The Role of Preanalytical Factors in Immunoassays

Introduction

Measurement of biochemical markers is an important aid to clinicians in the early detection, diagnosis, monitoring, and prognosis of disease. Specimen quality plays a key role in assuring accuracy of those measurements in clinical laboratory testing.

To gain efficiencies in workflow and decrease turn-around time (TAT), many laboratories have adopted new strategies and practices, including transitioning from:

- glass to plastic specimen collection tubes
- serum to anticoagulated plasma samples
- manual processing to lab automation, and
- sample collection by laboratory staff to non-laboratory personnel.

As laboratories automate more processes, less time is dedicated to sample inspection steps, which could monitor specimen quality. Preanalytical factors can be magnified by sensitive immunoassays and present an increasing challenge to quality clinical care.

Preanalytical Variables that Could Affect Results

As much as 84% of laboratory error can be attributed to the preanalytical phase of clinical laboratory testing, which is comprised of patient condition, as well as specimen collection, transport, processing, and placement on the analyzer.[1,2,3,4,5 3] Patient samples with circulating protein interferants such as human anti-mouse antibodies (HAMA) and rheumatoid factor (RF) may affect the results in certain assays and is an example of a potential source of error outside the control of the laboratory.[6,7] Knowledge of such factors is important when determining the appropriate interpretations of results.

The large majority of preanalytical errors are due to compromised sample quality[1,2,3,4,5] as affected by specimen collection, storage, transport, and processing. Common factors contributing to error include: incorrect labeling of tubes, insufficient blood draw volume, insufficient mixing, cellular contamination in plasma specimens, and inadequate clotting of serum specimens.

To maintain sample quality, each stage in sample preparation is important and it is critical that personnel performing blood collection adhere to all recommendations specified by blood collection tube manufacturers. Deviations from the manufacturers' recommendations must be validated in individual laboratories.

Factors Affecting Plasma Samples

While serum may provide the cleanest sample from an interference perspective, there are, at times, issues with being able to process the sample in a timely manner. Because urgent, critical decisions are based on STAT results, heparinized plasma samples have become the preferred sample type and are widely used. Laboratory Practice Guidelines, published by the National Academy of Clinical Biochemistry (NACB), recommend plasma for STAT analysis of cardiac markers.[8] Plasma provides the best opportunity for achieving desired TAT; however, there are variables that must be controlled to obtain the best possible sample for analysis.

Because a plasma sample contains anticoagulants, the cellular components (i.e., white blood cells, red blood cells, and platelets) are not trapped in a clot during the normal coagulation process of a serum sample. Following centrifugation, plasma samples can still contain trace amounts of cellular material, as well as latent fibrin. Gel separator tubes reduce the incidence of resuspension; however, small material, especially platelets, will remain above the plasma gel interface barrier. These factors can cause non-specific binding of the antibodies, leading to erroneous results.

Heparin as an Anticoagulant

Heparin, a negatively charged molecule used to inhibit clotting, can bind to some analytes, antibodies, and cellular material, and interfere with the antigen-antibody interaction in the test method.[9,10]

If a tube has insufficient blood volume, there is an excess of heparin. Maintaining an optimum, sample-to-additive ratio is important for effective heparin activity.[11,12,13] A key step in the sample handling process is ensuring that the blood draw sample volume is at least 90% of the stated volume on the collection tube.[11] Clinical and Laboratory Standards Institute (CLSI) has published guidelines for blood specimen handling. Heparin is also a commonly used pharmaceutical agent to inhibit clotting in critical care patients. Inadequate clearing of an intravenous line prior to blood collection can also create an excess in the sample.

Possible Mechanisms that Could Interfere with Heparin Anticoagulant Activity

There are mechanisms that could interfere with heparin anticoagulant action resulting in fibrin formation in a plasma sample.[12] These include:

1. The ability of heparin to bind to cell membranes/proteins, such as platelets. Heparin has a tendency to bind to plasma proteins and cell membranes, thus making its pharmacological action unpredictable. The presence of cellular proteins and membranes could result in binding of heparin; therefore, competing and interfering with anticoagulation.
2. Some patients upon re-exposure to heparin will exhibit heparin-induced thrombocytopenia (HIT). This condition can cause heparin-induced or facilitated

platelet aggregation resulting in low platelet counts. The activated platelets release platelet factor 4 (PF4) that allows clotting by neutralizing heparin.

Effect of Fibrin in Plasma and Serum Samples

Immunoassays are susceptible to interference by fibrin. Small amounts of fibrin (and other protein debris membranes or cell stroma) may affect sensitive immunoassays. The presence of gross amounts of fibrin in the specimen (serum or plasma) may cause blockage of instrument sample aspiration probes, leading to erroneous assay results.

Plasma Samples

Inadequate tube mixing may result in uneven distribution of the heparin additive throughout the specimen. This could lead to localized areas within the specimen where the anti-thrombin effect of the additive is insufficient to prevent the formation of fibrin. Thus, thorough mixing by gentle inversion (at least 8 times) immediately after blood is drawn in the tube is essential. A liquid anticoagulant was used in many glass tubes, facilitating easy mixing. Today the walls of the tube are coated with a powdered anticoagulant, which is not as easily mixed in the sample unless the required mixing occurs immediately after collection.

Since the heparin additive in specimens typically degrades over time, residual thrombin in the specimen can convert soluble fibrinogen to insoluble fibrin. Flocculent matter can frequently be observed in stored samples. Care should be taken to recentrifuge such samples prior to analysis.

Serum Samples

Inadequate clotting time, improper mixing, and failure to place the tube in an upright position can lead to incomplete clot formation. Following centrifugation, the resulting sample may appear satisfactory with a defined layer of cells at the base of the tube and a clear layer of serum above. Despite this appearance, the clotting process may not have been completed prior to transportation, centrifugation, and placement of the specimen on the analyzer. Further coagulation in the serum may subsequently occur, leading to the production of “latent” fibrin, which can interfere with the quality of a result.

For plastic tubes, thorough mixing by gentle inversion (at least 5 times) is essential to ensure even distribution of the clot activator throughout the specimen and to allow completion of the clotting process. Note that some cardiac patients will have therapeutic levels of anticoagulant in their blood that will increase clotting time in the tube and thus increase the potential for the formation of “latent” fibrin in the preanalytical phase.

Conclusions

Considering all of the above factors, serum appears to be the superior sample for immunoassays. Many laboratories use heparinized plasma for faster test turn around times, and to avoid prolonged clotting times in patients with high circulating levels of heparin. Regardless of which sample type is used, following the blood collection tube manufacturer's specimen collection and handling recommendations will help to reduce preanalytical laboratory error. In order to minimize laboratory error due to specimen quality, the key preanalytical actions are:

1. Adequately fill the collection tube to the full volume.
2. Ensure proper mixing immediately after collection.
3. Allow adequate clotting time (minimum 15 minutes, 30 minutes optimum) for serum specimens.
4. Proper centrifugation.
5. Avoid resuspension of separated samples, including tubes with a gel barrier.

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