

TABLE 6. INTERPRETATION OF THE THROMBOPLASTIN GENERATION TEST

| FACTOR DEFICIENCY | CLOTTING TIME CORRECTED BY ADSORBED PLASMA | NORMAL SERUM | PROTHROMBIN TIME |
|-------------------|--|--------------|------------------|
| VIII | yes | no | normal |
| IX | no | yes | normal |
| XI or XII | yes | yes | normal |
| anti-coagulant | no | no | may be normal |

thromboplastin in the patient's generation mixture. Correction of the generation clotting time by partial thromboplastin indicates defective platelet activity. The patient's platelets for this procedure may be prepared as follows:

- A. Centrifuge oxalated blood for 10 minutes at 1000 RPM immediately after collection.
 - B. Transfer the platelet-rich plasma to a siliconized or plastic test tube and note the volume of the plasma.
 - C. Centrifuge platelet-rich plasma at 3000 RPM for 15 minutes. A platelet button will be formed.
 - D. Pour off supernatant plasma and refrigerate until 20 minutes before use.
 - E. Resuspend platelets in 0.85% sodium chloride and centrifuge at 3000 RPM for 15 minutes.
 - F. Pour off the supernatant sodium chloride and resuspend the platelets in a volume of 0.85% sodium chloride, equal to one-third of the original plasma volume.
2. The presence of circulating anticoagulants may give abnormal results in this test.
 3. Oxalated plasma must be used if the plasma is to be adsorbed by barium sulfate. If citrated plasma is employed, aluminum hydroxide must be used as the adsorbing agent.

4. The thromboplastin generation test may also be performed on the Fibrinometer.

PARTIAL THROMBOPLASTIN SUBSTITUTION TEST

The partial thromboplastin substitution test may be performed if the PTT, or activated PTT, is abnormal in order to identify factor deficiencies in stage 1 or 2 of the coagulation process.

REFERENCE

Proctor, R.R., and Rapaport, S.I.: The partial thromboplastin time with kaolin, *Am. J. Clin. Path.*, 36, 212, 1961.

REAGENTS AND EQUIPMENT

1. Water bath, 37°C.
2. Calcium chloride, 0.025 M.

| | |
|----------------------------|--------|
| Anhydrous calcium chloride | 1.38 g |
| Distilled water | 500 ml |
3. Partial thromboplastin containing an activator (platelet substitute with an activator). Obtainable commercially.
4. Citrated normal control plasma.
5. Sodium chloride, 0.85% (w/v).
6. Sodium citrate, 0.1 M

| | |
|---|--------|
| Sodium citrate | 2.94 g |
| ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$) | |
| Distilled water | 100 ml |
7. Test tubes, 13 × 100 mm.
8. Stopwatch.
9. Adsorbed plasma (rich in factors V, VIII, XI, and XII). Prepare as follows:
 - A. Add 100 mg of barium sulfate to

each 1 ml of fresh oxalated normal plasma.

- B. Stir this mixture for 10 minutes at room temperature and refrigerate, or place on ice, for an additional 10 minutes.
- C. Centrifuge at 2500 RPM for 10 minutes and remove the supernatant plasma.
- D. Dilute the adsorbed plasma 1:5 with 0.85% sodium chloride (one part adsorbed plasma, four parts 0.85% sodium chloride).

As an alternative and recommended procedure, adsorbed plasma reagent may be obtained commercially.

10. Aged serum (rich in factors VII, IX, X, XI, and XII). Prepare as follows:
 - A. Incubate a tube of clotted normal blood at 37°C for 3 hours.
 - B. Add one part 0.1 M sodium citrate to nine parts whole blood to the preceding tube.
 - C. Allow the tube to incubate for 2 additional hours at 37°C.
 - D. Centrifuge for 10 minutes and remove the serum.
 - E. The serum may be used immediately or stored at -20°C.
 - F. Prior to use, dilute the aged serum 1:5 with 0.85% sodium chloride (one part aged serum, four parts 0.85% sodium chloride).

The aged serum reagent may be obtained commercially.

SPECIMEN

Citrated plasma: one part 0.11 M sodium citrate to nine parts whole blood.

PRINCIPLE

An activated PTT is performed on the patient's plasma diluted 1:1 with:

1. Adsorbed plasma, rich in factors V, VIII, XI, and XII.
2. Aged serum, rich in factors VII, IX, X, XI, and XII.
3. Sodium chloride, 0.85%.
4. Normal control plasma.

The test is also performed on undiluted patient's plasma. A clue as to the specific coagulation defect may be obtained by noting which reagent, adsorbed plasma, or aged serum, corrects the activated PTT. If a prothrombin time, which measures factors I, II, V, VII, and X, is also performed, further information as to the exact factor deficiency may be obtained. The activated PTT may then be repeated, diluting the patient's plasma 1:1 with plasmas deficient in one specific factor. The factor-deficient plasma, unable to correct the activated PTT, is a further check as to the exact coagulation deficiency.

PROCEDURE

1. Centrifuge patient's citrated blood at 2500 RPM for 10 minutes immediately after the blood has been collected.
 2. Remove the plasma from the cells immediately and place on ice.
 3. Incubate sufficient 0.025 M calcium chloride at 37°C.
 4. Maintain the partial thromboplastin with activator, at room temperature.
 5. Perform the activated PTT (as described in a previous section) on the following plasmas, using the dilutions indicated, and record the results. Each plasma must be tested in duplicate and the two results averaged.
 - A. Patient's plasma.
 - B. Normal control plasma.
 - C. Patient's plasma diluted 1:1 with 0.85% sodium chloride.
 - D. Normal control plasma diluted 1:1 with 0.85% sodium chloride.
- If any of the preceding patient tests are abnormal (all control values should be normal), proceed with the following dilutions, performing an activated PTT on:
- E. Patient's plasma diluted 1:1 with the normal plasma control.
- If the patient's activated PTT is corrected by the normal control plasma,

proceed with the following dilutions, performing an activated PTT on:

- F. Patient's plasma diluted 1:1 with adsorbed plasma.
 - G. Normal control plasma diluted 1:1 with adsorbed plasma.
 - H. Patient's plasma diluted 1:1 with aged serum.
 - I. Normal control plasma diluted 1:1 with aged serum.
6. For simplicity, record the results on a chart similar to the one shown.

| | Normal control plasma, 0.1 ml | Patient's plasma, 0.1 ml |
|----------------------------------|--|--------------------------------|
| Normal control plasma, 0.1 ml | | |
| Patient's plasma, 0.1 ml | | |
| 0.85% sodium chloride, 0.1 ml | | |
| Adsorbed plasma, 0.1 ml | | |
| Aged serum, 0.1 ml | | |

7. Interpretation of results. See Table 7.

DISCUSSION

1. If a factor deficiency is noted, the activated PTT should be performed, using the specific factor-deficient plasma indicated, in a 1:1 dilution with the patient's plasma. When the deficient factor(s) has been positively

identified, appropriate specific factor assays may then be performed.

2. In order for the patient's activated PTT to be considered as corrected, the corrected values must fall close to the normal plasma control value.

PROTHROMBIN TIME WITH SUBSTITUTIONS

The prothrombin time substitution test may be performed, along with the activated PTT, when the prothrombin time is prolonged, in order to detect a possible factor VII deficiency.

REFERENCE

Dade Reagents, Inc.: *Coagulation Procedures*, Dade Reagents, Inc., Miami, Fl., 1966.

REAGENTS AND EQUIPMENT

1. Water bath, 37°C.
2. Thromboplastin-calcium chloride mixture.
3. Citrated normal control plasma.
4. Adsorbed plasma. (See Partial Thromboplastin Substitution Test, Reagents and Equipment.)
5. Aged serum. (See Partial Thromboplastin Substitution Test, Reagents and Equipment.)
6. Sodium chloride, 0.85% (w/v).
7. Test tubes, 13 × 100 mm.
8. Stopwatch.

TABLE 7. PROBABLE COAGULATION DEFICIENCIES BASED ON PROTHROMBIN TIME AND ACTIVATED PTT TEST RESULTS

| APTT | PT | ADSORBED PLASMA APTT | AGED SERUM APTT | PROBABLE DEFICIENCY |
|------|----|-------------------------|--------------------|------------------------|
| N | N | N | N | No deficiency found |
| A | N | C | C | XI or XII |
| A | N | NC | C | IX |
| A | A | NC | C | X |
| A | A | C | NC | V |
| A | N | C | NC | VIII |
| A | A | NC | NC | II |

APTT = Activated partial thromboplastin time. PT = Prothrombin time. N = Normal result. A = Abnormal (prolonged) result. C = Corrected. NC = Not corrected.