



University of Diyala/ College of Medicine
Department of Physiology
Physiology Lab

Coagulation Profile:

Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), and Thrombin Time (TT)



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Outlines

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- ***Prothrombin Time:*** Definition, Principle, Procedure, and clinical significance.

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- ***Activated Partial Thromboplastin Time*** Definition, Principle,, and clinical significance.

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- ***Thrombin Time*** : Definition, Principle, and clinical significance.

Introduction

- Prothrombin is a protein produced by the liver for clotting of blood. *Prothrombin production depends on adequate vitamin K intake and absorption.* During the clotting process, prothrombin is converted to thrombin. The prothrombin content of the blood is reduced in patients with liver disease.
- The Prothrombin Time (PT) is one of the four most important screening tests used in diagnostic coagulation studies. It directly measures effectiveness of the extrinsic coagulation pathway through analysis of the clotting ability of five plasma coagulation factors (prothrombin, fibrinogen, factor V, factor VII, and factor X). In addition to screening for deficiency of prothrombin, the PT is used to evaluate dysfibrinogenemia, the heparin effect and coumarin effect, liver failure, and vitamin K deficiency.

Prothrombin Time

- Prothrombin Time (PT) evaluates extrinsic and common pathways.
- **PT defined as** *the time taken by the plasma to clot in the presence of thromboplastin and calcium.*
- It is sensitive to Factors I, II, V, VII, X.
- PT evaluates patients suspected of having an inherited or acquired deficiency in these factors.
- *Frequently performed* : Pre-surgery screening and Monitor Coumadin therapy.

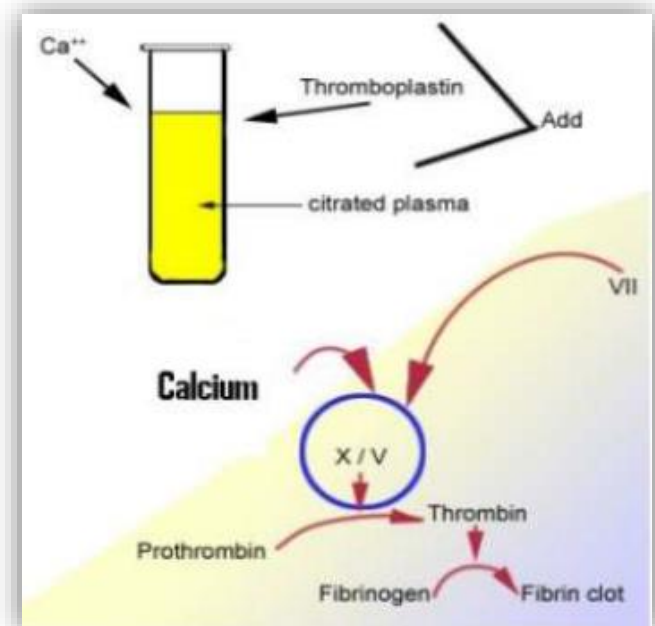
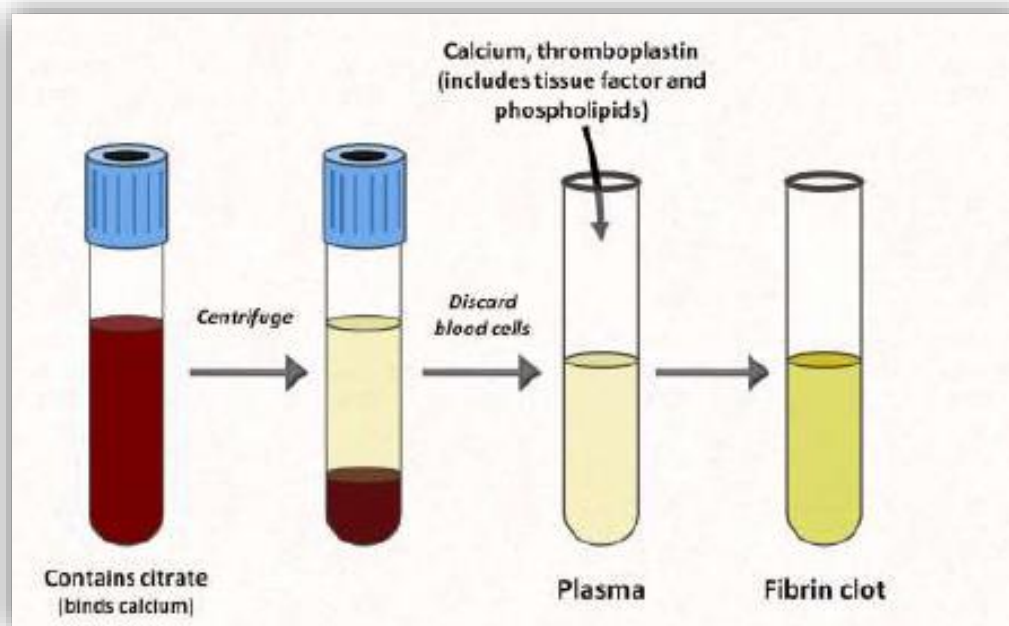
PT and INR

