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Preanalytical Errors that Occur After Specimen Collection

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Last reviewed for accuracy: 2/07

The more a procedure requires manual technique, the greater the potential for errors. Because laboratory technologists of decades past employed mostly manual methods to test blood, human error threatened the accuracy of test results through inaccurate reagent preparation, imprecise specimen and reagent pipetting, serum aliquot mix-ups, fluctuating temperature baths, and a multitude of other variables. Today, however, robotics and automation have eliminated many of these variables resulting in greater accuracy and precision in the results the modern laboratory reports.

However, one aspect of laboratory testing that cannot be automated is specimen collection and processing. This phase remains largely dependent upon specimen collection personnel adhering strictly to the established standards and principles of phlebotomy. Since the preanalytical phase is so complex and heavily dependent upon human variables, it comes as no surprise that up to 56% of all the errors that threaten the accuracy and precision of a blood test occur during the specimen collection and processing phases.[1,2] It has been estimated that specimen collection errors cost the average 400-bed hospital $200,000/year in recollections and medication errors.[3] Because errors that can occur during specimen collection and processing are staggering and complex, the preanalytical phase is the most difficult to control, especially at a time when phlebotomy responsibilities have been deployed to healthcare personnel outside of the laboratory. The challenge to today's laboratory managers and phlebotomy supervisors is to continually teach and enforce sound blood collection and handling practices in order to minimize the impact of the preanalytical phase on test results and, ultimately, patient management.

In this, the final article in our series in preanalytical errors, we will review the errors that phlebotomists can commit *after* the specimen is drawn and before it is tested that can alter the results and present a picture of the patient's health to the physician that is much different than the reality.

Errors Committed After Collection

The following is a partial list of the errors that can occur during specimen transportation and processing that can alter results. This is by no mean an all-inclusive list, but rather a list of those errors that occur most frequently, those that are easiest for collectors to correct and those that can have the greatest impact on the quality of care the patient receives.

- Delays in transportation and processing
  - Analyte instability
  - Bilirubin deterioration
- Specimen Processing Errors
  - Rimming clots

Improper centrifugation
Respinning gel separator tubes
- Specimen Storage after testing
  Gel-tube interference on therapeutic drugs

Specimen Transportation

Specimens that are drawn near to the testing station should be processed and delivered as soon after the collection as possible. Since near-patient testing requires little transportation time, specimens can be transported at room temperature unless the test requires immediate chilling of the specimen (e.g., ammonia, renin, etc.) In these cases, having a cooled transport block or cup of ice or ice slurry at the point of collection is necessary to preserve analytes that decay rapidly after collection. Most tests, however, do not require special transportation conditions in near-testing facilities. Specimens that will be tested for bilirubin, however, should be protected from light. (See "Bilirubin Deterioration" below.)

Testing stations that are distant to the point of collection require consideration for temporary storage and transportation conditions that preserve the analytes to be tested. (Temporary storage conditions will be discussed later under "Delays in Processing.") Transporting specimens from the drawing station to the remote testing facility must focus on two important considerations:

1. adherence to state and federal transportation regulations regarding biohazardous materials;
2. conditions that preserve the specimens and the analytes to be tested.

State and Federal Transportation Regulations

Specimens drawn at locations remote to the testing facilities, such as physician's offices, outpatient drawing stations and public health departments are usually transported to the testing facility by personnel from the testing facility or couriers contracted from the testing facility. In most cases, the responsibility of complying with the applicable state and federal regulations rests with whoever arranges or provides transportation. In the US, those who cause specimens to be transported by public carrier are to follow the US Department of Transportation's Dangerous Goods Shipping Regulations.[4] It is beyond the scope of this article to discuss federal or state transportation regulations. However, readers can refer to document 49 CFR (Title 49 of the Code of Federal Regulations) for more information.

