MIXING STUDIES

Principle

When the PT and/or APTT are abnormal, further testing may be done to identify the specific abnormality. Mixing studies are performed to differentiate a factor deficiency from a circulating inhibitor. The coagulation test with the abnormal result is repeated using several different dilutions of the patient's plasma and normal plasma. The testing is performed immediately and after incubation at 37°C. If the abnormal result is corrected by the addition of normal plasma, a factor deficiency is indicated, whereas no correction of the abnormal result indicates the presence of a circulating inhibitor.

Reagents and Equipment

1. Partial thromboplastin reagent
2. Calcium chloride reagent, 0.025M
3. Thromboplastin-calcium reagent
4. Normal control plasma or normal pooled plasma
5. Sodium chloride (NaCl), 0.85%
6. Test tubes, 12 x 75 mm
7. Fibrometer system
   a. Fibrometer
   b. Thermal prep block, 37 ± 1°C
   c. Automatic pipet
8. Coagulation cups, non-wettable surface
9. Fibro-tips
10. Micropipets: adjustable to deliver 100-1000 μL
11. Micropipet tips

Quality Control

A PT and/or APTT should be performed on the quality control materials to verify reagents and technique.
Specimen

Whole blood anticoagulated with 3.2% sodium citrate is the specimen of choice. The specimen should be processed to obtain platelet-poor plasma. Specimen collection and processing are discussed in detail in chapter 39.

APTT Procedure (Fibrometer Testing)

1. Prepare the dilutions of patient plasma and normal plasma as directed in Web Table 39-4.

2. Perform an APTT on each dilution immediately and again after two hours incubation at 37°C.
   a. Pre-warm a sufficient quantity of partial thromboplastin reagent and 0.025M calcium chloride reagent for the number of tests to be performed. Duplicate testing is recommended.
   b. Pipet 0.1 mL of each dilution into an appropriately labeled coagulation cup. Add 0.1 mL pre-warmed partial thromboplastin and mix.
   c. Using the automatic pipet (switch in the "on" position), add 0.1 mL pre-warmed 0.025M calcium chloride to the sample cup. The timer will be initiated with the dispensing of the reagent. Alternatively, the timer may be started by touching the timer plate.
   d. Record the clotting time.

Results

The clotting times for the various dilutions and time intervals are compared to determine if the patient's clotting time has been corrected. Clotting times will tend to increase with time due to the loss of labile factors; therefore, it is important to compare the patient's diluted sample results with the result obtained from the normal control plasma (Tube #1). A clotting time is considered prolonged if it is longer than the normal control plasma's clotting time (Tube #1).

If correction is observed, a factor deficiency is indicated. A circulating inhibitor is indicated by no correction of the prolonged test.

Comments

1. Normal pooled plasma is obtained from at least 20 normal donors.

2. Precision between duplicate measurements is said to be acceptable if the difference between duplicates is 10% or less of the mean of the duplicates.

3. The performance of the test after incubation reveals the time and temperature dependency of the inhibitor. Factor VIII inhibitors are time- and temperature-dependent. Inhibitors that are time- and temperature-dependent will exhibit correction immediately; however, on incubation, the result will become prolonged. Lupus anticoagulants tend to act immediately; however, they may exhibit time dependency.
4. The presence of a factor deficiency is indicated by the correction of the prolonged test result with as little as one part normal control plasma. The normal control plasma supplies the deficient factor.

5. The detection of a circulating inhibitor should be followed by a specific test to identify and quantitate the inhibitor. If a factor deficiency is detected, factor assays should be performed to identify and quantitate the activity of the specific factor.

References
