## BLEEDING TIME (BT) AND CLOTTING TIME (CT)

***Bleeding Time (BT)*** is the time interval between the skin puncture and spontaneous, unassisted (i.e. without pressure) stoppage of bleeding. The BT test is an *in vitro* test of platelet function.

***Clotting time (CT)*** is the time interval between the entry of blood into the glass capillary tube, or a syringe, and formation of fibrin threads.

**Note**
The BT and CT are two simple tests that are used as a routine before every minor and major surgery (e.g. tooth extraction), biopsy procedures, and before and during anticoagulant therapy, whether or not there is a history of bleeding.

## BLEEDING TIME (BT)

**[I] "Duke" Bleeding time (finger-tip; ear-lobe)**

- Since the skin of the fingertip is quite thick in some persons, a small cut in the skin of the earlobe with the corner edge of a sterile blade gives better results. The earlobe method is the original "Duke" method for BT.
- Ask your partner to fill the capillary tube with blood from the same skin puncture from where you are doing the BT (see below for CT).
- **Materials** •Equipment for sterile finger-prick. • Clean filter papers. • Chemically clean, 10–12 cm long, glass capillary tubes with a uniform bore diameter of 1–2 mm. • Stopwatch.

### PROCEDURES

1. Get a deep finger-prick under aseptic conditions to get free-flowing blood. Start the stop watch and note the time.
2. Absorb/remove the blood drops every 30 seconds by touching the puncture site with the filter paper along its edges, without pressing or squeezing the wound. Number the blood spots 1 onwards.
3. Note the time when bleeding stops, i.e. when there is no trace of blood spot on the filter paper. Encircle this spot and number it as well. This is the end point. (Do not keep the filter paper on the table and then press your wound on it).
4. Count the number of blood spots and express your result in minutes and seconds.

**Normal bleeding time** = 1–5 minutes.

- The test is simple and quite reliable in spite of the fact that the depth of the wound cannot be controlled.
- The BT is prolonged in purpura (platelet deficiency, or vessel wall defects) while it is usually normal in hemophilia.
- Lack of several clotting factors may prolong BT, though it is especially prolonged by lack of platelets.

### PRECAUTIONS

1. The skin site chosen for BT should be scrubbed well with alcohol to increase the blood flow.
2. The skin should be dry and the puncture should be 3–4 mm deep to give free-flowing blood. Do not squeeze.
3. Do not press the filter paper on the puncture site.
4. If bleeding continues for more than 10–12 minutes, stop the test and press a sterile gauze on the wound. Inform your teacher about the bleeding.

**[II] Another method** is to get a finger-prick and dip the finger in a beaker containing normal saline at 37°C. The blood drops will be seen falling to the bottom in a continuous stream. Note the time when bleeding stops.

**[III] "Ivy" Bleeding Time (Hemostasis Bleeding Time).**

This method is more reliable than the "Duke" method. However, it requires some practice to apply the BP cuff and maintain the pressure.

### Procedure

1. Clean the skin over the front of the forearm with 70% alcohol.
2. Apply a blood pressure cuff on the upper arm, raise the pressure to 40 mm Hg and maintain it there till the end of the experiment.
3. Clean the skin area once again. Grasp the underside of the forearm tightly, make a 1–3

mm deep skin puncture, about 5–6 cm below the cubital fossa. Note the time.

4. Remove the blood every 30 seconds by absorbing it along the edges of a clean filter paper by gently touching the wound with it, till the bleeding stops. This is the end-point.

**Note**
Instead of one prick, two lancet stabs may be given, 5 cm apart, one after the other, and the BT noted in them separately.

**Normal bleeding time** with this method is upto 9 minutes.

**[IV] Simplate method.** Though the "Duke" and the "Ivy" bleeding time methods are fairly reliable, it is not possible to control the depth of the wound made by a lancet or a blade. However, by careful standardization, it has become possible to do so. The most widely used technique uses a 'template' or an automated scalpel to control the depth and length of the wound—usually 1 mm deep and 9 mm long—and a blood pressure cuff inflated to 40 mm Hg to distend the capillary bed of the forearm.

**Normal bleeding time** = < 7 minutes.

**Note**
Although a BT of over 10 minutes has a slightly increased risk of bleeding, the risk becomes great when the BT exceeds 15 or 20 minutes.

**[V] Capillary fragility test of Hess (also called "Tourniquet" test).** This is an important test to assess the mechanical fragility of the capillaries (and formation of a platelet plug) by raising the pressure within them. It may reveal latent purpura.

1. Mark a 1 inch diameter circle on the front of the forearm, and using blue ink, mark any pink, purple, or yellow spots within the circle.
2. Apply a blood pressure cuff on the upper arm and note the systolic and diastolic pressures. Then, after a pause of about 2 minutes or so, raise the pressure to midway between systolic and diastolic levels and maintain it there for 15 minutes. Appearance of more than 10 new petechiae (pink or red spots in the skin) is a positive test, which may be seen in various types of purpura and vessel wall abnormalities.

**Comments**
The BT test is an in vivo test of platelet function, and "Ivy" method is probably the most reliable. However, a peripheral blood film is always examined for the number of platelets and their morphology. The students should note that platelets are involved both in BT and CT tests and one is normally affected without the other. If the BT is prolonged due to low platelet count (thrombocytopenic purpura), the platelets that are available are sufficient to give a normal clotting time.

## PLATELET COUNT

Despite their small size (2–4 μm) and being non-nucleated fragments of cytoplasm, the platelets contain a wide variety of chemical substances that play an important role in vasoconstriction, hemostatic plug formation, activation of factor X, conversion of prothrombin to thrombin, and in clot retraction that results in permanent sealing of a ruptured vessel. Thus they take part in almost all stages of hemostasis.

**Platelet counting.** There are two methods for this count: *direct method* and the *indirect method.* Automated counters are also available.

### A. DIRECT METHODS

You will require: • Microscope • RBC pipette • Counting chamber with cover slip • Equipment for fingerpick • Rees-Ecker diluting fluid—OR—Freshly prepared 1.0% ammonium oxalate solution.

### PROCEDURES

**I. Ammonium Oxalate Method.** This fluid destroys red cells but preserves platelets; it also acts as an anticoagulant.

1. Get a finger- prick and draw blood up to the mark 1.0. Suck the diluting fluid to the mark 101.
2. Mix the contents thoroughly and wait for 20 minutes. The red cells will be hemolyzed, leaving only the platelets. Mix the contents once again and charge the chamber on both sides.