



Laboratory Tests of Hemostasis (Part 1)

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Disclosures

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 - Amgen
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Learning Objectives:

Part 1: Laboratory Tests of Hemostasis

1. Describe analytical principles of routine coagulation screening tests and their clinical utilization.
2. Discuss methods and limitations of clot-based assays, immunoassays, and chromogenic assays.
3. Interpret abnormal coagulation screening test results and what additional testing is recommended.

Material To Cover

1. *The Hemostatic Balance*
2. *Overview of The Coagulation Cascade and Testing*
3. *Functional and Immuno-assays*
4. *The Prothrombin and Activated Partial Thromboplastin Times*
5. *Other Tests:*
 - *Specific Factor Assays*
 - *Anti-Xa Heparin Assay*
 - *Thrombin Time*
 - *Fibrinogen Assay*
 - *D-Dimer*
 - *Thromboelastography (TEG) and Thromboelastometry (ROTEM)*
6. *Interpretation of Prolonged PT and/or aPTT Results*
7. *Tests Of Thrombotic Disease*
8. *Heparin Induced Thrombocytopenia/Thrombosis (HITT): Pathophysiology*
9. *Antiphospholipid Antibody Syndrome*
10. *Laboratory Testing for Thrombophilia (Hypercoagulable State)*
11. *APC-Resistance—Screening Assay For Factor V Leiden*
12. *Conditions That Impact Tests for Thrombotic Risk Factors.*
13. *If/When to Do Hypercoagulable Work-up.*

The Hemostatic Balance

➤ Hemostasis is the balance between bleeding and clotting and involves both cellular and soluble enzymatic components of the blood and vasculature.