- **PT** is usually measured in seconds and is compared to a normal range that reflects PT values in healthy individuals.
- Because the reagents used to perform the PT test vary from one lab to another, the normal ranges also will fluctuate. To standardize results across different laboratories, a WHO committee developed and recommended the use of the **International Normalized Ratio (INR)**
- The **INR** was devised to standardize the results. Each manufacturer assigns an ISI value (International Sensitivity Index) for any tissue factor they manufacture. The ISI value indicates how a particular batch of tissue factor compares to an internationally standardized sample. The ISI is usually between 1.0 and 2.0.

$$\text{INR} = \left(\frac{\text{PT}_{\text{test}}}{\text{PT}_{\text{normal}}} \right)^{\text{ISI}}$$

Principle

- The calcium in whole blood is bound by sodium citrate, thus preventing coagulation. Tissue Thromboplastin, to which calcium has been added, is mixed with the plasma, and the clotting time is recorded.



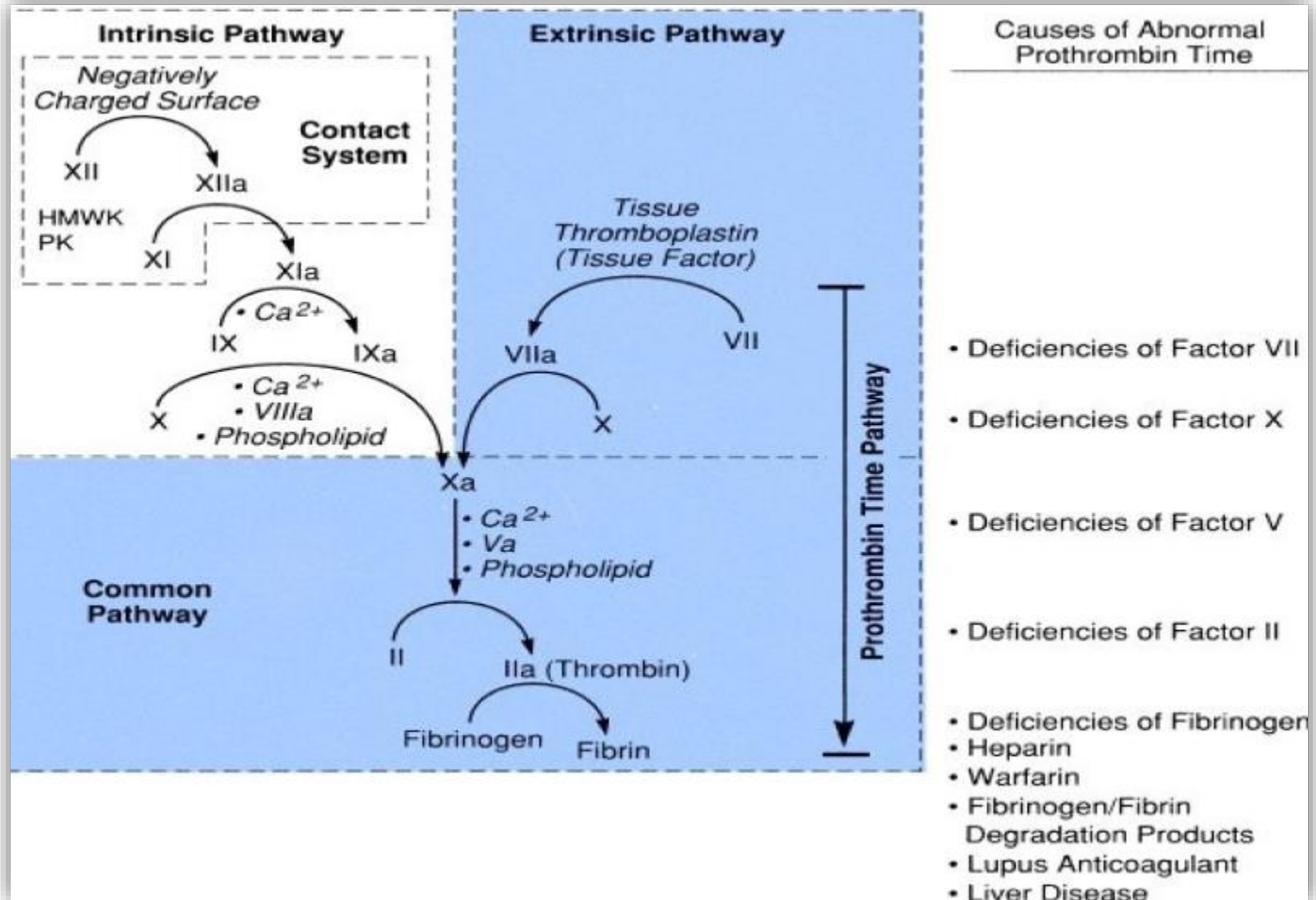
Materials and Instruments

- Thromboplastin-calcium reagent which is commercially available.
- Water bath (37°C)
- Test tubes (13× 100) mm
- Stopwatch
- Sodium citrate (3.8%) as anticoagulant. 0.5 ml of sodium citrate is added to 4.5 ml of the whole blood
- Centrifuge
- Micropipettes (0.1 ml and 0.2 ml)

Procedure

1. Centrifuge the anticoagulated blood at 1200-1500 g for 15 min.
2. Carefully, separate the plasma and store it at room temperature until it is ready for testing. Perform the test within 4 hours of blood collection.
3. Prepare thromboplastin-calcium reagent which is commercially available.
4. Add 0.2 ml of thromboplastin-calcium reagent into duplicate test tubes. Warm the two test tubes in a water bath for 10-15 min.
5. Incubate the plasma at 37C for 2-3 min.
6. Add 0.1 ml of the plasma into the duplicate test tubes that have 0.2 ml of thromboplastin-calcium reagent and simultaneously start the stopwatch.
7. Mix the contents of the tubes, remove the tube from the water bath(wipe and dry them from the outside). Gently , tilt the tube back and forth until a clot forms (seen), at that point timing is stopped.
8. Average the two results to get PT value. ***Normal range of PT*** 11.0–13.0 sec. ***The normal range for the INR*** is 0.8–1.2.
9. Normal value depends on the used thromboplastin, the exact technique, and whether visual or instrument end-point reading should determines its normal range.

Clinical Significance: Causes of Abnormal PT



Partial Thromboplastin Time (PTT) or Activated Partial Thromboplastin Time (aPTT or APTT)

Introduction

- The partial thromboplastin time (***PTT***) or activated partial thromboplastin time (***aPTT or APTT***) is a performance indicator measuring ***the efficacy of both the intrinsic and the common coagulation pathways.***
- Apart from detecting abnormalities in blood clotting, it is also used to monitor the treatment effects with heparin, a major anticoagulant.
- Kaolin cephalin clotting time (KccT) is a historic name for the activated partial thromboplastin time.

