing lesser amounts between two vials. (98% of all septicemias are a result of aerobic organisms or facultative anaerobes, i.e., anaerobic organisms that can tolerate aerobic environments). Therefore, if there is a problem with the venipuncture and the blood flow cannot be recovered before both bottles are filled, most of the causative organisms of septicemia will still be detected.

Wrong tube

Lithium heparin tubes

Although they look alike, phlebotomists should be aware that not all green-stopper tubes contain the same additive. Lithium heparin tubes and sodium heparin tubes can have the same color of stopper, but unless the phlebotomist reads the label, the patient could be at risk of being treated according to erroneous results. If drawing a lithium level on a patient, collectors should be sure that the tube being filled does not contain lithium heparin. If mistakenly filled and sent for testing, the result will likely show a falsely elevated of lithium in the patient. This is because the testing methods are unlikely to detect the difference between the lithium in the patient's blood and the lithium in the additive. As a result, the reported level can be as much as twice that of what was actually in the patient at the time of collection, moving the physician to reduce the patient's dosage.

Sodium heparin, however, does not contribute to the patient's reported sodium level to the same extent that lithium heparin contributes to lithium results. This is because sodium exists in much higher concentrations in the bloodstream and the contribution of sodium heparin to the result is likely to be clinically insignificant.

Gel serum separators

Gel-type serum separator tubes provide a simple and easy way to separate the serum or plasma from the red blood cells for easy access and storage. However, care should be exercised when drawing blood for therapeutic drug monitoring (TDM) into gel separator tubes, particularly when reduced sample volumes or prolonged storage may be required. The gel in some manufacturers' gel tubes interferes with the accurate analysis of some therapeutic drugs. Other tubes are inert. Therefore, CLSI highly recommends that facilities follow the manufacturer's restrictions when drawing therapeutic drug levels. CLSI also suggests that gel separator tubes should not be used for progesterone or tricyclic antidepressants.¹⁶

The Evils of Hemolysis

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When a specimen becomes hemolyzed during the collection process, it becomes evident only when the specimen is centrifuged and the serum is separated from the cellular components of blood. However, if the specimen collected is for a test for which separation is not necessary (e.g., CBC), hemolysis can go undetected and its effect on the results may never be known.

Because hemolysis is the rupturing of RBCs, gross hemolysis results in an RBC count that is falsely decreased for two reasons: 1) the physical destruction of cells and 2) the dilution effect that occurs when

the liquid content of the RBCs (hemoglobin) is released. For testing in which centrifugation is necessary (e.g., red-top tubes), hemolysis becomes evident by the red tinge it imparts to the serum. Not all hemolyzed specimens need to be re-collected, however, the necessity for which depends upon the test(s) that will be performed on the tinged serum.

Tests in which hemolysis falsely elevates results include potassium, LDH, AST, ALT, phosphorous, magnesium and ammonia. Values that are falsely lowered by hemolysis include RBC counts and hematocrit. For many tests, however, the interference of hemolysis is a function of the test methodology the testing facility is using to perform the assay. For example, one laboratory may employ a method of obtaining results for which hemolysis interferes, while another laboratory may employ a method immune from the interfering factor of free hemoglobin. For this reason, one should rely on the specimen rejection criteria established by the test methodology.

For specimens tested in facilities remote to the site of collection (i.e., long-term care facilities, physicians' offices without labs, etc.), healthcare professionals may not learn of the necessity for re-collection for a day or more. Often this creates hardships and impossibilities that cannot be overcome, underscoring the importance of utilizing good technique while collecting specimens to minimize the potential for this scenario.

References

- 1) Paxton, A. Stamping out Specimen Collection Errors. *CAP Today*. May, 1999.
- 2) Becan-McBride K. (ed) Preanalytical Phase an Important Requisite of Laboratory Testing. *Advance for Med. Lab. Prof.* Sept. 28, 1998:12-17.
- 3) Dale J. Preanalytic Variables in Laboratory Testing. *Lab Med.* 1998;29:540-545.
- 4) NCCLS. *Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture*. Approved Standard, H3-A5, Wayne, PA, 2003.
- 5) NCCLS. *Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens*. Approved Standard, H4-A5, Wayne, PA, 2004.
- 6) Kennedy C, Angermuller S, King R, Noviello S, Walker J, et al. A comparison of hemolysis rates using intravenous catheters versus venipuncture tubes for obtaining blood samples. *J Emerg Nurs* 1996 22(6):566-569.
- 7) Burns E, Yoshikawa N. Hemolysis in serum samples drawn by emergency department personnel versus laboratory phlebotomists. *Lab Med* 2002;5(33):378-80.
- 8) Grant M. The effect of blood drawing techniques and equipment on the hemolysis of ED laboratory blood samples. *J Emerg Nurs* 2003;29(2):116-21.
- 9) Cox S, Dages J, Jarjoura D, Hazelett S. Blood samples drawn from IV catheters have less hemolysis when 5-ml (vs 10-ml) collection tubes are used. *J Emerg Nurs* 2004;30(6):529-33.
- 10) Tenabe P. Letter to the editor. *J Emerg Nurs* 2004;30(2):106-8.
- 11) Calam R, Cooper M. Recommended "order of draw" for collecting blood specimens into additive-containing tubes. *Clin Chem.* 1982;28:1399.
- 12) NCCLS. *Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays; Approved Guideline*. H21-A4, Wayne, PA 2003.
- 13) Yawn, BP, Lodge C, Dale J. Prothrombin Time: One tube or two? *Am J Clin Pathol.* 1996; 105: 794-797.
- 14) Reneke J, Etzell J, Leslie S, Valerie L, Gotfried E. Prolonged Prothrombin Time and Activated Partial Thromboplastin time due to underfilled specimen tubes with 109mmol/L (3.2%) citrate anticoagulant. *Coag and Transf. Med.* 1997; 9, 6: 754-757.
- 15) Becan-McBride K, Garza D, *Phlebotomy Handbook*. Upper Saddle River, NJ: Prentice Hall; 2002.
- 16) NCCLS. *Procedures for the Handling and Processing of Blood Specimens*. Approved Standard, H18-A3 Wayne, PA, 2004.