- 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:
  INR = (PT patient in seconds/ PT normal plasma in seconds) ISI

Uses

- **Screening test to evaluate coagulation disorders:** It measures coagulation factor I, II, V, VII and X. Deficiency of any one of these factors leads to prolongation of PT. It should be used along with PTT.
- **To monitor oral anticoagulant therapy.**
- **To evaluate liver function:** Liver disease can result in deficiency of the coagulation factors. Hence PT should be performed before a *liver biopsy* and *prolonged* PT is a contraindication for liver biopsy.

Interpretation: **Prolonged PT is seen in:**

- Liver disease
- Administration of oral anticoagulants like coumarin
- Vitamin K deficiency
  - Obstructive jaundice
  - Hemorrhagic disease of the newborn
- Deficiency of factors I, II, V, VII and X
- Disseminated intravascular coagulation (DIC)

Precautions

- The ratio of anticoagulant to blood must be 1:9.
- Avoid hemolyzed and clotted blood samples.
- Test should be performed within 2 hours of collection.
- Correction for PCV must be done.
- Test should be done at 37°C.
- Always run a control sample with the patient sample.

Prothrombin time can also be measured by the instrument called coagulometer. Semi-automated and fully automated coagulometers are now available in the market. These coagulometers should be properly calibrated and monitored regularly.

Activated Partial Thromboplastin Time (Partial Thromboplastin Time)

Principle: Activated partial thromboplastin time (APTT) is the time taken for citrated plasma to clot in the presence of a surface activator (kaolin), phospholipid and calcium. Partial thromboplastin time (PTT) is a measure of the **intrinsic** and **common coagulation pathways.**

Reagents Required

- Citrated platelet poor plasma of the patient
- Platelet substitute-commercially available phospholipid
- Surface activator (kaolin)
- Calcium chloride
- Normal control plasma