

- **Primary vascular disorders:** Ehlers- Danlos syndrome.
- **Platelet-vessel wall interactions:** von Willebrand disease
- **Others:** Afibrinogenemia, severe hypofibrinogenemia, uremia, aspirin.

TESTS FOR COAGULATION COMPONENT

Coagulation or Clotting Time (Lee-White Method)

It measures the time taken for the fresh blood to clot.

Procedure

- Draw 3 mL of venous blood with aseptic precautions.
- Label 3 test tubes as No. 1, 2 and 3 and keep in a water bath at 37°C.
- Deliver 1 mL of blood into each of the above 3 test tubes and start the stop watch.
- After 3 minutes, take out tube No 1, tilt it every 30 seconds till a clot develops.
- Note the time when the tube can be inverted completely.
- Next examine the tube No 2 every 30 seconds, exactly the same way as tube No 1, till the clot forms and note the time.
- Finally, invert the third test tube as above till blood clots. Stop the watch.
- Record the time from the moment blood is delivered into the test tube to the complete clotting in the third tube.
- The clotting time of the third tube is reported as the clotting time.

Normal: 4-11 Minutes

Disadvantages

Not a sensitive test: It is one of the oldest tests and is not sensitive as it fails to detect mild/moderate procoagulant defects. Hence, it is obsolete now and not recommended as a screening test. PTT is a more sensitive test for assessment of the coagulation cascade. Clotting time is prolonged only with severe deficiency of factor VIII, IX or fibrinogen (afibrinogenemia) and in heparin therapy.

Misleading: Normal value may be obtained in mild-to-moderately severe hemophilia A and B and it does not exclude major factor deficiency.

- **Other use:** Following the clotting time, the tubes can be left in the water-bath and examined after one hour for *clot retraction test*.

Specimen Collection for Coagulation Studies

- Care should be taken not to hemolyse the sample either by excessive suction during venipuncture or while delivering the blood through the needle into the vial.

- Ratio between blood and anticoagulant should be exactly 1:9. Any variability of ratio affects the results of coagulation studies. Vacuum tubes drawing exactly 4.5 mL blood in 0.5 mL of 3.2% disodium citrate are preferred.
- All the glassware should be clean and dry.

Quick's One Stage Prothrombin Time

Principle: Prothrombin time (PT) is the time taken by citrated plasma to clot after the addition of tissue thromboplastin and calcium. It tests *extrinsic and common pathway* of coagulation system.

Reagents Required

- Thromboplastin reagent
- Test plasma: Centrifuge the patient's blood sample at 4500 RPM for 15 minutes and collect the platelet-poor plasma in a clean test tube.
- Control plasma from a normal person.
- Calcium chloride solution
- Water-bath at 37°C
- Stopwatch or a coagulometer.

Procedure

- Incubate all the reagents and test tubes at 37°C for 15 minutes.
- Pipette 0.1 mL of plasma into a test tube and add 0.1 mL of thromboplastin suspension. Incubate the tube at 37°C water-bath.
- Add 0.1 mL of calcium chloride solution to the tube after 1 minute and mix.
- Immediately start the stop watch and leave the tube in water-bath for a minimum of 8 to 9 seconds.
- Gently tilt the tube and look for clot. As soon as the clot appears, record the time.
- The same procedure is repeated for the control sample.

Normal Range: 11-16 Seconds

Reporting of Prothrombin Time

Prothrombin time may be reported in different ways:

- Patient PT and control PT in seconds
- Ratio of patient PT to control PT
- International normalized ratio (INR): Due to the inherent variation in the sensitivity of thromboplastin reagents, it is advisable to report the PT in international normalized ratio (INR). It provides a uniform scale in spite of using different sources of thromboplastin. WHO recommended that each thromboplastin should have ISI [International sensitivity index] value. INR is calculated using the formula:
$$\text{INR} = (\text{PT patient in seconds} / \text{PT normal plasma in seconds})^{\text{ISI}}$$