
**FIG. 127.** Simplate bleeding time device.

incision made is 5 mm long and 1 mm deep. A Simplate bleeding time device is also available in the form of two blades in one housing (for duplicate testing).

*REFERENCE*

General Diagnostics: Simplate Bleeding Time Device, General Diagnostics, Morris Plains, New Jersey, 1977.

*REAGENTS AND EQUIPMENT*

1. Blood pressure cuff.
2. Simplate bleeding time device.
3. Stopwatch.
4. Circular filter paper.
5. Alcohol sponges.
6. Butterfly bandage.

*PRINCIPLE*

A uniform incision, 5 mm long and 1 mm deep, is made on the forearm, and the length of time required for bleeding to cease is recorded.

*PROCEDURE*

1. Place a blood pressure cuff on the patient's arm, above the elbow. Increase the pressure to 40 mm of mercury and hold this exact pressure for the entire procedure.
2. Cleanse an area on the volar surface of the forearm with an alcohol sponge.
3. Remove the tear-away tab on the Simplate and place it firmly on the forearm, either perpendicular or parallel to the fold of the elbow. (Make certain the area is free of scars, surface veins, and bruises.)
4. Depress the trigger and start the stopwatch. Remove the device approximately 1 second after making the incision. (The incision should be made within 30 to 60 seconds after the blood pressure cuff has been inflated to 40 mm mercury.)
5. Blot the blood from the puncture site on a clean section of the filter paper every 30 seconds. The filter paper should not touch the wound at any time.
6. When bleeding ceases, stop the watch and release the blood pressure cuff. Record results. The normal range for this procedure is 2.3 to 9.5 minutes.
7. Place a butterfly bandage over the puncture site and advise the patient to keep the bandage in place for 24 hours.

*DISCUSSION*

1. Some patients may receive slight scarring at the incision site and should be so informed prior to performing this procedure.

## COAGULATION TIME OF WHOLE BLOOD

The whole blood clotting time theoretically measures all stages of coagulation in the intrinsic system. Its usefulness as a screening test is limited, however. In the coagulation of blood in this procedure most of the time is consumed in the production of the prothrombin activator (plasma thromboplastin). It requires only a matter of seconds to convert prothrombin to thrombin, and fibrinogen to fibrin. Therefore, moderate deficiencies in stages 2 and 3 of the coagulation process do not significantly prolong the clotting time. The coagulation time is influenced mainly by defects in stage 1 of the clotting process.

Moderately severe hemophilia, afibrinogenemia, and severe fibrinolytic states cause a prolonged clotting time. The presence of an anticoagulant, such as heparin, also causes an abnormally long clotting time. This procedure is, therefore, used primarily to monitor heparin therapy, which is employed in the treatment of thromboembolic disorders. In such cases, the patient's normal clotting time is first determined, and then initial therapy with heparin is begun and monitored with the whole blood clotting time. The therapy may then be changed to the coumarin drugs and monitored by means of the prothrombin time. Heparin is faster-acting than the coumarin drugs, but the therapy is more difficult to maintain.

There are a number of modifications of the in vitro test for the coagulation time of whole blood. They are all based on the same principle and the same basic techniques. The size of the tubes, the amount of blood used, and the temperature at which the determination is performed are the main variables between the different modifications employed. Each of the modified tests, therefore, shows a slight variation in the normal values. Once a technique is chosen, the test must be run under those same conditions each time. The normal values for the test described below are 5 to 15 minutes.

### Lee and White Method

*REFERENCE*

Ortho Diagnostics: *Coagulation Procedures*, Ortho Diagnostics, Raritan, New Jersey, 1968.

*REAGENTS AND EQUIPMENT*

1. Water bath, 37°C.
2. Glass test tubes, 13 × 100 mm.
3. Stopwatch.
4. Syringe (10 ml) and 20-gauge needle.

*SPECIMEN*

Fresh whole blood, 4 ml.

*PRINCIPLE*

The coagulation time of whole blood is the length of time required for a measured amount of blood to clot under certain specified conditions.

*PROCEDURE*

1. Label three 13 × 100-mm test tubes, #1, #2, and #3.
2. Perform a clean, untraumatic venipuncture using a 20-gauge needle and withdraw 4 ml of blood. Start the stopwatch as soon as the blood enters the syringe.
3. After obtaining 4 ml of blood from the patient, remove the needle from the syringe, and carefully place 1 ml of the blood in test tube #3, then 1 ml in tube #2, and lastly, 1 ml in tube #1. The last 1 ml of blood may be discarded.
4. Place the three tubes in a 37°C water bath.
5. At exactly 5 minutes, tilt tube #1 gently to a 45° angle. Repeat this procedure every 30 seconds, until the tube can be completely inverted, without spilling the contents (that is, until the blood is completely clotted).
6. Record the time it took the blood in tube #1 to clot.
7. Thirty seconds after the blood in tube #1 is clotted, proceed with tube #2, and repeat the preceding procedure, tilting the tube every 30 seconds, until a clot is formed. Record the results. Repeat this procedure for tube #3.
8. Since agitation and handling speed up coagulation, the coagulation time is determined by the clotting time of tube #3.

*DISCUSSION*

1. It is important to place exactly 1 ml of whole blood in each tube. Amounts greater than 1 ml prolong the clotting

time. Less than 1 ml of blood in the tube yields a shortened clotting time.
2. Poor venipuncture technique, causing hemolysis or causing tissue thromboplastin to mix with the blood, shortens the clotting time.
3. Incubation at 37°C is important if the normal values for the test have been determined using this technique. Temperatures lower than 37°C retard the clotting time.
4. Bubbles entering the syringe when the blood sample is being obtained increase the rate of coagulation.
5. Unnecessary agitation of the blood shortens the coagulation time.
6. At the completion of the Lee and White clotting time, it is suggested that one tube remain in the 37°C water bath to be checked after 2 and 4 hours for clot retraction. Also, the tube may be allowed to remain in the water bath overnight, and checked the next day for abnormal clot lysis.
7. The Lee and White clotting time may also be performed using siliconized glass test tubes in place of the plain glass test tubes used in the previously described method. Using the same procedure, the normal clotting time (using siliconized tubes and tilting the tubes every 5 minutes) is 20 to 60 minutes. This method is more sensitive to coagulation deficiencies than the unsiliconized test tube procedure. However, because of the time involved, it is not a practical method for the routine hematology laboratory.

## CLOT RETRACTION

When blood coagulation is complete, the clot normally undergoes contraction, where serum is expressed from the clot, and the clot becomes denser. Thrombosthenin, released by the platelets,is responsible for clot retraction. In addition, the number of platelets present also affects the clot retraction time. If the platelet count is below 50,000 per cu mm, poor clot retraction may occur. In rare instances where the platelet count is normal, there may be poor clot retraction due to an abnormality present in the platelets. Normally, clot retraction begins within 30 seconds after the blood has clotted. At the end of one hour, there should be appreciable clot retraction, and almost complete retraction by the end of 4 hours. Clot retraction should be complete within 24 hours. An abnormal clot retraction time is found in Glanzmann's thrombasthenia.

### *REFERENCE*

Cartwright, G.E.: *Diagnostic Laboratory Hematology*, Grune & Stratton, Inc., New York, 1963.

### *REAGENTS AND EQUIPMENT*

1. Water bath, 37°C.
2. Glass test tubes, 13 × 100 mm.

### *SPECIMEN*

One of the tubes containing 1 ml of whole blood, used in the Lee and White clotting time, or 3 ml of whole fresh blood, placed in a 13 × 100-mm glass test tube.

### *PRINCIPLE*

Whole fresh blood is placed in a 37°C water bath, and inspected at 1, 2, 4, and 24 hours for the presence of a retracted clot.

### *PROCEDURE*

1. If a Lee and White clotting time was not performed, obtain 3 ml of blood and dispense carefully into a 13 × 100-mm glass test tube.
2. Place tube of blood in the 37°C water bath and allow the blood to clot.
3. As soon as the blood has clotted, inspect the clot at 1, 2, 4, and 24 hours for the formation of a retracted clot. The clot should be firm and retracted from the sides of the tube. It generally