In order to preserve analyte stability during transportation to remote testing facilities, those who collect and process specimens must take into consideration the analyte being tested and its stability over time. Of primary importance for chemistry tests is the separation of serum or plasma from the cells within two hours of collection.[5] Of primary importance for coagulation tests is to assure that the specimen is transported and tested within four hours if an activated partial thromboplastin time (aPTT) is to be performed on the specimen.[6] Since the affects of time and temperature on analyte stability also apply to near-testing collections, a thorough discussion of transportation and storage conditions follows.

Delays in processing

As soon as a blood specimen is removed from the circulatory system, significant and irreversible changes begin to take place. Additives that manufacturers put into collection tubes are developed to preserve the concentration of the analytes to be tested. Some work to prevent clotting, some work to hasten clotting, but all are dedicated to preserving the blood so that when it is tested, the result reported is as close as technology can get to the concentration of that analyte in the patient. In a sense, from the moment the specimen enters the tube, a race against time begins to test analytes before they no longer represent the patient's status. Specimen collection personnel play a major role in winning that race.

Analyte stability:

As long as serum or plasma remain in contact with red blood cells, significant and irreversible changes take place for many analytes.[5,6,7] Some of these changes are accelerated by refrigeration. To prevent these alterations, all tubes for chemistry testing should be centrifuged as soon as possible after collection and the serum/plasma removed from contact with the cells. According to the Clinical and Laboratory Standards Institute, (CLSI, formerly NCCLS), unless evidence exists that longer contact times do not contribute to the inaccuracy of the result, serum or plasma should be separated from the cells within two hours. Although some analytes are stable in contact with the cells for up to 72 hours at room temperature, many analytes, including the following, are not: [1,7]

albumin	glucose
ALT	HDL cholesterol
AST	iron
calcium (ionized)	LDH
chloride	potassium
CO_2	phosphorous
creatinine	total protein
folate	Vitamin B12

If these analytes are scheduled to be tested, or if there is a potential for them to be tested during the storage of the specimen, the serum/plasma should be removed from the cells within two hours of collection. This can be accomplished by collecting the specimen into a gel separator tube and centrifuging the tube within two hours, or by physically removing the serum/plasma from the tube within two hours and transferring it to a properly labeled transfer tube with a fitted cap to prevent spilling. According to CLSI, once the serum/plasma is removed, it should be kept at room temperature no longer than 8 hours without refrigeration and no longer than 48 hours without freezing in order to preserve the concentration of the analytes.

For complete blood counts (CBCs), EDTA tubes can be refrigerated up to 24 hours without affecting cell counts. For sed rates, unrefrigerated EDTA specimens should be tested within 4 hours, or 12 hours if the specimens are refrigerated.[8] Reticulocyte counts drawn into EDTA tubes are considered stabile for up to 6 hours at room temperature, 72 hours if kept refrigerated.[9]

Sodium citrate tubes for coagulation studies can remain at room temperature after collection. aPTT should be tested within four hours.[6] If the patient is heparinized, specimens for aPTT testing should be centrifuged and the plasma removed from the cells within one hour after collection. aPTT results obtained more than 4 hours after collection cannot be considered reliable. Protimes, however, are more forgiving; they are stable for up to 24 hours at room temperature, even uncentrifuged.

This information must be applied when those who process specimens receive calls from physicians or those who relay their orders to add tests to previously drawn specimens. *It is not possible to accommodate all requests to add on tests.* For example, if a lavender-top tube was drawn on a hospitalized patient during the morning draw for a CBC, and a request to add a reticulocyte count is received later that afternoon, the individual who handles the request should not assume that it can be done. If the tube has set out for more than six hours at room temperature, as is the case in many laboratories, a reticulocyte count cannot be accurately performed. In such cases, a fresh specimen must be collected. Failure to apply the knowledge of analyte stability when additional tests are requested for previously drawn specimens puts the patient at risk of being treated or diagnosed according to an incorrect result.

The phlebotomist or specimen processor is often the patient's last line of defense against inaccurate results and their consequences.

Adhering to the principles of analyte stability and recollecting specimens when appropriate protects patients from being treated, diagnosed and/or managed according to inaccurate laboratory results.

