INTRODUCTION

It is important for laboratories investigating patients for possible bleeding disorders to be able to identify the presence of antibodies that prolong laboratory tests such as APTT.

One group of antibodies that can cause dramatic prolongation of APTT is the heterogenous group collectively termed anti-phospholipid antibodies (APA), sometimes referred to in the literature as lupus anticoagulants (LAC). It is now clear that so called anti-phospholipid antibodies are a heterogenous family of antibodies that react with epitopes on proteins that are themselves complexed with negatively charged phospholipids. Many such antibodies require beta-2-glycoprotein 1, a protein that binds to phospholipids. Others may be directed against prothrombin.

It is important to note that these antibodies may interfere with coagulation reactions in the laboratory, prolonging phospholipid-dependant tests such as APTT and occasionally PT, but they are not associated with bleeding except in a few rare cases where there is significant acquired prothrombin deficiency. Paradoxically, these antibodies are clearly associated with venous and arterial thrombosis by mechanisms not well understood.

In centres attempting to diagnose bleeding disorders, it is necessary to be able to detect such antibodies using specific tests in the investigation of patients with prolonged APTTs. Several guideline documents and reviews of results using different techniques have been published that review the laboratory detection of lupus anticoagulants (LAC), including those listed in the references at the end of this section.

It is important to recognize that different APTT techniques frequently do not have the sensitivity to detect the presence of such antibodies, and the heterogenous nature of the group has led to recommendations that more than one test can be required to confirm the presence of lupus-type anticoagulants.

The criteria for presence of lupus anticoagulants are as follows:

1. Prolongation of a phospholipid-dependant coagulation test.
2. Evidence of an inhibitor demonstrated by mixing studies.
3. Confirmation of the phospholipid-dependant nature of the inhibitor.
4. Lack of specific inhibition of one coagulation factor (such as FVIII:C, FIX:C, or FXI).
SAMPLE PREPARATION

It is important to ensure that the number of residual platelets in the test plasma is kept to a minimum, particularly if plasma is frozen and thawed prior to analysis. One way to remove the maximum number of platelets is to filter the centrifuged plasma (prepared as for other clotting tests) through a 0.22-µ filter. Alternatively, the plasma can be centrifuged twice, as described in Section 4. The aim is to reduce the platelet count to <10 x 10⁹/l in the plasma under analysis. Since filtration may affect other coagulation assays and may be expensive, the preferred option is to remove the platelet-poor plasma from the cells after the first centrifugation (minimum of 1700 g for minimum of 10 minutes) and then centrifuge the secondary container with plasma for a second time under the same conditions. After the second centrifugation, the plasma is carefully removed, leaving the bottom layer undisturbed, since this will contain the majority of the few additional platelets not removed during the first centrifugation. Plasma prepared by double centrifugation in this way will normally have residual platelet counts well below 10 x 10⁹/l and are suitable for deep-freezing prior to analysis for lupus anticoagulant testing. These comments apply to the preparation of control plasmas as well as test plasma.

MIXING STUDIES

Details of mixing studies used to indicate the possible presence of an inhibitor are given in Section 14.

SPECIFIC TESTS TO DETECT LAC AND APA

There are an increasing number of specific tests for the presence of lupus anticoagulants including the dilute Russell’s viper venom time (DRVVT) and kaolin clotting time (KCT), which is also termed the Exner test. Other snake venom–based specific tests, such as the Textarin time or taipan snake venom time, have been used, as well as the dilute thromboplastin time. Details of these tests are included in the references listed below.

Even when relatively specific tests such as DRVVT and others are employed, it is important to confirm the phospholipid-dependent nature of the inhibitor using confirmatory tests.

A method is given below for the DRVVT, for which abnormal results are more specific than APTT for the presence of anti-phospholipid antibodies.

The recently published ISTH guidelines update (Pengo et al. 2009) recommends that the two phospholipid-dependent tests that should be used are the DRVVT and the APTT, and that where abnormality occurs, the test should be repeated in the presence of excess phospholipid. They point out that use of washed platelets as a source of excess phospholipid in this way can be problematic in view of the lack of standardization and the possibility
of batch-to-batch variation between preparations. We include a method for preparation of washed platelets here for centres that may continue to use this approach. The ISTH document does not recommend use of the KCT or the dilute thromboplastin time, or a number of less-well-characterized snake venom based assays.

REFERENCES

