Pre-analytical Variables in Laboratory Testing

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Abstract

Pre-analytical variables play an important role in the quality of specimens that are obtained for laboratory testing. Offering reliable laboratory testing is essential for diagnosis, prognosis and patient care management. There are three phases to laboratory testing: pre-analytical, analytical and post analytical. The pre-analytical phase accounts for the majority of errors made in laboratory test results. Healthcare providers must recognize the importance of good specimen collection and processing and the effect it has on laboratory testing and patient outcomes. Understanding the principles of proper blood collection is critical to good laboratory practice and preventing potential laboratory test errors.

Introduction

Many of the clinical decisions that physicians make about a person’s health status is determined by a blood test. To further the goal of high quality healthcare, it is essential to have quality blood specimens received in the laboratory for reliable laboratory testing. The laboratory testing cycle includes three steps in the total testing process. These steps are:

1. Pre-analytical, which includes specimen collection, handling, transport and processing.
2. Analytical, or the analysis of the sample.
3. Post analytical, which includes the reporting of the data that is then interpreted for clinical management.

The focus of this article will concentrate on the pre-analytical variables that can occur from the time the test is ordered, blood collection is performed, to the time the sample arrives in the laboratory for processing and testing.
Scenario

A physician on staff at the hospital inquires about his complete blood count (CBC) result that was sent to the laboratory. He had received a call from the lab with critical values. His CBC result had a below normal white blood cell count (WBC) and an abnormal differential, which was put in for review for blast type cells. A second specimen was drawn and sent to the lab and all results were within normal range. Why did the two tests differ?

There are many variables that can contribute to the quality of specimens received in the laboratory. In the total testing process, most errors occur in the pre-analytical phase, with a reported error rate of 46-70 percent.1,2 The more common causes of pre-analytical errors are proper collection tube volume (13 percent), patient identification (9 percent), incorrect collection tube (8 percent), test request error (7 percent) and the remaining at 18 percent include hemolysis, incorrect anticoagulant choice, clotted specimens and poor processing.1,3 For a complete chart of pre-analytical errors associated with blood collection, refer to Table 1.

In the process of collecting blood, patient identification is the most important step. The identification should involve active and direct communication. A patient should be asked to spell their last name followed by their first name. A second identifier is required4 and it should be patient specific, which could be a birth date or medical record number. This procedure should be followed for both inpatients (who should be wearing an identification band) and outpatients. If this first basic step is not followed, it could have unwanted clinical outcomes or serious consequences can occur.

Patient preparation is also important. The patient should be asked if they have had anything to eat or drink within the last 10 to 12 hours. It is acceptable to have water. All laboratory normal values are determined on a basal state, which is defined as resting and fasting (absence of food and liquids). Many analytes that are measured require a fasting state. Some examples of fasting analytes are glucose, cholesterol triglycerides and vitamins. Alcohol intake, caffeine, exercise and stress can also cause a different clinical outcome.

Proper venipuncture technique should include selecting the correct site. A tourniquet is place on the patient’s arm 3-4 inches above the antecubital area. The tourniquet should be left on for no longer than one minute.4 Prolonged tourniquet application causes hemoconcentration, resulting in an accumulation of analytes in the circulatory system whereby biochemical changes occur in this trapped blood. Along with hemoconcentration, hemolysis can occur. Hemolysis is the destruction of the red blood cells and can affect many analytes such as potassium and lactic acid.

### Table 1: Pre-analytical Variable Errors

**Specimen Collection Variables**
- Poor requisition request
- Patient identification error
- Patient not properly prepared for the test
  - Fasting
  - Non-fasting
- Sample collection technique not proper
  - Wrong anticoagulant
  - Improper tube
  - Tourniquet time
  - Cleansing the arm
  - Vein selection
  - Tube volume
  - Inverting
  - Order of Draw
  - Mislabling
  - No labels
- Errors in transport and handling
  - Protect from light
  - Specimen storage
  - Blood sample clotted

**Biological Variables**
- Age
  - Adult
  - Pediatric
  - Elderly
- Gender
  - Male
  - Female
- Race
  - Caucasian
  - Black

**Behavioral Variables**
- Diet
- Stress
- Exercise
- Alcohol intake
A site should be selected for venipuncture once the tourniquet is placed. The median cubital vein is preferred. The next choice is the cephalic vein. This vein tends to roll and should be anchored well. The last choice is the basilic vein. This vein should be used with caution because of its location to the median nerve and brachial artery.