Principle

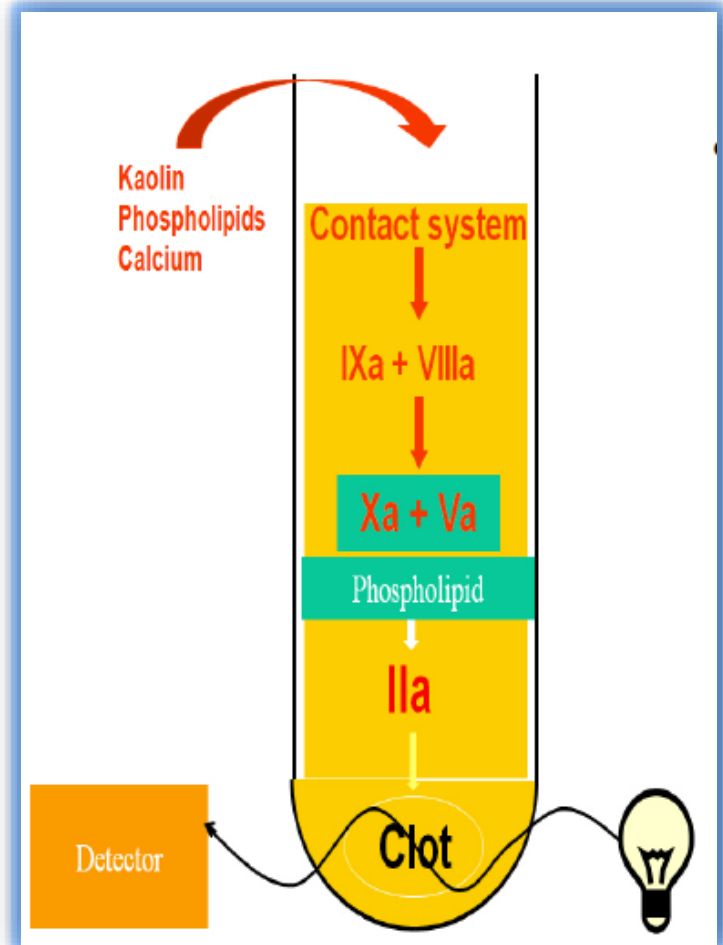
- The calcium in a whole blood sample is bound by sodium citrate, thus preventing coagulation.

- In the APTT test, partial thromboplastin (a phospholipid substitute) and an activator (to ensure maximum activation) are added to the plasma allowing the coagulation cascade to begin.

- During incubation, Factors XII, PK (prekallikrein) and XI are activated, building up the levels of XIa in the reaction tube. Once CaCl_2 is added, the rest of the coagulation cascade is allowed to continue and timing of the event is obtained. ***The time required for the plasma to clot is the activated partial thromboplastin time.***

Principle

- After centrifugation, the Plasma contains all the intrinsic coagulation factors except calcium (removed during anticoagulation) and the platelets (removed during centrifugation).



Clinical Significance

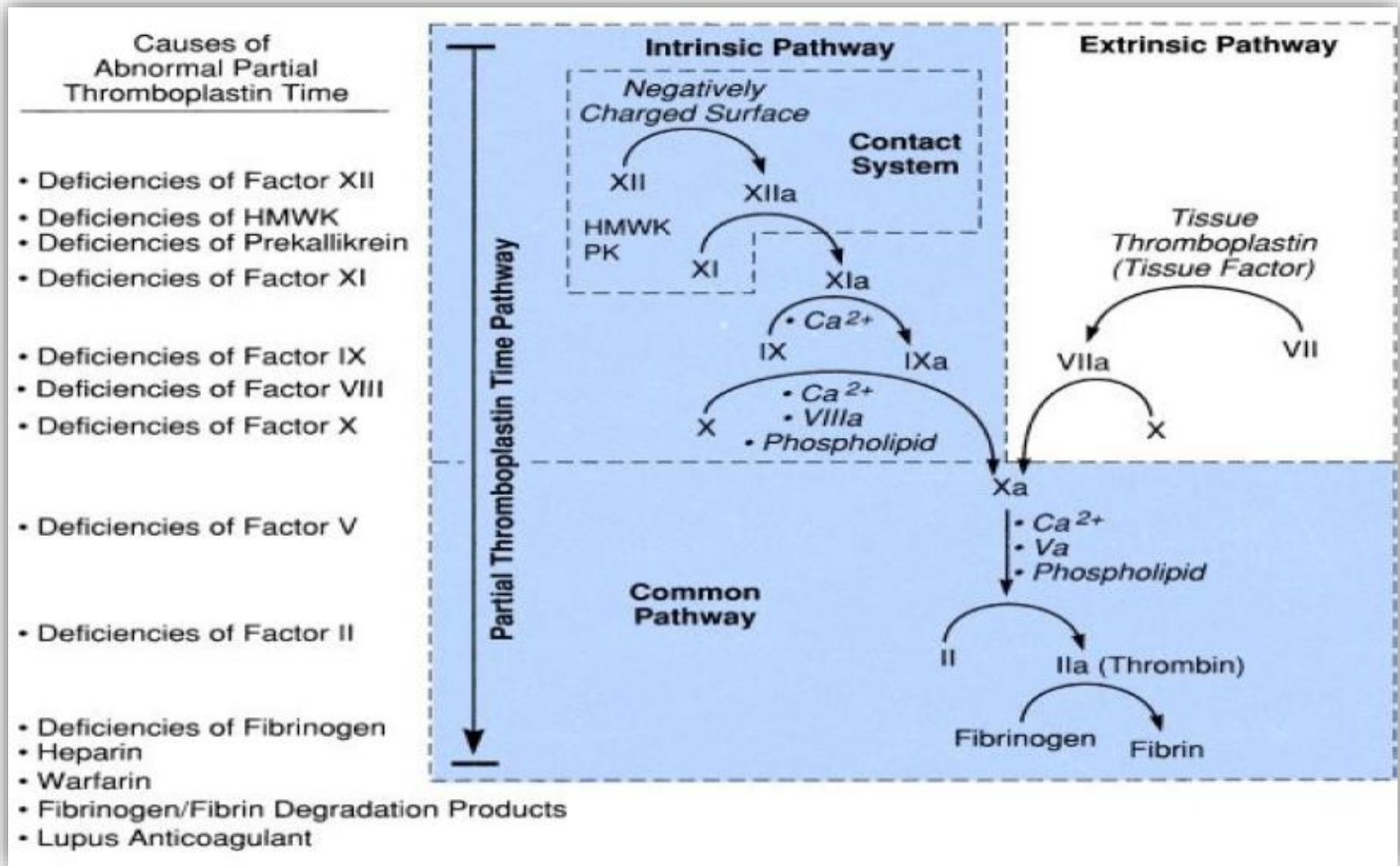


Normal Range • 25 – 39 sec.

***Prolonged APTT
may indicate:***

- Use of heparin.
- Coagulation factor deficiency , e.g hemophilia.
- DIC.
- Liver disease.

Clinical Significance: Causes of Abnormal APTT



Thrombin Time (TT)

