

# Experiment 11: Determination of Bleeding Time and Clotting Time

## BLEEDING TIME

### Aim

To determine the bleeding time of the given subject by Duke's method.

### Apparatus Required

Filter paper, stop clock, lancet, cotton, spirit.

### Procedure

The tip of the left ring finger is pricked with aseptic precautions (3–4 mm). The blood should flow freely without squeezing. The time of puncture is noted. With a filter paper, the blood is gently blotted every 30 seconds. The successive blots become smaller. This procedure is repeated until no blot appears on the filter paper. The time is noted again. The number of blots on the paper is counted. Number of blots  $\times$  30 seconds will be the bleeding time.

### Discussion

Bleeding time is the time interval between the skin puncture and the cessation of bleeding—the time in minutes which it takes for a standardized skin wound to stop bleeding. Significance: Cessation of bleeding from a small wound as that inflicted during this procedure can be affected by vascular spasm and formation of platelet plugs. This test therefore measures the capillary and platelet functions in haemostasis.

*Normal:* 1–5 minutes (by Duke's method).

### *Factors which affect the Bleeding Time*

1. Size and nature of the injury
2. Condition of the vessel wall
3. Number of platelets.

### *Conditions where Bleeding Time is Prolonged*

1. Decrease in the number of platelets—thrombocytopenic purpura
2. Functional platelet defect:
  - a. Drugs like aspirin, penicillin
  - b. von Willebrand's disease
  - c. Uraemia, cirrhosis, leukaemia
3. Vessel wall defects:
  - a. Prolonged treatment with corticosteroids
  - b. Allergic purpuras

- c. Infections with haemolytic streptococci, bacterial endocarditis
- d. Deficiency of vitamin C
- e. Senile purpura.

### Other Methods

1. In Duke's method, the edge of the ear lobe can also be used
2. *Ivy's method:* The cuff of the BP apparatus is applied to the upper arm and the pressure is raised to 40 mm Hg. The front of the fore arm is used. The normal bleeding time by this method is up to 9 min.
3. The finger is pricked and dipped in a beaker containing normal saline at 37°C. The blood drops will be seen falling to the bottom of the beaker in a continuous slow stream. The time is noted when the bleeding stops.
4. Capillary fragility test of HESS or tourniquet test. The bleeding time may be performed by Duke's method or Ivy's method; Ivy's method is a recommended method, but in this lab, we follow Duke's method for the sake of convenience.

### Result

The bleeding time of the given subject is.....

## CLOTTING TIME

### Aim

To determine the clotting time of the given subject by Wright's method.

### Apparatus Required

Capillary glass tube 15 cm long with a bore of 0.8 mm, stop clock, lancet, cotton, spirit.

### Procedure

The tip of the left ring finger is pricked under aseptic precautions and the time of puncture is noted. The prick must be deep enough to allow free flow of blood without squeezing.

The blood is drawn into the capillary tube by dipping one end of the tube in the blood drop. The blood fills the tube by capillary action. After 2 minutes, a small bit of the tube is broken every 30 seconds until a fine thread of fibrin appears between the broken ends. The time is again noted and the interval between the prick and the appearance of fibrin threads gives the clotting time.

Student's Notes

## Discussion

Clotting time is the time interval between the skin puncture and formation of fibrin thread. Significance: The clotting time is generally not affected by deficiency of platelets as only few platelets are required to provide enough platelet factor 3 for normal coagulation. This test assesses the intrinsic and common pathways of coagulation because the trigger for coagulation in this test is the surface activation of blood that comes into contact with the glass surface of the test tube (therefore for comparability, we should use standard size tubes and constant blood volumes so that the same amount of surface activation occur each time).

Normal: 2–8 minutes.

### Conditions where Clotting Time is Prolonged

1. Haemophilia (bleeding time is normal)
2. Christmas disease
3. Afibrinogenemia
4. Vitamin K deficiency
5. Liver disease
6. Anticoagulant therapy
7. Newborn baby.

## OTHER METHODS

1. *Lee and white method*: 1 ml of blood is placed in 8 mm diameter tube. The tube is tilted at definite time intervals until there is no displacement of fluid blood, when the tube is inverted.
2. *Drop method*: A drop of blood is placed on a glass slide. Clotting is indicated by the absence of any change in the shape of the drop when the slide is held vertical.

Lee and White's method is the most accurate and recommended method. But since it involves larger quantities of blood and venous sampling, this method will 'only be demonstrated to you. For individual exercises you will do the Wright's capillary tube method.

## Result

The clotting time of the given subject is.....

## Tests of Hemostasis

The first line tests for each of the following mechanisms of haemostasis are:

1. Vascular integrity—bleeding time
2. Platelet function—bleeding time and platelet count
3. Coagulation mechanism – Clotting count
  - PTT
  - One stage prothrombin time fibrinogen assay

In this course, you will learn to perform the following tests of haemostasis.

1. Bleeding time
2. Clotting time
3. Platelet count.

## QUESTIONS

1. What happens to bleeding time in each of the following disorders of haemostasis and why?
  - a. Thrombocytopenia
  - b. von Willebrand's disease
  - c. Haemophilia
  - d. Thrombasthenia
  - e. Pseudoxanthoma elasticum (vascular disorder)
  - f. Scurvy.
  - g. A. fibrinogenemia
2. List 4 conditions where clotting time is increased.
3. What happens to the following tests of haemostasis in each of the following conditions?

	Platelet count	Bleeding time	Clotting time
Thrombocytopenia			
Haemophilia			
Obstructive jaundice			
Heparin therapy			
von Willebrand's disease			
Thrombasthenia			

4. What is the normal platelet count?
5. What are the functions of platelets?
6. Name two conditions where platelet counts may be abnormally
  - a. High
  - b. Low
7. What are the clinical effects of a low platelet count?
8. Explain the term thrombasthenia. What is its clinical significance?
9. Name the different methods by which you can determine clotting time.
10. Name the diseases where bleeding time and clotting time are altered; explain briefly the reasons for the alterations.
11. Why the bleeding time is normally shorter than clotting time?
12. What is haemostasis? Name the different mechanisms involved in it.
13. What is the natural anticoagulant present in the human body?

Student's Notes

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14. Name some of the anticoagulants used clinically.
15. What is thrombosis?
16. What is an embolus?
17. Enumerate the various coagulation factors.
18. What is prothrombin time and what is its significance?
19. Name some other tests used in coagulation disorders.

Student's Notes

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