Bilirubin deterioration

Light is deadly to some analytes, especially bilirubin, which can deteriorate up to 50 percent after only one hour of exposure.[10] To prevent this, specimens to be tested for bilirubin should be protected from light immediately after they are collected and especially after centrifugation. For newborns, bilirubin levels can be preserved in amber, light-blocking capillary collection tubes. For specimens drawn by venipuncture, protection from light immediately after collection can be accomplished by wrapping the specimen in aluminum foil or by encasing the tube in a light-tight canister.

Specimen Processing Errors

Rimming clots

Occasionally, specimens emerge from centrifugation with clotted serum. This presents a problem to the individual processing the specimen and preparing it for delivery to the testing facility. Often, preparing a specimen with clotted serum for testing requires the processor to "rim" the clot. Rimming is accomplished by removing the stopper of the tube, inserting wood applicator sticks and removing the fibrin clot by wrapping it around the stick(s) and removing it physically. Not only does this expose the processor to bloodborne pathogens, but also risks hemolyzing the specimen.

Clotted serum often occurs when the specimen has not been allowed enough time to clot prior to centrifugation. Complete clotting can take up to 30 minutes. Collectors who find themselves rimming clots should allow more time for specimens to clot before spinning. A quick inversion of serum tubes as a check for clotting is not an effective means of assuring that complete clotting has occurred.

Improper centrifugation

Test results can also be altered if specimens are not centrifuged properly. Serum or plasma must be free of red blood cells and platelets within two hours after collection. If tubes are not spun long enough or fast enough, these cells can remain in the serum or plasma without being visible and radically alter results prior to testing. The following actual case study underscores the effect that improper centrifugation can have on test results and patient management.

Case Study

A 58-year old Caucasian male diagnosed with a myeloproliferative disorder was drawn in his physician's office for a basic metabolic profile. The patient's potassium, tested on a Vitros 500, produced a reproducible result of 7.6 mEq/L. The physician was notified of the level immediately, called the patient at home and instructed him to go directly to the hospital laboratory for recollection and repeat testing. Six hours after the patient was initially tested, a second specimen was drawn by laboratory personnel and subsequently tested for potassium. The second collection yielded a result of 5.4 mEq/L. Neither specimen was hemolyzed.

At issue is the significant difference between the potassium levels of the same patient drawn in the physician's office, then transported to the laboratory versus the potassium level drawn by hospital laboratory personnel and tested immediately. The collection tubes filled in both cases were in-dated gel tubes with a clot activator; both draws were performed in the antecubital fossa without difficulty; both specimens were tested at the same laboratory on the same instrument by the same technical personnel. The specimen drawn and centrifuged at the physician's office was transported to the laboratory by a laboratory courier in a cooled and insulated carrier bag according to proper procedure.

There were four variables within the two specimens: 1) the personnel who drew the specimen (physician's office staff versus hospital laboratory staff), 2) the first specimen had to be transported from the physician's office to the testing facility whereas the second draw took place at the testing facility; 3) the specimen from the office draw was centrifuged in a fixed-angle centrifuge while the specimen drawn in the laboratory was centrifuged in a swivel-head centrifuge; 4) the passing of six hours between draws.

In order to explain the discrepancy, those preanalytical errors known to produce inaccurate potassium levels were investigated. Errors committed during the collection that can affect potassium levels include the following:

- hemoconcentration due to excessive tourniquet time (longer than one minute);
- drawing specimens in an incorrect order of draw (EDTA before a tube to be tested for potassium);
- having the patient pump his fist prior to the puncture;

- the drawing of blood from a site prepped with povidone iodine.
- prolonged serum/cell contact (greater than 2 hours before centrifugation/separation);
- refrigeration of uncentrifuged gel tubes;
- inadequate gel interface
- inadequate centrifugation

Although the nurse who drew the first specimen in the physician's office was not available to question as to the techniques employed in regards to tourniquet time, order of draw, the pumping of the patient's fist, the physician attested that proper technique respective to these factors is employed routinely. Without being able to completely rule out collection variables, the laboratory was left to focus on processing and transportation variables that could have contributed to the spurious results.

