CLOTTING TIME (CT) COAGULATION TIME

[I] CAPILLARY BLOOD CLOTTING TIME (WRIGHT'S CAPILLARY GLASS TUBE METHOD)

(While your partner is doing BT on your finger prick, you can proceed with your CT.

1. Absorb the first 2 drops of blood on a separate filter paper and allow a large drop to form. Now dip one end of the capillary tube in the blood; the blood rises into the tube by capillary action. This can be enhanced by keeping its open end at a lower level.
2. Note the time when blood starts to enter the tube. This is the zero time.
3. Hold the capillary tube between the palms of your hands to keep the blood near body temperature (in winter, you may blow on it).
4. Gently break off 1 cm bits of glass tube from one end at intervals of 30 seconds, and look for the formation of fibrin threads between the broken ends. The end-point is reached when fibrin threads span a gap of 5 mm between the broken ends ("rope formation"). Note the time.

   Normal clotting time = 3–6 minutes.

Comments

(i) The clotting of blood with this method involves both the intrinsic and the extrinsic systems of clotting. There is injury to the blood (coming in contact with glass, intrinsic pathway), and the injury to the tissues (extrinsic pathway).

(ii) The CT is prolonged in hemophilia and other clotting disorders, because thrombin cannot normally be generated. Yet, the BT, which reflects platelet plug formation and vasoconstriction, independently of clot formation, is normal.

[II] Drop Method

This method is less accurate than the above method. Place a large drop of blood from a skin puncture on a clean and dry glass slide. Draw a pin through the drop every 30 seconds, and note the time when fibrin threads adhere to the pin and move with it out of the blood drop. The time elapse between placing the blood drop on the slide and the formation of fibrin threads is the clotting time.

Normal clotting time = 2–4 minutes.

- In the original Duke's Drop Method for CT, two drops of 4–5 mm diameter are placed on a glass slide. The slide is tilted at 30 second intervals. The end-point is absence of change in the previous shape when the slide is held vertical.

[III] *VENOUS BLOOD CLOTTING TIME* (LEE AND WHITE TEST-TUBE METHOD)

A. Single test-tube method. This is the most widely used method for the determination of clotting time.

1. Draw 5 ml venous blood by a clean, non-traumatic venepuncture. Note the time when blood starts to enter the syringe. This is the zero time. Transfer the blood to a chemically clean and dry test tube.
2. Holding the test tube in a water bath at 37°C, take it out at 30 second intervals and tilt it. The end-point is when the tube can be tilted without spilling the blood.

Normal clotting time with this method = 5–10 minutes.

B. Multiple test-tube method. The CT can be determined more accurately by using 3 test tubes rather than one only.

1. Rinse 3 test tubes of 8 mm diameter with normal saline, drain them and place them in a metal rack kept in water at 37°C. Transfer 1.5 ml blood into each test tube.
2. Take out the first tube after 1 minute, tilt it to 45° and return it to the rack. Repeat every 30 seconds until clotting occurs, i.e. where the test tube can be tilted without spilling the blood. Note the time.
3. Repeat the tilting on the second test tube and note the time when clotting occurs, (this happens a few seconds later because tilting the tube hastens clotting). The third tube acts as a control and a check on the end-point in the 2nd test tube.

   If a siliconized test tube is used at the same time, a delayed clotting time (40–70 minutes) can be shown.

Normal clotting time with this method = 5–10 minutes.

- The CT depends on the condition of the glass itself, and even on the size of the test tube. Therefore, a high-degree of standardization is needed.

Comments

This method is more reliable than the capillary blood CT method, because there is no admixture of blood with tissue fluid which contains tissue thromboplastin (extrinsic system). Thus, this method tests only the intrinsic system of blood clotting. However, this method is nonspecific because the CT can theoretically increase due to deficiency of any of the factors in the intrinsic system. But, in actual practice, a prolonged CT nearly always means hemophilia in which the CT may exceed 1 hour in severe cases.

CLOT RETRACTION TIME (CRT)

Transfer the larger test tube containing clotted blood to an incubator at 30°C. Normally, the clot starts to shrink (retract) in about 30 minutes (leaving behind straw-colored serum), becomes half its size in 2–3 hours, and complete in 24 hours. Note if there is any digestion of the clot or discoloration of serum.

Comments

Clot retraction (tightening or consolidation) depends on the release of many factors from the platelets, and so the CRT depends on the platelet count. The fibrin-stabilizing factor causes more and more cross-linking bonds between nearby fibrin fibers. Their spicules also release contractile proteins—actin, myosin, and thrombosthenin. The contraction (retraction) of the clot is activated by thrombin, and calcium ions stored in the endoplasmic reticulum, Golgi apparatus, and mitochondria.

In addition to forming a meshwork and entrapping blood cells and plasma, the fibrin fibers also adhere to the edges of the wound. When the clot contracts, it stitches the edges of the wound and thus prevents further loss of blood.

CLOT LYSIS TIME (CLT)

The dissolution (dissolving; also called fibrinolysis) of a clot is the process by which a clot becomes fluid so that the trapped red cells sink to the bottom of the test tube.

Normally, the clot lysis time is about 72 hours. If it occurs within 24 hours, it is considered abnormal.

PROTHROMBIN TIME (PT)

The patient's blood is quickly oxalated (or citrated) to remove calcium ions so that prothrombin cannot be converted to thrombin. The sample is then centrifuged. Then to the oxalated plasma, a large excess of calcium ions (as calcium chloride solution) and rabbit brain suspension (to provide tissue thromboplastin; tissue factor, TF) is added. The excess calcium neutralizes the effect of oxalate and the TF converts prothrombin to thrombin via the extrinsic clotting pathway (i.e. factor VII).

The time required for clotting to occur is called the prothrombin time (PT).

Normal PT = 15–20 seconds.

Clinical Significance. Since the potency of tissue thromboplastin (TF) may vary, blood from a normal person is used as a control when the test is used for controlling anticoagulant dose, or in a hemorrhagic disease. Bleeding tendency is present when the prothrombin level falls below 20% of normal (normal plasma prothrombin = 30–40 mg/dl). Prolonged PT suggests the possibility of deficiency of factors II (prothrombin), V, VII and X (See Q/A 17). Prothrombin level is low in vitamin K deficiency and various liver and biliary diseases.

TESTS FOR OTHER CLOTTING FACTORS

Tests similar to prothrombin time have been devised to estimate the quantities of other clotting factors. In each test, excess of calcium ions and all the other factors, except the one being tested, are added to the oxalated blood (or plasma) all at once. The time required for clotting to occur is determined in the same way as for prothrombin time. The clotting time will be prolonged if the factor being tested is deficient. The time itself is used to quantitate the concentration of the factor.

QUESTIONS

Q.1 What is the clinical importance of doing BT and CT?

BT and CT are important in the following situations: