

## MANUAL PROTINE

The Prothrombin time is a useful screening procedure for deficiencies in factors II, V, VII and X. Deficiencies in factor I, although rare, may also be detected. This test may be used to follow the course of anticoagulant therapy in patients receiving coumarin (warfarin) drugs. Factors II, VII, IX and X are inhibited by warfarin drugs, with factor VII showing decreased activity first. Common causes of prolonged Prothrombin time are vitamin K deficiency, certain liver diseases, specific coagulation deficiencies, and warfarin drug therapy. The normal Prothrombin time is generally 11 to 13 seconds. These values, however, differ according to the method and reagents used in the performance of the test. Therefore, each laboratory should determine its own set of normal values.

One stage Prothrombin time method.

Reference: Quick, A.J. 1970. Bleeding Problems in Clinical Medicine.  
W.B. Saunders Company, Philadelphia.

### Reagents and Equipment:

1. Heat block, 37°C
2. Thromboplastin-calcium chloride mixture
3. Control samples (normal and abnormal)
4. Test tubes, 12mm x 75mm
5. Stopwatch

### Specimen:

Citrated plasma: one part Sodium Citrate to nine parts whole blood

### Principle:

The calcium in whole blood is bound by sodium citrate, thus preventing coagulation. Tissue Thromboplastin, to which calcium has been added, is mixed with the plasma, and the clotting time is noted.

### Procedure:

1. Centrifuge anticoagulated blood at 2,500 rpm for 10 minutes as soon as possible after collection.
2. Pipet 0.2 ml of thromboplastin-calcium mixture into a set (4 – 6) of 12 x 75 test tubes. Warm the test tubes in the 37 C heat block for at least 1 minute, until they have reached 37 C. The incubation period for this mixture is not critical once it reaches 37 C. (Thromboplastin reagent is good for 20 minutes at 37 C)
3. Incubate a portion of the plasma for approximately 2 to 3 minutes, until it reaches 37 C. *Plasma should be incubated for no longer than 10 minutes after reaching 37 C.*
4. Forcibly inject 0.1 ml of patient's plasma into the test tube containing 0.2 ml of thromboplastin-calcium mixture and simultaneously start the stopwatch.
5. Remove the tube from the heat block and mix the contents of the tube. Hold the tube so that contents can be monitored for the formation of the clot.

Gently tilt the tube back and forth until a clot forms, at which point, the timing is immediately stopped.

(If the Nichrome wire loop method is preferred, pass the wire loop through the mixture at the rate of two sweeps per second until a formed clot adheres to the loop.)

6. Each test and control plasma should be performed in duplicate. The results should agree with each other within  $\pm 1.0$  seconds when the Prothrombin time is below 20 seconds. Duplicate tests on a Prothrombin time above 20 seconds will not agree as closely, but should agree within  $\pm 2.0$  seconds.
7. Report the patient's results along with the results of your normal control.

## MANUAL ACTIVATED PARTIAL THROMBOPLASTIN TIME

The activated partial thromboplastin time (APTT) is the single most useful procedure available for routine screening of coagulation disorders in the intrinsic system. It measures those coagulation factors present in the intrinsic system except for platelets and factor XIII. (Factor VII is not measured because it is in the extrinsic system). The APTT is also the method of choice for monitoring heparin therapy. The normal range for the APTT may vary widely from one laboratory to another and is dependent on the reagents used and the clot detection method employed. It is, therefore, very important that each laboratory determine its own normal range for the specific lot number and type of reagents used. Generally speaking, the normal mean value for the APTT will usually fall between 30 and 40 seconds.

Reference: Proctor, R.R., and Rapaport, S.I. 1961. *The Partial Thromboplastin Time with Kaolin*. American Journal of Clinical Pathology.

### Reagents and Equipment:

1. Heat block, 37°C
2. Calcium chloride, 0.025M (commercially available)
3. Partial thromboplastin containing an activator (commercially available)
4. Control samples (normal and abnormal)
5. Test tubes, 12mm x 75mm
6. Stopwatch

### Specimen:

Citrated plasma: one part Sodium Citrate to nine parts whole blood

### Principle:

The calcium in a whole blood sample is bound by sodium citrate, thus preventing coagulation. The plasma, after centrifugation, contains all intrinsic coagulation factors except calcium and platelets. In the APTT test, partial thromboplastin (a phospholipid substitute) and an activator (to ensure maximum activation) are added to the plasma allowing the coagulation cascade to begin. During incubation, Factors XII, PK and XI are activated, building up the levels of XIa in the reaction tube. Once  $\text{CaCl}_2$  is added, the rest of the coagulation cascade is allowed to continue and timing of the event is obtained. The time required for the plasma to clot is the activated partial thromboplastin time.

