Pre-analytical and Analytical Variables and Coagulation Tests

Introduction
The nature of clot formation, with an interaction between platelets and labile coagulation factors, means there is a greater need to consider and understand the role of preanalytical factors. Such factors include, phlebotomy, collection tubes, sample transportation, centrifugation and processing, as well other interfering substances.

A puzzling or dissimilar result on a repeat study, paves the way for the clinician to question the interplay of such variables, leading to earlier rectification of the problem thus avoiding unnecessary work-up of the patient. In this regard a responsible laboratory will ensure that all such risks are minimised in the verification of an outgoing result.

Phlebotomy
The simple initial step of following correct patient identification procedures in the labelling of the specimen can save a lot of hardship. Transcription errors at phlebotomy and at the work bench have the potential for erroneous patient results and clinical mismanagement.

Syringes, especially the larger sizes, can significantly increase the risk of clot initiation, as shear force can activate platelets and introduce significant shortening of APTT results. In vitro haemolysis is also higher with the use of needles and syringes compared to vacuum tubes.

The order for multiple tube draws should be an important part of the phlebotomy procedure. The recommended order, if all are utilised, is blood culture, citrate, serum separator, heparin, EDTA and fluoride oxalate tube (see photo above).

If blood is being collected using a butterfly kit (scalp vein), a serum discard tube should be obtained first to ensure the appropriate blood:citrate ratio, since the tubing contains a dead space. Prolonged tourniquet application past one minute should be avoided, if possible. This can cause haemoconcentration, and subsequent activation of coagulation factors.

Traumatic and/or prolonged phlebotomy leads to increased haemostatic activation inevitable in blood collection, producing artefactual changes in PT, APTT, platelet dysfunction and other coagulation assays (see Table 1).

Underfilling and overfilling of the tube (minimised by the vacuum tubes) is known to prolong or shorten the PT/APTT respectively. The correct ratio is 1 part citrate to 9 parts blood collected in a 3.2% (0.109 Molar) tube. Once collected, adequate mixing of the blood and anticoagulant should take place by gentle inversion 3-4 times, but over vigorous mixing can result in platelet activation with shorter clotting times.

Table 1. Haemostatic problems in handling and collection of specimens

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Cause</th>
<th>Explanation</th>
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<tbody>
<tr>
<td>Short PT</td>
<td>Chilling in refrigerator or placing on ice.</td>
<td>Chilling to 4°C activates factor VII. The practice of transporting citrate specimens on ice or storing in refrigerators is discouraged.</td>
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<tr>
<td>Shortened PT and APTT</td>
<td>Slow collection or excessively vigorous mixing.</td>
<td>Haemolysis caused by vigorous mixing or slow collection leads to platelet and coagulation factor activation.</td>
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<tr>
<td>Prolonged PT and APTT</td>
<td>Short draw (less than 2.7ml blood) Failure to gently mix specimen 3-4 times immediately after collection.</td>
<td>Blood clots form when anticoagulant and blood do not mix rapidly. Even a small clot affects results.</td>
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<tr>
<td>False negative LA and decreased Factor levels</td>
<td>Inadequate centrifugation with platelet contamination of plasma.</td>
<td>Specimen is centrifuged so that plasma has a platelet count &lt;10,000/nl (platelet-free plasma). Platelets release phospholipids, coagulation factors, and platelet factor IV. Phospholipids neutralize lupus anticoagulants, platelet factors, and platelet factor IV neutralizes heparin.</td>
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<tr>
<td>Falsely elevated VWF/VIII</td>
<td>Prolonged tourniquet application.</td>
<td>Tourniquet causes venous stasis, which falsely elevates the concentration of large molecules. The tourniquet may be applied for up to 1 minute</td>
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Specimen Transportation and Processing

Coagulation factors V and VIII are labile so delays between the sample collection and processing can result in the artefactual prolongation of the PT and APTT. It is recommended that PTs be performed within 24 hours of collection, and APTTs within four hours of collection. These should be transported and stored at ambient temperatures, 18ºC to 24ºC but it is always best to test the specimens as soon as possible, preferably within four hours for both PTs and APTTs.

Likewise factor assays should be transported at ambient temperatures preferably to prevent cold activation of FVIII/VWF. The separated plasma may be frozen at -20ºC for up to two weeks, or at -70ºC for up to six months in a freezer and thawed at +37ºC, mixed gently by inversion and tested immediately or up to two hours after thawing, though for large sample numbers and to prevent prolonged thawing, testing in small batches is preferable.

A quick thaw at +37ºC minimises the formation of cryoprecipitate which will contain FVIII/VWF. In the latter case re-warming and recentrifugation is recommended. Plasma samples exhibiting a lipaemic appearance can be ultracentrifuged to separate the lipaemic portion for optimal results, otherwise a fasting sample should be requested. Haemolysed and icteric samples may also constitute a problem when an optical clot detection instrument is used and hence a specimen re-collect is often advised in the former case and an alternative method utilised in the latter situation.

Proper centrifugation conditions are needed for plasmas when coagulation testing is involved. Plasma tubes should be centrifuged at a speed and time that will consistently provide platelet-poor plasma (platelet count <10,000/ml). This may be achieved by centrifuging at 1,500 G, for no less than 15 minutes at room temperature, using a swing bucket centrifuge to minimise remixing of plasma and platelets. Otherwise if platelet poor plasma is not obtained, excess platelets in the plasma may result in false negative lupus anticoagulant assays and also lower APTT results in heparinised patients.

Disease States

Changes in the haematocrit can occur with anaemia and polycythaemia and may thus alter the blood to anticoagulant ratio, ultimately effecting higher or lower ionised calcium concentration. Since the citrate volume is 0.5ml and the ratio of citrate to blood 1:9, more than 4.5 mls of blood is necessary when faced with a higher concentration of red cells and the following formula 60/100-hct x 4.5 is used to calculate the extra volume needed.

Patient Characteristics

Though not strictly considered preanalytical, patient variables need to be taken into account when judging results. Such variables includes gender, age, blood type (e.g. patients with blood type O have lower Von Willebrand factor levels), diurnal rhythm, diet, smoking, alcohol use, medications, menstrual cycles, pregnancy, physical and emotional stress. Knowledge of such variables may be helpful in the final interpretation of the result.

Summary

In summary, this article has examined the preanalytical and analytical variables that can impact on coagulation tests but, with correct specimen identification, appropriate collection methods, proper transport, centrifugation, processing and storage the influence of such variables may be minimized or totally excluded thus ensuring an accurate result for precise patient diagnosis and management.

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