When the site has been determined, the site should be cleansed with alcohol in concentric circles working outward. The alcohol should be allowed to dry for 30 to 60 seconds. The drying process creates a barrier to bacterial contamination. If alcohol is left on the site, it can cause hemolysis and could cause a burning sensation for the patient when entering the vein. The site should not be touched after it has been cleaned. This can cause specimen contamination. The next step is to perform proper phlebotomy technique. Probing should be avoided, which again can cause hemolysis and poor specimen quality. When obtaining the specimens, the Order of Draw should be followed. Refer to Table 2 for the Clinical and Laboratory Standards Institute (CLSI) recommended Order of Draw.4

Following the correct order will ensure that there is no cross-contamination of additives in the blood drawing tubes. All blood collection tubes should be filled until the vacuum is exhausted. This ensures that the correct volume of blood to the additive ratio is accurate. For coagulation testing, it is imperative that correct blood-to-anticoagulant ratio (one part sodium citrate to nine parts blood) is followed.

In the final steps of venipuncture, all blood collection tubes should be mixed properly. The blood collection tubes should be inverted immediately after the draw. Poor mixing will produce specimens with clots. Vigorously shaking the tubes can also cause hemolysis. The last step is to properly label the specimen. Blood collection tubes should be labeled after the blood is in them. The label should contain the patient’s full name (first and last), birth date or medical record number, date, time and the initials of the drawer. For a blood bank specimen, a full signature is usually required.

Once the blood is collected, the blood should be sent to the laboratory in a timely manner for processing, handling and testing. Serum specimens should be fully clotted before they are centrifuged. Specimens that were drawn with a clot activator should be allowed to clot for 30 minutes. Patients that are on anticoagulants may take a little longer for clot formation to occur. Tubes should be allowed to clot upright and at room temperature. Serum specimens that are not fully clotted and are centrifuged will contain a fibrin mass that was not consumed in the clotting process. This mass can cause erroneous test results. Tubes should be centrifuged only once. Repeated centrifugation can cause hemolysis and elevated potassium levels.

Some specimens need special handling. Certain analytes may be required to be kept cold. Examples are ammonia, lactic acid, catecholamines and acetone. Other analytes need to be kept warm or at body temperature. Examples are cold agglutinins and cryoglobulins. Analytes that need to be protected from light are bilirubin, folate, porphyrins, Vitamin A, Vitamin B6 and beta-carotene.

In conclusion, pre-analytical errors can be minimized or prevented to improve laboratory testing. Decreasing pre-analytical errors will increase test reliability and enable clinicians to have optimal clinical management for patient care. In the scenario presented, there are many variables that could be considered for the erroneous results. Some of the errors that could have been made are: wrong patient was drawn, the tube contained a clot, the tube was not at the correct volume or the tube was not handled properly. When the first specimen was drawn, it was discovered that the specimen was three days old. A laboratory test result is only as good as the specimen received.

Table 2: Order of Draw

<table>
<thead>
<tr>
<th>Blood cultures: sterile specimen</th>
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<tbody>
<tr>
<td>• Light blue: sodium citrate for coagulation. Tube should be full and well mixed.</td>
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<tr>
<td>• Red tops: glass or plastic</td>
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<tr>
<td>• Gel separator with/without clot activator</td>
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<tr>
<td>• Green: heparin or plasma chemistry</td>
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<tr>
<td>• Lavender: EDTA for hematology</td>
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<td>• Gray: oxalate/fluoride for glucose testing</td>
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References

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