

PT prothrombin time:

- ▣ **Principle:**

Test measures the clotting time of plasma in presence of an optimal concentration of tissue extract (Thromboplastin).

- ▣ It is useful in assessment of deficiency of factors involved in extrinsic clotting system and common pathway

- ▣ **Factor: I,II,V,VII and X**

▣ Thromboplastins were originally tissue extracts obtained from different species and organs containing tissue factor and phospholipid → the majority of animal thromboplastins now in use are extracts of rabbit brain or lung

PT procedure:

- ▣ 200 μ l from working reagent
- ▣ incubate for 5 min at 37°C
- ▣ 100 μ L from citrated plasma
- ▣ Start stop watch
- ▣ Determine the coagulation time.

Normal range :13 – 17 seconds

The common causes of prolonged PT are:

- ▣ Administration of oral anticoagulant drugs
- ▣ DIC
- ▣ Vitamin K deficiency
- ▣ Liver disease

APTT Activated partial thromboplastin time:

- ▣ **Principle:** This Test measure the clotting time of plasma after the activation of contact factors XII but without added tissue thromboplastin
- ▣ Indication for deficiency of factors involve in intrinsic pathway I,II,V,VIII,IX,X,XI and XII

APTT procedure:

- ▣ Incubate at 37°C the APTT reagents(R1& R2)
- ▣ Pipette into a dry tube:
100μL citrated plasma+100μL from R1
- ▣ Mix and incubate for 5 min at 37°C
- ▣ Add 100 μl from R2
- ▣ On addition of R2 start stop watch and determine the coagulation time.

Normal range: 22 – 30 seconds

The common causes of prolong APTT:

- ▣ DIC
- ▣ massive transfusion with stored blood
- ▣ liver disease
- ▣ administration of heparin
- ▣ hemophilia A and B

High PT& High APTT:

the problem is in the Common Pathway

High PT& normal APTT:

the problem is in the Extrensic Pathway

Normal PT&High APTT:

the problem is in the Intrinsic Pathway

Fibrinogen (factor I)

FDPs or D dimer → to be sure that fibrinolysis process is working well because Excessive fibrinolysis results in accumulation of fibrin degradation products in the blood That may interfere with the coagulation pathway & platelets functions.

(FDPs normal range is less than $10\mu\text{g} / \text{ml}$)

- ▣ **Fibrinolysis** : to get rid from fibrin clot after the healing done by Plasminogen when it becomes active it becomes plasmin this activation is done by:

1- **intrinsic activation** by factor VII and kallikren

2- **extrinsic activation** by TPA (tissue plasminogen activator)

FDPs & D dimer test

Principle:

fibrinogen and fibrin are broken down by plasmin (proteolytic enzyme) to produce D,E (LOW Mwt) & X,Y (High Mwt) then removed by the liver

Normal range: less than 10 $\mu\text{g/ml}$

- ▣ Now they measure D i.e D dimer kit → that reflect FDP
- ▣ Using Latex that is sensitized to (coated by Ab against) fragments D.

High fibrinogen:

1-DIC

2- deep vein thrombosis

3-pulmonary embolism

4- pregnancy

D dimer & FDPs kits

In D dimer kit:


- ▣ Using latex coated with Anti D
- ▣ no need serum dilution
- ▣ Sample is EDTA or Trisodium citrate
- ▣ (qualitative - Semi quantitative)

In FDPs kit :

- ▣ we use latex coated with Anti D, E
- ▣ need serum dilution
- ▣ Sample :Tri sodium citrate or special tube has antifibrolytic agent and thrombin

FDPs kit Procedure:

Sample preparation:

- ▣ Leave the blood sample in the tube for $\frac{1}{2}$ hour
- ▣ Centrifuge it
- ▣ Get the **serum**
- ▣ Dilute the serum 1:5 (1 part of serum  4 parts of diluent buffer
- ▣ 100 μL of serum + 400 μL buffer .
- ▣ Mix it .

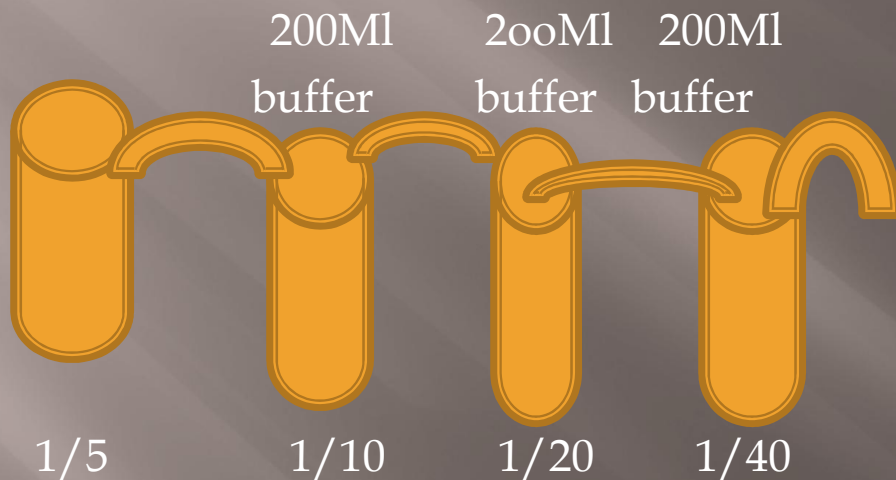
FDPs procedure:

- ▣ 20 μ l of +ve control + 20 μ l of latex
- ▣ 20 μ l of -ve control+20 μ l of latex
- ▣ 20 μ l of sample+20 μ l of latex
- ▣ Mix 2 min
- ▣ -ve(no agglutination) is normal(no fibrin clot) mean less than 10 μ g/ml
- ▣ If +ve (agglutination) more than 10 μ g/ml
⇒ do the Semi quatitative test

Semi quantitative test:

tube 2,3,4 have 200 μ l of buffer

- ▣ 400 μ l of buffer+100 μ l of serum in the first tube



1 drop of tube 1/10+1 drop of latex

1 drop of tube 1/20+1 drop of latex

1 drop of tube 1/40 +1 drop of latex

Look for agglutination

If the agglutination in tube 1/10 it means more than 10 less than 20 μ g/ml

If the agglutination in tube 1/20 it means more than 20 less than 40 μ g/ml

If the agglutination in tube 1/40 it means more than 40 μ g/ml