Red blood cells have 25 times as much potassium as serum. Hence, as is well known, serum potassium levels can rise significantly when serum remains in contact with red cells, a process that is accelerated at refrigerated temperatures. CLSI states that specimens to be tested for potassium and other analytes sensitive to red cell exposure should be centrifuged and separated from the cells within two hours.

Conversations with the physician ruled out any delays in the centrifugation of the office-drawn specimen as well any refrigeration prior to centrifugation. The gel interface created by centrifugation in the office's fixed-angle centrifuge, however, was angled so that the interface was distributed disproportionately, leaving a thick interface on one side of the tube and a very thin interface on the other. Cell exposure to the serum could not be ruled out. However, it was thought that any exposure was unlikely to be attributable to the degree of discrepancy between the two results.

One variable remained that could not be discounted: the speed and duration of centrifugation. Platelets release potassium during the clotting process. If platelets remain in the serum in high concentrations due to inadequate centrifugation, they can contribute a significant amount of potassium to the measured result. The World Health Organization's recommendations are to spin plasma gel tubes at 2000-3000 relative centrifugal force (rcf) for 10 minutes to position platelets fully beneath the gel interface.[5] (rcf=G force =1118 x r(rpm/1000)^2 where *r* = the distance between the axis of rotation and the base of the container in millimeters.)

Excessive rcf can traumatize platelets and RBCs and also cause elevated potassium levels. One way to avoid the contribution of potassium from platelets is to draw potassium levels in heparinized gel tubes. However, because platelets drawn in heparinized tubes are not bound up in the clot, centrifugation at a higher rcf than serum gel tubes is required to yield platelet-free plasma. The World Health Organization recommendation for centrifuging serum gel tubes is 10 minutes at 1500 rcf.

Although the centrifuge at the testing facility produced an evenly dispersed gel interface (by virtue of its swivel head feature) and achieved the rcf recommended by the gel tube manufacturer for optimum separation, the centrifuge at the physician's office generated a rcf thought to be significantly less. The presence of platelets in the serum drawn in the physician's office, therefore, could have contributed to the discrepant results.

Because the patient's myeloproliferative disease resulted in platelet counts as high as 984,000/uL, this increases the likelihood that platelets released significant amounts of potassium during the clotting process as well as contaminated the serum of the inadequately centrifuged specimen resulting in the patient's pseudohyperkalemia.

In summary, although deviations from accepted standards of collection in the physician's office could not be completely ruled out as contributors to this patient's spurious results, inadequate centrifugation of the initial specimen is more likely to have rendered platelet-rich serum, which released significant concentrations of potassium during transportation. Facilities who experience similar spurious results should consider drawing potassium levels on their patients into heparinized tubes to avoid the release of potassium from platelets that occurs during clotting and to centrifuge specimens according to tube-manufacturer's specification, keeping in mind that heparinized tubes require a greater rcf than serum tubes.

As you can see, proper centrifugation is critical to accurate chemistry results. Those who process specimens should adhere to the centrifugation requirements of the tube manufacturer.

Respinning tubes

Occasionally, additional serum/plasma from a serum tube will be required than what was originally obtained during centrifugation. Be aware, however, that spinning tubes (with or without a gel separator) a second time in an attempt to harvest more serum may not be a good idea.[5] If the original tube has been refrigerated since centrifugation, or more than several hours have passed since collection, the serum from a second spin will be significantly different in the concentration of some analytes than the serum of the first spin because of prolonged contact with cells. Respinning stored specimens, even those stored only a couple hours,

yields an altered specimen. Combining the serum from the first and second spin creates a hybrid serum sample that may not represent the patient's actual condition.

As mentioned above, not all analytes are affected by contact with the cells, but phlebotomists and specimen processors must have a working knowledge of those analytes that are affected and avoid spinning serum tubes a second time.