➤ Primary Hemostasis/Cellular Components

- Vascular endothelial cells
- Platelets

➤ *Secondary Hemostasis/Fluid Phase Components*

- *Coagulation proteins: (Focus for today)*

Coagulation
Processes

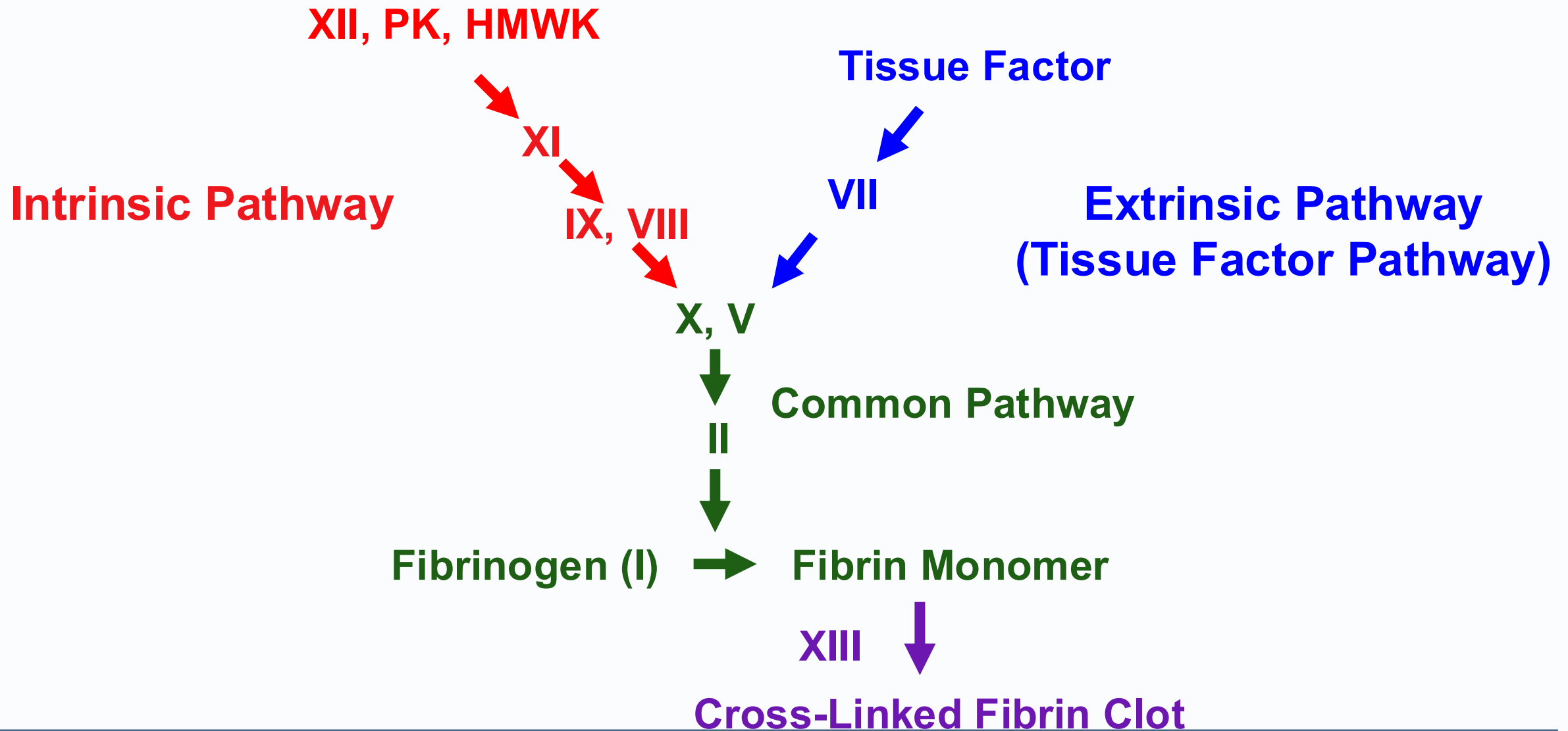
Physiologic
Anticoagulation

Hemorrhage

Thrombosis



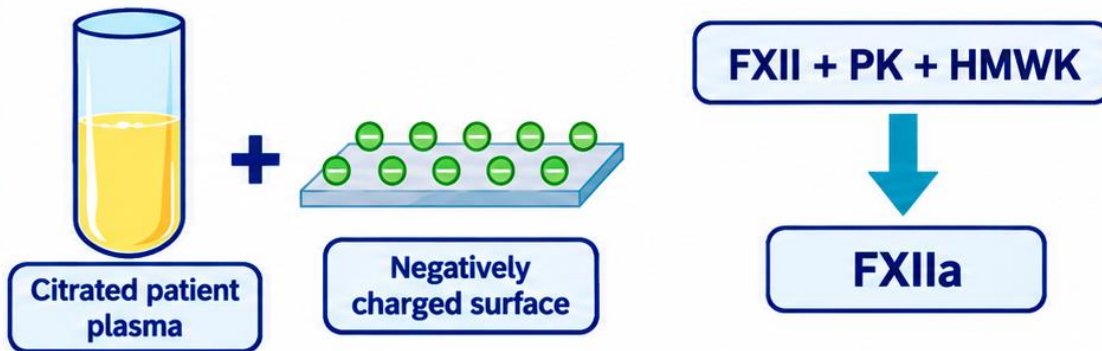
Contemporary Representation of the Coagulation Cascade



Initiation of the Intrinsic and Extrinsic Pathways

Intrinsic Pathway

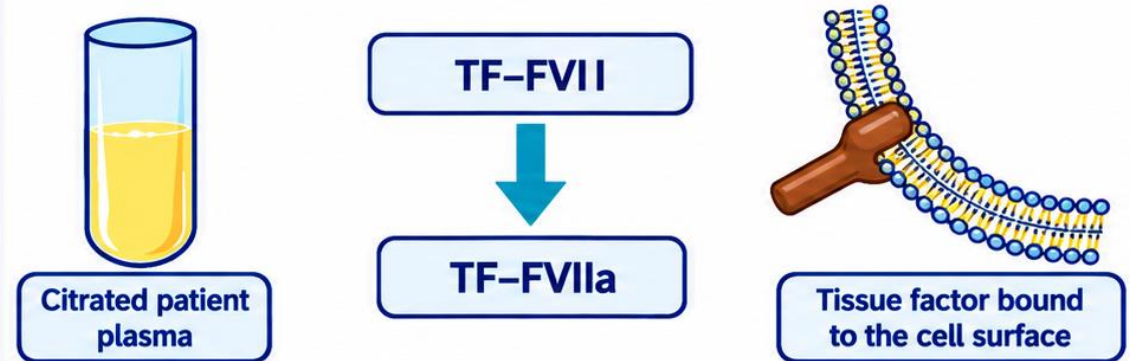
Initiated by a negatively charged surface



Contact activation

Extrinsic Pathway

Initiated by tissue factor



Tissue-factor activation

Contact activators, used *in vitro*, are not physiological.

Endogenous Contact-System Activators

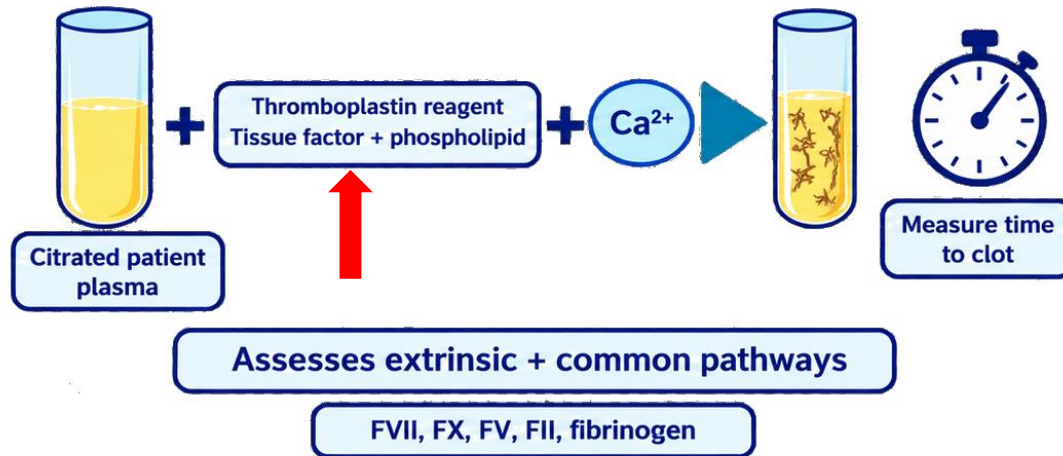
Activator	Source in vivo	Mechanism of contact activation
Inorganic polyphosphate (polyP)	Dense granules of activated platelets (also microbial polyP)	Anionic polymer binds and triggers FXII autoactivation to FXIIa; accelerates FXI and prekallikrein activation; strongest characterized endogenous activator, drives pathologic thrombosis
Extracellular DNA / NETs	Neutrophil extracellular traps, dying/damaged cells	Negatively charged nucleic acid scaffold promotes FXII autoactivation, linking neutrophil activation to coagulation (immunothrombosis)
Extracellular RNA	Released from injured/dying cells	Anionic surface supports a subset of contact-pathway reactions and FXII activation
Mast cell heparin	Degranulating mast cells	Polyanionic glycosaminoglycan activates FXII; implicated in bradykinin-mediated angioedema
Collagen / subendothelial matrix	Exposed after vascular/endothelial injury	Provides negatively charged surface for FXII binding and FXIa generation
Misfolded / aggregated proteins (e.g., amyloid)	Protein aggregation states	Aggregate surfaces serve as contact activators of FXII
Ruptured atherosclerotic plaque material	Disrupted plaque	Exposed anionic/matrix components activate FXII, linking atherothrombosis to contact pathway
Microbial / bacterial surfaces	Pathogen surfaces, microbial polyP	Provide anionic interface for FXII activation, coupling contact system to innate host defense
Plasma kallikrein (fluid-phase)	Circulating zymogen prekallikrein	Reciprocally cleaves FXII → FXIIa, forming feed-forward amplification loop (not surface-dependent)
Plasmin (fluid-phase)	Fibrinolytic system	Proteolytically cleaves and activates FXII
Fibrin	Formed clot	Amplification loop promoting further FXII activation
Endothelial cell-surface complexes	gC1qR / uPAR / cytokeratin-1 receptors	FXII/prekallikrein bound to HK activated in presence of Zn^{2+} ; prekallikrein also activated FXII-independently by PRCP or HSP90

References for Table

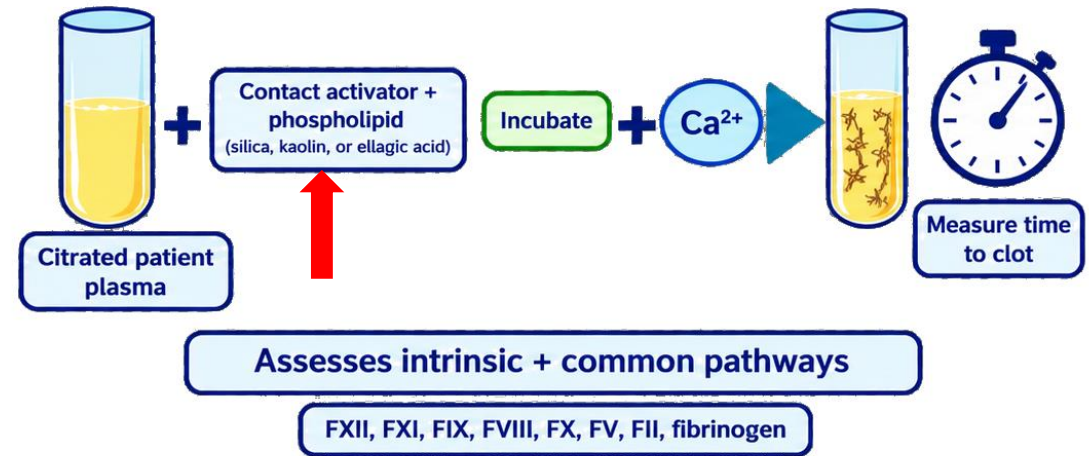
- Naudin, C., et al., Factor XII Contact Activation. *Semin Thromb Hemost*, 2017. 43(8): p. 814-826.
- Long, A.T., et al., Contact system revisited: an interface between inflammation, coagulation, and innate immunity. *J Thromb Haemost*, 2016. 14(3): p. 427-37.
- Gajsiewicz, J.M., S.A. Smith, and J.H. Morrissey, Polyphosphate and RNA Differentially Modulate the Contact Pathway of Blood Clotting. *J Biol Chem*, 2017. 292(5): p. 1808-1814.
- Cohn, D.M. and T. Renne, Targeting factor XIIa for therapeutic interference with hereditary angioedema. *J Intern Med*, 2024. 296(4): p. 311-326.

Prothrombin Time and Activated Partial Thromboplastin Time

Prothrombin Time (PT)



Activated Partial Thromboplastin Time (aPTT)



- Functional Assays: Clot-Based Assays.
- Good screening assays for moderate to severe factor deficiencies.
- Based on a functioning coagulation cascade.
- Results in fibrin clot.
- Subject to exogenous and intrinsic interferences.

- The key differences in the assays is source of phospholipid and use of an “activator” versus Tissue Factor.”
- Micronized/colloidal silica and ellagic acid are the two activators most commonly used aPTT reagents.

