Clotting time & Bleeding time

1-Clotting time (CT)

The time required for blood to form a clot, it test clot formation which depend on coagulation factors (figure 1).

• The normal coagulation time in glass tubes is 3 to 10 minutes.

• The whole blood clotting time is a rough measure of all intrinsic clotting factors in the absence of tissue factors.

• Used in diagnosis of hemophilia (prolonged time to stop).

• Its chief application is in monitoring anticoagulant therapy.

First method uses Capillary tube

Equipment

• Capillary tubes of uniform size (non-heparinized) • A petri dish.

• Alcohol swabs. • Cotton wool. • Plasticine. • A water bath set at 37 C.

Procedure

1-Clean finger with alcohol swap, prick it with lancet and note the time that the prick is made.

2-Wipe away the first drop of blood. Then while the blood is still flowing freely place one end of a capillary tube in the blood. Holding the tube horizontally let it fill by capillary action, fill more than one tube.

3-Close the end of the capillary tube with plasticine. Place the tube in the water bath. Two minutes after making the puncture, break a capillary tube and separate the two halves slowly.

4-Repeat the procedure at 60 second intervals with the remaining tubes.

5-When the blood forms a continuous thread like clot between the broken ends of the tube, the end point has been reached, note the time.

6-The time from pricking the finger to the appearance of the clot thread is the clotting time.

Results

• Usually the clotting time measured by this method is in the range 3 - 12 minutes

• Prolong clotting time seen in deficiencies in the intrinsic coagulation pathway

• Example hemophilia due to deficiency of Factor VIII 8

Second method

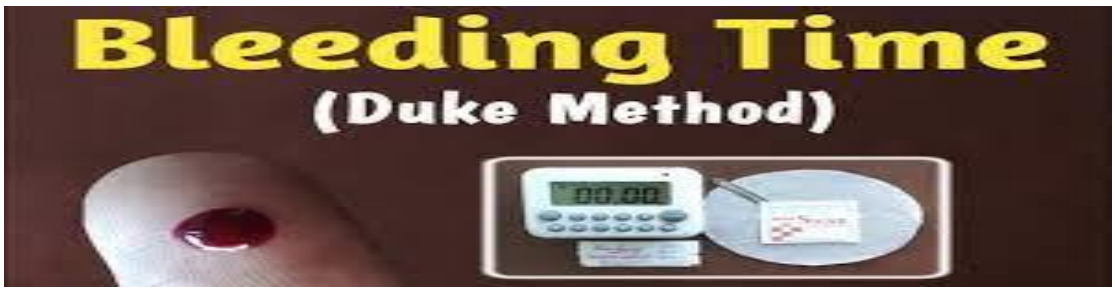
Clotting Time using Test Tube Method

• Place 2 ml blood into non-heparinized test tube incubated in water bath

• Every 30 second invert gentle to check for clot formation

• Time from pricking finger to clot formation is clotting time

• Normally 6 -10 min by this method.



2- Bleeding time (BT)

Definition:

The bleeding time is the time required for a small cut in the blood vessels to stop bleeding. When a blood vessel is injured, blood comes out for some time and then it stops because of the formation of platelet plug, therefore this test

makes to measure platelet plug formation and condition of vessels. The duration of bleeding is the bleeding time. Normal value for bleeding time is 1-3 minutes.

Significance:

The bleeding time is mainly used in the diagnosis and treatment of the haemorrhagic diseases. The bleeding time is also useful just before operations such as tonsillectomy.

The bleeding time may be performed by following methods: Duke Method, Ivy Method and Macfarlane Method.

Requirements:

Cotton, needle, piece of filter or bloating paper, stop watch.

Procedure:

Duke method for bleeding time:

1) The finger tip of the subject is sterilized with spirit and a bold prick is made with a sterile needle to have free flow of blood.

2) The stop watch is started and time is recorded.

3) A piece of bloating paper is folded into half and exactly at every 30 seconds interval. The blood coming out from the puncture is wiped.

4) The above step is repeated until blood ceases to flow.

5) The time at which blood ceased to flow is recorded.

6) The bleeding time is determined from the recorded time data.

Report:

The bleeding time of the subject is found to be minutes.

Reference:

Hoffbrand AV, Steensma DP. Hoffbrand's essential haematology. John Wiley and Sons; 2019 Dec 31.

Kumar, S. S., VK, J., George, J., & Mukkadan, J. K. (2013). Bleeding time and clotting time in healthy male and female college students of Karukutty village, Kerala. *Health Prospect*, *12*(1), 7-9.

Figure 1 Coagulation factors

Name	Description	Function
Fibrinogen (Factor I)	MW = 340,000 Da; glycoprotein	Adhesive protein that forms the fibrin clot
Prothrombin (Factor II)	MW =72,000 Da; vitamin K-dependent serine protease	Activated form is main enzyme of coagulation
Tissue factor (Factor III)	MW = 37,000 Da; also known as thromboplastin	Lipoprotein initiator of extrinsic pathway
Calcium ions (Factor IV)	Necessity of Ca^{++} ions for coagulation reactions described in 19th century	Metal cation necessary for coagulation reactions
Labile factor (Factor V)	MW =330,000 Da	Cofactor for activation of prothrombin to thrombin
Proconvertin (Factor VII)	MW = 50,000 Da; vitamin K-dependent serine protease	With tissue factor, initiates extrinsic pathway
Antihemophilic factor (Factor VIII)	MW = 330,000 Da	Cofactor for intrinsic activation of factor X
Christmas factor (Factor IX)	MW = 55,000 Da; vitamin K-dependent serine protease	Activated form is enzyme for intrinsic activation of factor X
Stuart-prower factor (Factor X)	MW = 58,900 Da; vitamin K-dependent serine protease	Activated form is enzyme for final common pathway activation of prothrombin
Plasma thromboplastin antecedent (Factor X)	MW = 160,000 Da; serine protease	Activated form is intrinsic activator of factor IX
Hageman factor (Factor XII)	MW = 80,000 Da; serine protease	Factor that normally starts aPTT-based intrinsic pathway
Fibrin stabilizing factor (Factor XIII)	MW =320,000 Da	Transamidase that cross-links fibrin clot
High molecular weight kininogen (Fitzgerald, Flaujeac, or William factor)	MW = 110,000 Da; circulates in a complex with factor XI	Cofactor
Prekallikrein (Fletcher factor)	MW = 85,000; serine protease	Activated form that participates at beginning of aPTT-based intrinsic pathway