### Procedure:

1. Centrifuge anticoagulated blood at 2,500 rpm for 10 minutes as soon as possible after collection.
2. Incubate a sufficient amount of 0.025M  $\text{CaCl}_2$  at 37 C.
3. Pipet 0.1 ml of normal control plasma (or patient's plasma) into a 12 x 75 test tube.
4. Pipet 0.1 ml of the partial thromboplastin (containing activator) into the test tube containing the control (or patient's) plasma. Mix the contents of the tube quickly and place in a 37 C heat block for 5 minutes.

5. After exactly 5 minutes, forcibly inject 0.1 ml of the prewarmed  $\text{CaCl}_2$  and simultaneously start the stopwatch. Mix the test tube immediately and allow the test tube to remain in the heat block, gently tilting the tube every 5 seconds.
6. At the end of 20 seconds, remove the test tube from the heat block. Hold the tube so that contents can be monitored for the formation of the clot. Gently tilt the tube back and forth until a clot forms, at which point, the timing is immediately stopped.
7. Control and patient plasma specimens must always be run in duplicate, and the two results averaged to obtain the final value. The two results should check within  $\pm 10\%$  of each other. If they do not agree, another test should be performed. In certain abnormal states, clot formation is markedly prolonged. If formation of the clot has not started by the end of 2 minutes, the test may be stopped and the results reported as "greater than 2 minutes."
8. Report the patient's results along with the results of your normal control.

### **PROTIME AND APTT MIXING STUDIES (MANUAL METHOD)**

To determine when a factor deficiency exists or when an inhibitor is present. If a factor deficiency is present, the 50:50 mix should correct the PT and/or APTT. Most inhibitors will prevent significant correction of the 50:50 mix. The most common inhibitors are Lupus-type inhibitors. Heparin contamination may also act as an inhibitor.

Reference: Williams 5<sup>th</sup> ed. pg. L85-L86.

#### Reagents and Equipment:

1. Heat block, 37°C
2. Reagents: for PT and/or APTT testing (all appropriate quality control performed)
3. Test tubes, 12mm x 75mm
4. Stopwatch or timer

#### Specimens:

Patient: Citrated plasma (patient must exhibit an abnormal PT and/or APTT)

Normal Plasma: Pooled citrated plasma (do NOT use the normal control)

#### Principle:

When prolonged PT and/or APTTs are recorded for a patient that is not taking any anticoagulant therapy, a factor deficiency or inhibitor may be present. To determine if a factor deficiency exists, "normal plasma" is combined in equal quantities with the patient's plasma and the PT and/or APTT is repeated. If the mixture of samples results in a fully corrected clotting time, the patient is confirmed to have a factor deficiency. If the mixture of samples results in a partial correction or no correction at all, the patient may have an inhibitor. Further studies will need to be accomplished on the patient to confirm either possibility.

#### Procedure:

Note: If your patient has BOTH abnormal PT and APTT results, you only need to perform mixing studies on the APTT. A Factor VII deficiency or inhibitor has already been ruled out by the results and in order to detect an antiphospholipid antibody, the APTT must be performed.

1. Prepare the Normal Plasma: Obtain 2-4 coagulation specimens in Na-Citrate from individuals who are NOT on anticoagulant therapy and whose tube has been filled to the proper level. The pooled sample must have a PT and/or APTT run on it as a reportable result and to confirm its normal status.
2. Mix an aliquot of Patient sample with an equal amount of Normal Plasma (200µL of each works well to allow replicate tests if necessary)
3. Perform the PT and/or APTT test in the same manor as previous.

#### Report:

1. Report the type of test(s) performed (PT and/or APTT) and both the Patient result(s) and the Patient With Normal Plasma result(s).
2. Determine if a factor deficiency or inhibitor may be present.