Identifying an inhibitor of a coagulation factor is a challenging task. This process requires several steps and is not straightforward. In this article, I will attempt to simplify this complex process.

The first step is to understand how specific and nonspecific antibodies affect coagulation. A specific antibody that is directed against a specific coagulation factor may result in bleeding. However, the risk of bleeding depends on which protein the coagulation inhibitor is directed against. For example, the most common severe acquired coagulation protein inhibitor is an antibody directed against Factor VIII. Patients with Factor VIII experience bleeding and prolonged activated partial thromboplastin time (aPTT). A nonspecific antibody may inhibit several factors and may not result in bleeding but in a thrombotic state. For example, lupus anticoagulant (LA) and antiphospholipid antibody are antibodies that are directed against epitopes of proteins bound to phospholipids. The persistence of LA in the presence with other antibodies, such as beta2-glycoprotein 1 (B2GPI), indicates a patient at thrombotic risk. Therefore, being able to distinguish between the different types of antibodies will help determine the risk to the patient of bleeding or a thrombotic event.

The type of commercial reagents plays a significant role in antibody assessment. How sensitive is the reagent in detecting specific factor levels? Is your reagent sensitive or insensitive to LA? Knowing the answers to those questions will help interpretation of the results.

Reagent Sensitivity

It is important to know how sensitive your reagent is in detecting factor levels. In other words, what levels of factor will prolong the prothrombin time (PT) and/or aPTT? Most reagents should have a prolonged screening test when the level of factor falls below 30% in activity. If reagents are insensitive to a specific factor at a certain low level, PT or aPTT results could falsely show results within the normal reference range.

To test factors for sensitivity:
- Dilute normal plasma with factor-deficient plasma at different levels
- Run a PT or an APTT on the sample
- Compare the results with the upper limit of the normal range

**Keywords:** non-specific inhibitor, specific inhibitor, lupus anticoagulant, mixing study, factor sensitivity
It is important to be aware that one may detect a normal PT or APTT with an abnormal percentage factor level. For example: to test for Factor IX sensitivity, take assayed pooled normal plasma and make dilutions in IX-deficient plasma (Table 1). The normal range for aPTT is 25.5 to 35.5 seconds.

For this aPTT reagent, a specimen from a patient could yield a normal aPTT result when, in reality, it has 20% Factor IX. The reagent does not yield an abnormal screening test result until the level of Factor IX is approximately 15%, making this reagent insensitive to low levels of Factor IX. Insensitive reagents that cannot detect low factor levels can put a patient at risk for bleeding and mismanaged care.

A factor deficiency or a mild inhibitor to a specific factor could also be missed if the coagulation laboratory is unaware of the sensitivities of the assays. Diluting pooled normal plasma with specific factor-deficient plasma that is representative of varying levels of factor activity, with corresponding PT and/or aPTT values, can provide the laboratory with a ballpark figure of where the laboratory screening tests will detect an abnormal test result from a patient. The next issue is to know whether you are testing with an aPTT reagent that is lupus sensitive or lupus insensitive; this is related to the concentration of phospholipid in your reagent. An LA manifests itself in the laboratory as an antibody to phospholipids. Hence, if you have a reagent with a low concentration of phospholipid, it will mask the presence of the antibody; the reagent is, therefore, LA insensitive. Many laboratories like to use this type of reagent because LAs can be thought of as a nuisance when screening patients. This reagent eliminates unnecessary testing of a prolonged aPTT, when a patient is not at risk for bleeding.

An LA-sensitive reagent has a low concentration of phospholipid, which allows the LA to present itself. Many laboratories use this type of reagent when they are seeking an LA. Hence, if you have both reagents, a strong and quick way to distinguish between a factor deficiency and an inhibitor would be to run them both. If the aPTT is normal with the LA-insensitive reagent and prolonged with the sensitive reagent, this is a strong start in identifying an inhibitor.

### Mixing Study

Another important test in coagulation testing is the mixing study, which determines if the patient has a factor deficiency or the presence of a factor-inhibiting antibody. This test involves a mixture of equal volumes of the plasma of the patient and pooled normal plasma. The aPTT or PT of the mixture is repeated immediately after the mix or after incubating the mixture. Incubation is used to help demonstrate a possible weak inhibitor.

If the plasma of the patient is suspected of being factor deficient, adding the pooled normal plasma will add back the deficient clotting factor, and the PT or aPTT will correct itself. If there is no correction in the corresponding PT or aPTT, the plasma of the patient contains an inhibitor, which prevents the ability of the pooled normal plasma to correct itself. The mixing-study procedure varies between laboratories. Some labs run just an immediate mixture and others do an incubated mixture. Some incubate for 1 hour and others incubate for 2 hours—some labs mix a 1:1 dilution, others mix a 1:4 dilution. But at what point can we consider the sample to be corrected?

No standard rule exists to determine if a mixing study has corrected itself. Some labs use an aPTT within 2 seconds of the upper limit of their normal range as the criteria for a corrected result, whereas other labs look at the aPTT result compared with the result of the normal pooled plasma. A few tools (such as the Rosner Index) have been developed to aid laboratories in determining whether the mixing-study results have corrected themselves. However, there are no standard rules or guidelines. Every laboratory must strictly adhere to its standard policies and procedure.

### Table 1. Variables in Testing for Factor IX Sensitivity

<table>
<thead>
<tr>
<th>Factor IX Activity</th>
<th>Dilution of PNP</th>
<th>IX-Deficient Plasma</th>
<th>aPTT Sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>NA</td>
<td>NA</td>
<td>33.2</td>
</tr>
<tr>
<td>75%</td>
<td>750μl + 250μl</td>
<td>250μl</td>
<td>33.9</td>
</tr>
<tr>
<td>50%</td>
<td>500μl + 500μl</td>
<td>500μl</td>
<td>34.2</td>
</tr>
<tr>
<td>35%</td>
<td>350μl + 650μl</td>
<td>650μl</td>
<td>34.8</td>
</tr>
<tr>
<td>20%</td>
<td>200μl + 800μl</td>
<td>800μl</td>
<td>35.0; normal</td>
</tr>
<tr>
<td>15%</td>
<td>150μl + 850μl</td>
<td>850μl</td>
<td>37.0; abnormal</td>
</tr>
</tbody>
</table>

PNP, pooled normal plasma; NA, not applicable.
is in a medically urgent situation. The coagulation profile results will most likely be as follows: PT equals 34.0 seconds (reference range = 10-13 seconds), aPTT = 85 seconds (reference range = 25-35 seconds). After mixing the plasma sample of the patient in a 1:1 ratio with normal pooled plasma and then immediately testing the mixture, the PT equals 32 seconds and the aPTT equals 79 seconds. Because this is clearly not a correction, the influence of an inhibitor must be considered. PT and aPTT are prolonged, so the inhibitor may be affecting a factor in the common pathway (Factors II, V, or X). However, some inhibitors are so strong that they can cross over and also inhibit factors in the intrinsic and extrinsic pathways (Factors VIII, IX, and XI). The treatment for patients with these inhibitors involves intravenous gamma immunoglobulin or even chemotherapy to eradicate the B-cells producing the inhibitor antibodies.

Another conundrum is how to deal with a partial correction? For instance, let us examine a partial correction in a patient with a very prolonged aPTT of 87 seconds. The 1:1 mix is performed, and the result corrects to 40 seconds. The upper limit of the normal range is at 36 seconds, and the pooled normal plasma time is 32 seconds. In this case, factor assays and a dilute Russell viper venom test (DRVV) are indicated.

The DRVV is based on the activation of Factor X by the venom derived from the Russell viper in combination with dilute phospholipids. The coagulant protein in RVV is an enzyme (serine protease) that directly activates Factor X in the presence of Ca++, bypassing the intrinsic and extrinsic pathways. The activated Factor X then activates prothrombin (Factor II) in the presence of Factor V and phospholipid. The screen uses a low concentration of phospholipid, which is sensitive to LA, giving a prolonged result. The confirmed portion alters the concentration of the phospholipid by increasing it, which will result in a shortened time. Results can be reported out as a ratio. A positive ratio is used to confirm the presence of an LA, thus raising the concern for a patient with clinical thrombosis rather than for clinical bleeding.

Conclusion

Testing for the presence of inhibitors can be complicated and requires consistent, robust laboratory practices. It is important to determine the sensitivities of the assays used in the coagulation lab to obtain valid cut-offs values for specific instrument-reagent combinations and to be able to distinguish normal from abnormal values. LM