Specimen Storage after testing

Plunger-type serum separators

Some facilities separate serum from cells with plunger-type serum separators. Although such devices are a convenient way to accomplish separation, users should be cautioned about storing serum in these devices. Since they may not provide an adequate barrier for storage, CLSI suggests that if you are using the plunger-type serum separators, serum/plasma is to be removed from the device immediately unless documentation states otherwise. The problem is that these separators do not provide an adequate barrier from the cells over time. Facilities that use these devices can, however, accomplish adequate separation by pulling the plunger up slightly in the tube to create an air barrier between the bottom of the plunger-type serum separator and the cells.

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Analyte stability before centrifugation and separation from cells

(See next page for stabilities *after* centrifugation and separation from cells)

ANALYTE	ROOM TEMP	REFRIGERATED
ALBUMIN	AT LEAST 6 HOURS[1]	
ALT	24 HOURS[1]	
AST	24 HOURS[1]	
BICARBONATE	6 HOURS[1]	7 DAYS[4]
BILIRUBIN	3 DAYS[4]	
CALCIUM (ionized)	2 HOURS[1]	
CHLORIDE	6 HOURS[1]	
CREATININE	24 HOURS[1]	
GLUCOSE	2 HOURS[1]	< 2 HOURS[1]
GLUCOSE (in fluoride tube)	1 WEEK[4]	1 WEEK[4]
HDL CHOLESTEROL	6 HOURS[1,2]	
IRON	8 HOURS[1]	
LDH	2 HOURS[1]	
MAGNESIUM (ionized)	6 HOURS[1]	5 DAYS[1]
PHOSPHOROUS	3 HOURS[1,2]	
POTASSIUM	2 HOURS[1,3]	< 2 HOURS[1]
PROTHROMBIN TIME	24 HOURS[5]	7 HOURS[3]
aPTT	4 HOURS[5]	4 HOURS
aPTT (patient on unfractionated heparin)	1 HOUR[5]	1 HOUR[5]
SODIUM	7 DAYS[4]	1 DAY[3,4]
TOTAL PROTEIN	48 HOURS[1]	
TRICYCLIC ANTIDEPRESSANTS	N/A	6 DAYS[1]

Stability of whole blood analytes

TEST	ROOM TEMP	REFRIGERATED
WBC (normal or elevated counts)	3 DAYS TO 1 WEEK[8]	1 WEEK[4]
WBC (low counts)	<1 DAY[8]	1 WEEK[4]
AUTOMATED DIFFERENTIAL	<1 DAY[8]	N/A
RBC	AT LEAST 1 WEEK[4,8]	AT LEAST 1 WEEK[4]
HGB	AT LEAST 1 WEEK[4,8]	AT LEAST 1 WEEK[4]
HCT	1-2 DAYS[4,8]	5 DAYS[4]
PLT	<1 DAY[4] AT LEAST 4 DAYS[8]	AT LEAST 24 HOURS[4]
MCV	1-2 DAYS[4,8]	AT LEAST 24 HOURS[4]
MCH	AT LEAST 1 WEEK[8]	N/A
SED RATE	4 HOURS[6]	12 HOURS[6]
RETIC	6 HOURS[7]	72 HOURS[7]

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Analyte stability after separation from cells[4]

ANALYTE	ROOM TEMP	REFRIGERATED
ALT	1 DAY	3 DAYS
ALK PHOS	4 HRS	N/A
AMYLASE	1 WEEK	2 MONTHS
AST	3 DAYS	1 WEEK
CALCIUM	7 DAYS	10 DAYS
BILIRUBIN	2 DAYS	7 DAYS
FE/TIBC	N/A	1 WEEK
FREE T4	1 WEEK	N/A
GGT	1 MONTH	1 MONTH
HCG	N/A	4 DAYS
LDH	2-3 DAYS	N/A
TSH	N/A	4 DAYS
T3	2 WEEKS	N/A
URIC	3 DAYS	7 DAYS

Note: Some facilities may have conducted studies that demonstrate longer stabilities. List your own facility's exceptions below: