Preanalytical Variables in the Chemistry Laboratory

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Abstract: There are many factors that contribute to accurate test results in the chemistry laboratory. These factors can be broken down into three areas: preanalytical, analytical and post analytical. Preanalytical variables account for 32-75% of laboratory errors, and encompass the time from when the test is ordered by the physician until the sample is ready for analysis. The focus of this article will be preanalytical variables that can occur during a venipuncture and specimen processing and how they relate to testing in the clinical chemistry laboratory.

Scenario: A patient has been in the cardiac intensive care unit for 3 days. For the past 2 mornings, he has had his cardiac enzymes drawn into a BD SST™ tube to monitor his condition since his heart attack. On this particular morning, his tube of blood is drawn and sent to the clinical chemistry lab for analysis. However, when the tube is processed and ready for analysis, the technologist running the chemistry analyzer notices that the specimen is very gelatinous and will need to be re-processed before the sample can be run on the analyzer. What could have happened to the quality of this specimen?

There are many variables that can contribute to the quality of a chemistry specimen. This article will investigate the variables that may have contributed to the gelatinous specimen in the case of the cardiac patient, as well as the other variables that are important to specimen quality. The focus will be on the preanalytical phase of the blood collection and sample handling, up until the time that the sample is to be run on the chemistry instrument.

Following the above BD SST™ tube from time of collection until it is ready for analysis, the preanalytical variables that can contribute to the quality of the sample are as follows:

Patient Identification: It is important to identify a patient properly so that blood is being collected from the correct person. Drawing blood from the wrong person, or labeling the correct patient’s sample...
with a different patient’s label can certainly contribute to laboratory error. Perhaps in the opening scenario, the patient in the next bed, with an extremely prolonged clotting time, was drawn and labeled as the cardiac patient.

When identifying the patient, have them provide their full name, address, identification number and/or date of birth. Hospital inpatients should be wearing an identification band with the above information, which the phlebotomist should confirm before the venipuncture. Blood should not be drawn from a patient without a band. A nurse, physician, relative or guardian should identify patients that are unable to speak or identify themselves.

Patient Preparation: Prior to collecting specimens for chemistry, certain patient variables need to be considered. For certain chemistry analytes, such as glucose and cholesterol, patients need to be fasting (absence of food and liquids) for at least 12 hours prior to venipuncture. Other analytes, such as cortisol and adrenocorticotropic, have diurnal variations, where the analyte is at its highest level in the morning, and the levels gradually decrease during the course of the day.

Selecting the Site: Selecting the appropriate site for venipuncture can contribute to a better quality sample. The preferred site is the median cubital vein. This vein is usually the easiest to access. Generally, there is less need to probe to find the vein, which in turn should cause less trauma during the venipuncture. This will usually be the most comfortable for the patient. If the median cubital vein cannot be used, the next choice would be the cephalic vein. The last vein to consider for venipuncture is the basilic vein. This vein is in close proximity to the median nerve and brachial artery, and extreme caution must be used so that only the basilic vein is being punctured.

Site Preparation: Prior to venipuncture, the site should be cleansed with alcohol. Cleansing starts at the center of the vein, and should continue outward in concentric circles. Before performing the venipuncture, the alcohol should be allowed to air dry. This will help to ensure that the specimen is not contaminated with alcohol, as this can lead to hemolysis. Hemolysis can result in the spurious elevation of such analytes as potassium, lactate

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dehydrogenase (LD), iron and magnesium in the chemistry lab. Allowing the alcohol to dry completely will also cause less burning and pain to the patient.

**Tourniquet Application and Time:**
The tourniquet should be applied approximately three to four inches above the venipuncture site. The tourniquet should be on the arm no longer than one minute. A good rule of thumb to determine the one-minute tourniquet time is to remove the tourniquet when blood starts to flow into the first tube of blood being drawn. Prolonged tourniquet time can lead to an increase in various chemistry analytes, including serum protein, potassium and lactic acid due to hemoconcentration of blood at the puncture site.

**Proper Venipuncture Technique:** During phlebotomy, avoid probing to find the vein and achieve blood flow. Excessive probing and/or “fishing” to find a vein can result in a poor quality sample, including hemolysis. As mentioned previously, hemolysis can affect several chemistry analytes.

**Order of Draw:** Following the correct order of draw during venipuncture will help to ensure accurate test results.
The BD and CLSI (Clinical and Laboratory Standards Institute, formerly NCCLS) recommended order of draw for evacuated blood collection tubes is as follows:

An example of improper order of draw that can lead to an incorrect chemistry result is drawing an EDTA tube prior to an BD SST™ or heparin tube for chemistry testing. The potential cross contamination of K2 or K3 EDTA on the needle from the lavender top tube to the chemistry tube can lead to an elevated potassium result. This in turn can require a recollection of the sample, or possible misdiagnosis or treatment of the patient.

**Order of Draw for Multiple Tube Collections**
Reflects change in NCCLS recommended Order of Draw (NCCLS H3-A5, Vol 23, No 32, 8.10.2)

<table>
<thead>
<tr>
<th>Closure Color</th>
<th>Collection Tube</th>
<th>Mix by Inverting</th>
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</thead>
<tbody>
<tr>
<td>BD Vacutainer® Blood Collection Tubes (glass or plastic)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Blood Cultures - SPS</td>
<td><strong>8 to 10 times</strong></td>
<td></td>
</tr>
<tr>
<td>• Citrate Tube*</td>
<td><strong>3 to 4 times</strong></td>
<td></td>
</tr>
<tr>
<td>or</td>
<td></td>
<td></td>
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<tr>
<td>• BD Vacutainer® SST™ Gel Separator Tube</td>
<td><strong>5 times</strong></td>
<td></td>
</tr>
</tbody>
</table>
| • Serum Tube (glass or plastic) | **5 times (plastic)**
| none (glass) |
| or | |
| • Heparin Tube | **8 to 10 times** |
| or | |
| • BD Vacutainer® PST™ Gel Separator Tube With Heparin | **8 to 10 times** |
| or | |
| • EDTA Tube | **8 to 10 times** |
| or | |
| • Fluoride (glucose) Tube | **8 to 10 times** |

*When using a winged blood collection set for venipuncture and a coagulation (citrate) tube is the first specimen tube to be drawn, a discard tube should be drawn first. The discard tube must be used to fill the blood collection set tubing’s “dead space” with blood but the discard tube does not need to be completely filled. This important step will ensure maintenance of the proper blood-to-additive ratio of the blood specimen. The discard tube should be a nonadditive or coagulation tube.

**Proper Tube Mixing:** All tubes with additives need to be inverted to mix the additive evenly with the blood. Plastic serum tubes and BD SST™ tubes contain clot activator and should be inverted 5 times to mix the activator with the blood and help the specimen clot completely. In the opening case study, improper mixing of the tube after venipuncture could have contributed to the gelatinous serum sample that was seen in the laboratory. Other additive tubes, such as heparin, need to be inverted 8-10 times to mix the anticoagulant with the blood and prevent clotting. Be sure that tubes are not being shaken vigorously, as this can lead to a hemolyzed sample.

**Proper Tube Handling and Specimen Processing:** Once the blood collection tubes have been drawn in the correct order, to the proper fill volume and mixed thoroughly, the next step toward accurate test results is processing the tubes properly. This section will look at serum and plasma tubes separately, as both specimen types have their own special handling requirements.

**Serum Samples**
Serum specimens, namely red top tubes and BD SST™ gel tubes, need to clot completely prior to centrifugation and processing. Blood specimens in red top tubes should clot for 45 to 60 minutes, and those in BD SST™ tubes should be allowed to clot for 30 minutes to ensure complete
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collection in order to maintain the stability of the analyte. A slurry of ice and water is recommended for chilling the tubes of blood. Examples of specimens that need to be chilled or transported on ice include adrenocorticotropic hormone (ACTH), angiotensin converting enzyme (ACE), acetone, ammonia, catecholamines, free fatty acids, lactic acid, pyruvate and renin.

Other analytes are photo-sensitive, and need to be protected from light in order to remain stable and to ensure that the laboratory reports an accurate result. This can be done by wrapping the tube of blood in aluminum foil. The most common example of a light-sensitive analyte is bilirubin. Other chemistry analytes that need to be light-protected include beta-carotene and erythrocyte protoporphyrin.

Stability for Whole Blood, Serum and Plasma: A whole blood specimen that is going to be spun down should be centrifuged and the serum or plasma removed from the red blood cells within two hours after the venipuncture. Once the serum has been removed or separated from the red blood cells (in the case of a gel barrier tube), the sample will be stable at room temperature for eight hours, and up to 48 hours at 2-4 degrees C. After 48 hours, the serum specimen should be frozen at –20 degrees C in an aliquot tube.

Paying close attention to the preanalytical variables associated with blood collection will help to ensure accurate test results in the chemistry department, as well as all areas of the clinical laboratory. As was evident from the opening case study, there are often several variables that can potentially contribute to erroneous test results. Our cardiac patient’s blood could have been drawn from the wrong patient, had improper tube handling or his blood may have not clotted long enough. Therefore, it is important to remember that a better quality sample during the preanalytical phase of blood collection will yield a better test result.

References
4. BD Evacutainer Blood Collection System Package Insert 6/2/04

Clot formation. Blood from patients who are receiving anticoagulant therapy, such as heparin or coumadin, may take longer to clot. Tubes should be allowed to clot at room temperature, upright in a test tube rack, with the closures on the tubes. In the gelatinous sample that was presented at the beginning of this article, perhaps the blood was not clotted completely prior to centrifugation because a cardiac patient is often heparanized. Spinning the tube too soon may result in a gelatinous and/or fibrinous serum sample that will require respining.

Plasma Samples
Blood specimens collected in plasma tubes, such as the plain heparinized green top tubes and the BD PST™ tubes with heparin and gel do not require clotting prior to centrifugation. This allows the tube of blood to be drawn, mixed and centrifuged immediately, resulting in a quicker turn-around-time for test results.

Centrifugation: The next step in sample processing is the centrifugation of the blood collection tubes. Both BD SST™ and BD PST™ tubes are centrifuged at the same speed and for the same amount of time. In a swinging bucket centrifuge (preferred type of spin for gel separation tubes), the tubes should be spun for ten minutes at a speed of 1100 to 1300 relative centrifugal force (RCF). A fifteen-minute spin at the same speed is required for spinning tubes in a fixed- angle centrifuge. Serum and plasma tubes without gel can be spun at a speed of 1000 RCF for ten minutes.

It is important to spin gel tubes for the recommended time. The gel barrier in the tubes needs time to move and form a solid barrier between the red cells and the serum or plasma. Also, in BD PST™ tubes, the white blood cells and platelets that remain in the plasma need adequate time to spin out of the plasma. If the BD PST™ tubes are spun for less than the recommended 10 minutes, these cells and platelets may remain in the plasma and could cause interference with some chemistry analytes. It is recommended that BD SST™ tubes should not be re-centrifuged after their initial centrifugation. Re-spinning the tubes can result in elevated potassium values, as excess serum that has been in contact with the red cells will be expressed from underneath the gel barrier.

Special Handling of Blood Specimens:
Certain chemistry analytes will require the tube of blood to be chilled after
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