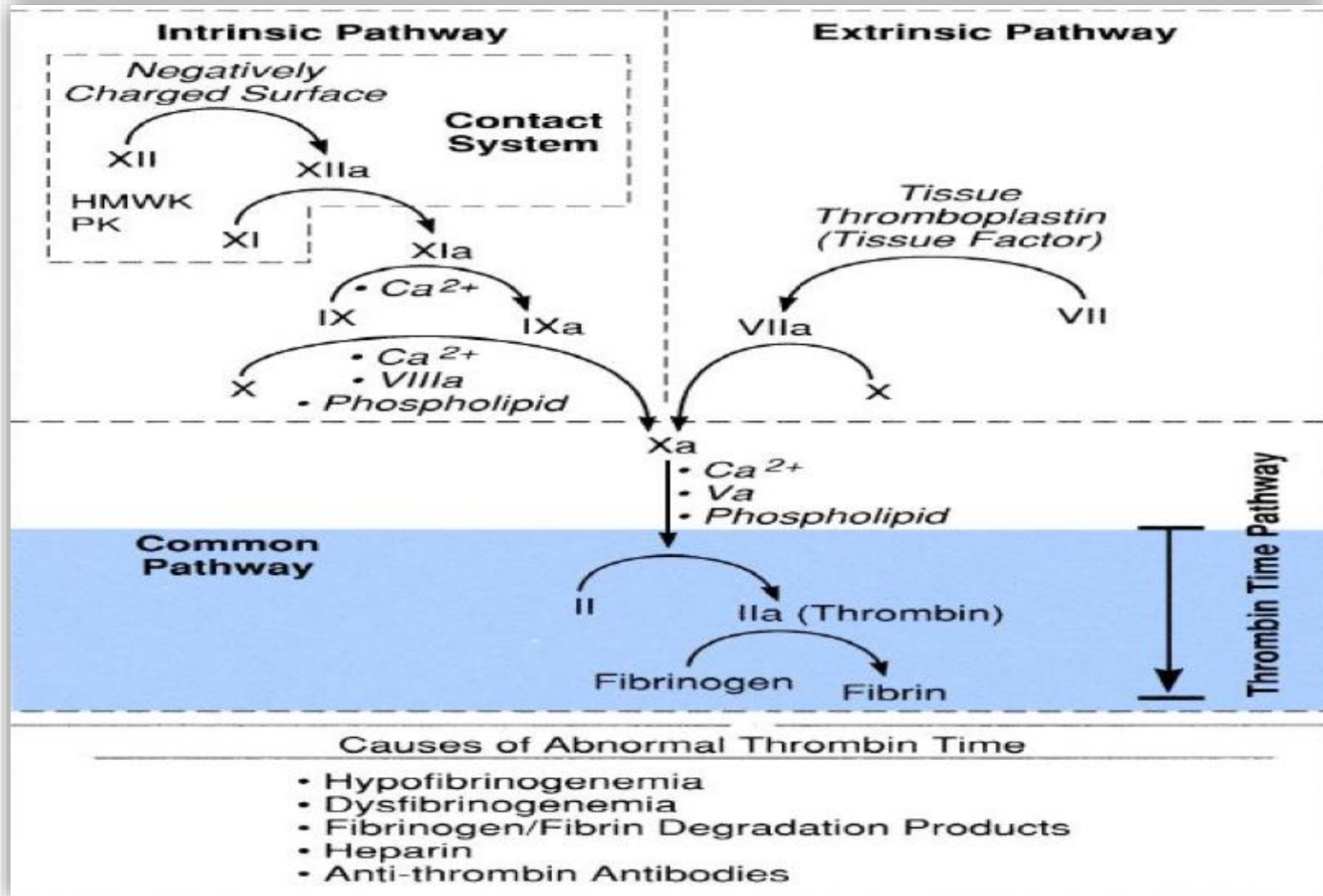
Thrombin Time (TT): Definition and Principle

- TT is a blood test which measures the time it takes for a clot to form in the plasma from a blood sample containing anticoagulant after an excess of thrombin has been added.
- Thrombin is added to the samples of plasma. If the plasma does not clot immediately, a quantitative (fibrinogen deficiency) or qualitative (dysfunctional fibrinogen) defect is present.
- If a patient is receiving heparin, a substance derived from snake venom called reptilase is used instead of thrombin.
- Reptilase has a similar action to thrombin but unlike thrombin, it is not inhibited by heparin.

Clinical Significance

- **The thrombin time** is used to *diagnose bleeding disorders and to assess the effectiveness of fibrinolytic therapy.*
- The time between the addition of the thrombin and the clot formation is recorded as **the thrombin clotting time.**
- *Normal range:* 10-15 sec. If reptilase is used, the reptilase time should be between 15 -20 sec.
- *Thrombin time can be prolonged by :* heparin, factor XIII deficiency and fibrinogen deficiency or abnormality.

Clinical Significance



Screening Test	Normal	Defective
Platelet count (PC)	150-450/cumm	• P.C.
Bleeding time (BT)	2-7 minutes	• Platelet function
		• Vascular function
Prothrombin time (PT)	12-14s	• Extrinsic pathway
		• (FVII def.)
		• Common Path. (FX, V, II, I def.)
Activated Partial Thromboplastin time (APTT)	35-45s	• Intrinsic
		• Common path (FX, V, II, I def)
Clot stability (CS)	Stable at 2 hr, 24 hrs	• F, XIII def.

References

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**Vitamin K
dependent factors
are:**

II, V, IX, & X

Hemophilia A:

Caused by the
deficiency of
Factor VIII

Hemophilia B:

Caused by the
deficiency of
Factor IX

Thanks for Your Attention