

The Importance of Preanalytical Factors in Immunodiagnostic Testing

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The quality of patient care depends on the quality of all the information that a physician uses in making treatment decisions. As clinicians come to rely more and more on biochemical markers for early detection, diagnosis, and prognosis, the accuracy of the

clinical laboratory result is an increasingly important component of quality patient care.

In the laboratory, the interval between the time a patient's blood is drawn until the result is reported to the physician can be divided into three phases: preanalytical, analytical, and postanalytical.

The preanalytical phase involves collecting the specimen and preparing it for analysis. This includes everything from the time the order is received until the sample is ready for analysis (Table 1).

The analytical phase includes all the steps involved in the actual analysis of the sample. The postanalytical phase consists of reviewing the results for anomalies, reporting the results to the physician, and storing the sample.

Table 1. Components of the preanalytical phase.

- Patient status
- Specimen collection
- Specimen transport to the laboratory
- Specimen processing
- Specimen delivery to the instrument for analysis

Historically, clinical laboratories have focused on the analytical phase when monitoring the quality of results. This has involved monitoring assay performance, lot-to-lot variation and other characteristics, typically through the use of statistical quality control. However, it is estimated that 60 percent of errors in the laboratory occur in the preanalytical phase¹, so it is worthwhile to consider common sources of these errors.

Sources of preanalytical errors

Several factors related to the status of the patient can affect the quality of the analytical result. In certain disease states, substances in the patient's blood may interfere with the analytical process; a common example of such an interfering substance is elevated bilirubin. In addition, the patient may be taking medications that can cause assay interference. The patient may also have circulating endogenous antibodies (heterophilic antibodies, autoimmune antibodies and others) that can interfere with immunoassays. The laboratory cannot

control these sources of error but it must be aware of them and consider them whenever the analytical result seems inappropriate.

Most preanalytical errors are, in fact, associated with the quality of the sample. Haemolysis is the most common cause of error. Other causes include insufficient sample volume, inadequate or incorrect labeling of the tube, and poor clotting of serum specimens. Since many of these sources of error are the result of specimen mishandling, attention to the proper procedures for handling blood collection tubes is critical to result quality. Each step in the process of preparing the sample for analysis is crucial for maintaining sample integrity. Lack of adherence to proper technique can result in haemolysis, or in incomplete clotting leading to interference from fibrin. It is very important that laboratories carefully follow the recommended procedures supplied by the manufacturer of the collection tubes.

Fibrin interference

Residual fibrin, long recognized as a possible interferent in the clinical laboratory, may be present as a result of improper specimen handling during and after collection. It can be present in primary tube samples either as a visible clot, which may physically occlude the instrument sample probe or, more insidiously, as an invisible microfibrin or as strands. Fibrin strands, though invisible, may directly affect some assays, especially immunoassays 2-5. Unlike interference from heterophilic antibodies or rheumatoid factor, fibrin interference is usually not reproducible and disappears with time as the fibrin settles out of the sample.

Care taken during the preanalytical phase can help to reduce the presence of fibrin strands in the processed specimen. Important considerations in the preanalytical phase that can have an effect on fibrin formation are shown in Table 2.

Table 2. Preanalytical phase considerations that can affect fibrin strand formation.

- Recognition of disease or therapy that may affect clotting time
- Selection of the appropriate tube type
- Collection sequence when multiple tubes are collected
- Collection tube mixing
- Time allotted for clotting
- Centrifugation
- Transportation to the instrument

Recognition of factors affecting coagulation

The preanalytical phase begins with the patient and the recognition that diseases or therapies may impact on specimen clotting. A number of conditions and treatments can affect how readily clots form. Pregnant women and dialysis patients, for example, often exhibit prolonged clotting times, and many patients treated for a variety of conditions receive anticoagulants which inhibit clotting. It is important for laboratory personnel to be aware of these possibilities and, whenever possible, to obtain information about the patient. If the ability of the patient's blood to form clots is inhibited, specimen collection must be handled accordingly: either a plasma specimen can be used so that clot formation is not necessary, or increased time can be allowed for clot formation during specimen processing. If these steps are not taken, fibrin strands may form after specimen processing and compromise result quality.

Sample collection

The specimen collection protocol and attention to technique can have a major effect on the quality of the analysis. Problems often occur in critical care situations, where specimens may be collected by procedures other than the usual venipuncture. Using an intravenous (IV) catheter or a syringe for blood collection

followed by transfer of the blood into a collection tube can result in a higher incidence of haemolyzed samples than is commonly seen using routine venepuncture 6,7. These procedures can also affect the rate of clot formation. IV lines typically contain heparin to prevent clots from occluding the line, and the heparin can affect the ability of the sample to clot when it is transferred to a serum tube. Even if plasma is used for testing, sample collection by syringe may allow microclots to begin forming before the blood is transferred to a tube containing anticoagulant.

In the case of multiple-tube collection during a single phlebotomy, the order of collection as described in the collection tube product labeling should be observed; the device for puncturing the tube stopper can become contaminated with additive from the previous tube. Serum tubes without additives should be drawn first followed by those with clot activator. Plasma tubes should be drawn after serum tubes since small amounts of heparin or EDTA contaminating a serum tube may slow clotting.

It is important to mix tubes thoroughly immediately after collection. Most tubes contain additives that must be completely dispersed throughout the tube to be effective. Blood drawn into tubes containing heparin, for example, may begin to form microclots because the localized anticoagulant concentration may be low in some regions of the tube prior to mixing. Adequate mixing is important even in serum tubes. Current serum tubes are plastic rather than glass and contain an additive to enhance clotting. If these tubes are not mixed well, clot formation will not be adequately initiated and will take much longer to complete.

Sample processing

Serum tubes may not be allowed adequate clotting time before centrifugation. BD (Becton, Dickinson and Company) recommends a clotting time of 60 minutes for serum tubes and 30 minutes for gel barrier tubes, for example. Under pressure to provide a rapid result, laboratories all too often shorten the time allotted for the specimen to clot. This increases the likelihood of fibrin strand formation after centrifugation and separation, particularly if the sample is slow to clot because the patient is pregnant or on anticoagulant therapy.

Adequate centrifugation is required to ensure that all clots or fibrin strands are removed. For primary tubes to work optimally with sampling probes on automated instrumentation, the upper surface of the barrier gel or clot should be horizontal. Use of a fixed-angle centrifuge rotor and the resulting slanted clot or barrier gel can result in fibrin strands being disturbed when the tube is removed from the centrifuge and placed upright. In addition, level-sense errors are more likely to occur when the volume of serum is low in tubes spun in fixed-angle rotors.

Finally, care must be taken in the transportation of the sample to the analyzer. If samples are transported from a remote site, centrifugation in the laboratory prior to analysis is a good practice. During transportation over long distances, there are many opportunities for the sample to be disturbed and for fibrin strands to be resuspended. Consequently, centrifugation in the laboratory can prevent analytical problems, even if the specimen was originally centrifuged at the collection site. Even with samples that have been transferred to a secondary transport container, centrifugation before analysis can often reduce problems from fibrin strands that may have formed after the initial processing. An alternative to centrifugation is the use of separation filters that may be inserted into the collection tube or secondary tube to capture fibrin strands and force them to the bottom of the tube.

Conclusion

Many of the steps of sample handling prior to analysis can affect the quality of the sample, and deviation from recommended best practices can lead to erroneous results. While fibrin can never be totally eliminated from primary tubes, care taken during the preanalytical phase can minimize errors. Such a precaution will reduce the necessity of repeating tests or redrawing the patient, and contribute to overall cost savings and improved patient care.

References

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