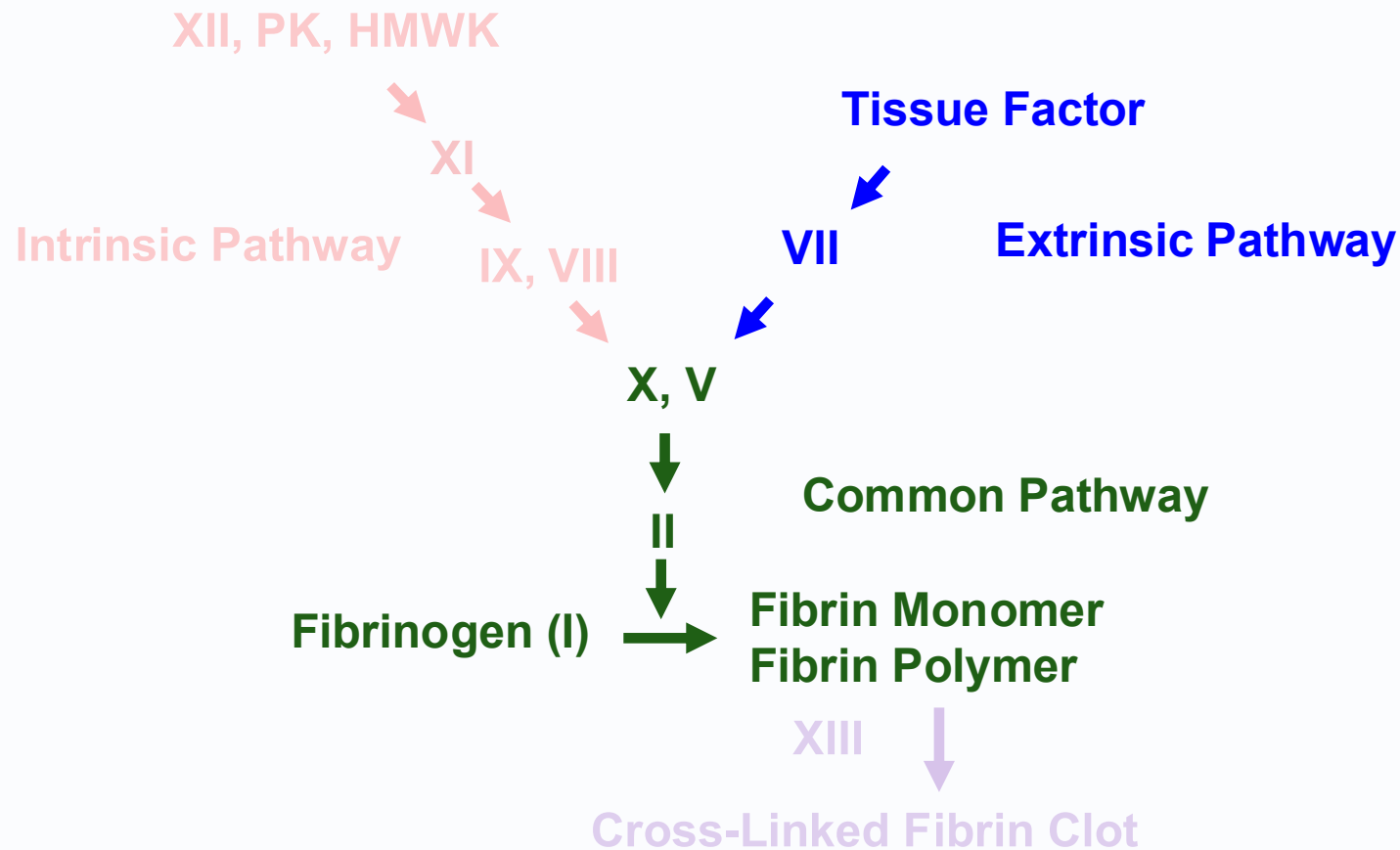
Lupus Anticoagulant

- The lupus anticoagulant prolongs the aPTT because aPTT reagents contain a low, rate-limiting amount of anionic phospholipid, so LA antibodies (mainly against β 2-glycoprotein I or prothrombin bound to that surface) interfere with phospholipid-dependent complex assembly.
- The PT stays normal and there is no bleeding *in vivo* because thromboplastin reagents and activated platelet/cell membranes provide abundant phospholipid that overwhelms the antibody — a matter of phospholipid quantity, not a chemically different "reagent versus natural" phospholipid.
- Lupus anticoagulant–hypoprothrombinemia syndrome (LAHPS), in which acquired anti-prothrombin antibodies reduce circulating factor II activity. (Rare).
 - Wongkrajang P, et al. Comparison of two algorithms of APTT-based lupus anticoagulant assay, two Dilute Russell viper venom time reagents, and silica clotting time. PLoS One. 2026 Jun 26;21(6):e0352430. doi: 10.1371/journal.pone.0352430. PMID: 42361075; PMCID: PMC13308853.
 - Rasmussen KL, et al. Unexpected, isolated activated partial thromboplastin time prolongation: A practical mini-review. Eur J Haematol. 2020; 104: 519–525. <https://doi.org/10.1111/ejh.13394>
 - Ieko M, et al Lupus anticoagulant-hypoprothrombinemia syndrome and similar diseases: experiences at a single center in Japan. Int J Hematol. 2019 Aug;110(2):197-204. doi: 10.1007/s12185-019-02674-2. Epub 2019 Jun 4. PMID: 31165409.

Uses of the PT/aPTT

- The PT and PTT/aPTT were developed to screen for factor deficiencies.
 - Still widely used for this indication.
- Currently, much of the use is for monitoring and titrating the doses of anticoagulation.
- The PT used for warfarin and other Vitamin K inhibitors.
 - Owren & Aas first described a relationship of the prolongation of the PT by Dicumarol (an early Vitamin K inhibitor) and the risk of bleeding.
 - Owren, P. A., & Aas, K. (1951). *Scandinavian Journal of Clinical and Laboratory Investigation*, 3(3), 201-208.
- The aPTT has traditionally been the standard test to monitor and titrate heparin.
 - Adopted in the 1950's.
 - This is being replaced with the anti-Factor Xa assay for heparin and low molecular weight heparin. (To be discussed further below).

Prothrombin Time



➤ Measures:

- Extrinsic and Common Pathways
- Endpoint is Fibrin Polymer

➤ Major Uses:

- Hemostasis Screening
- Monitoring Warfarin Anticoagulation

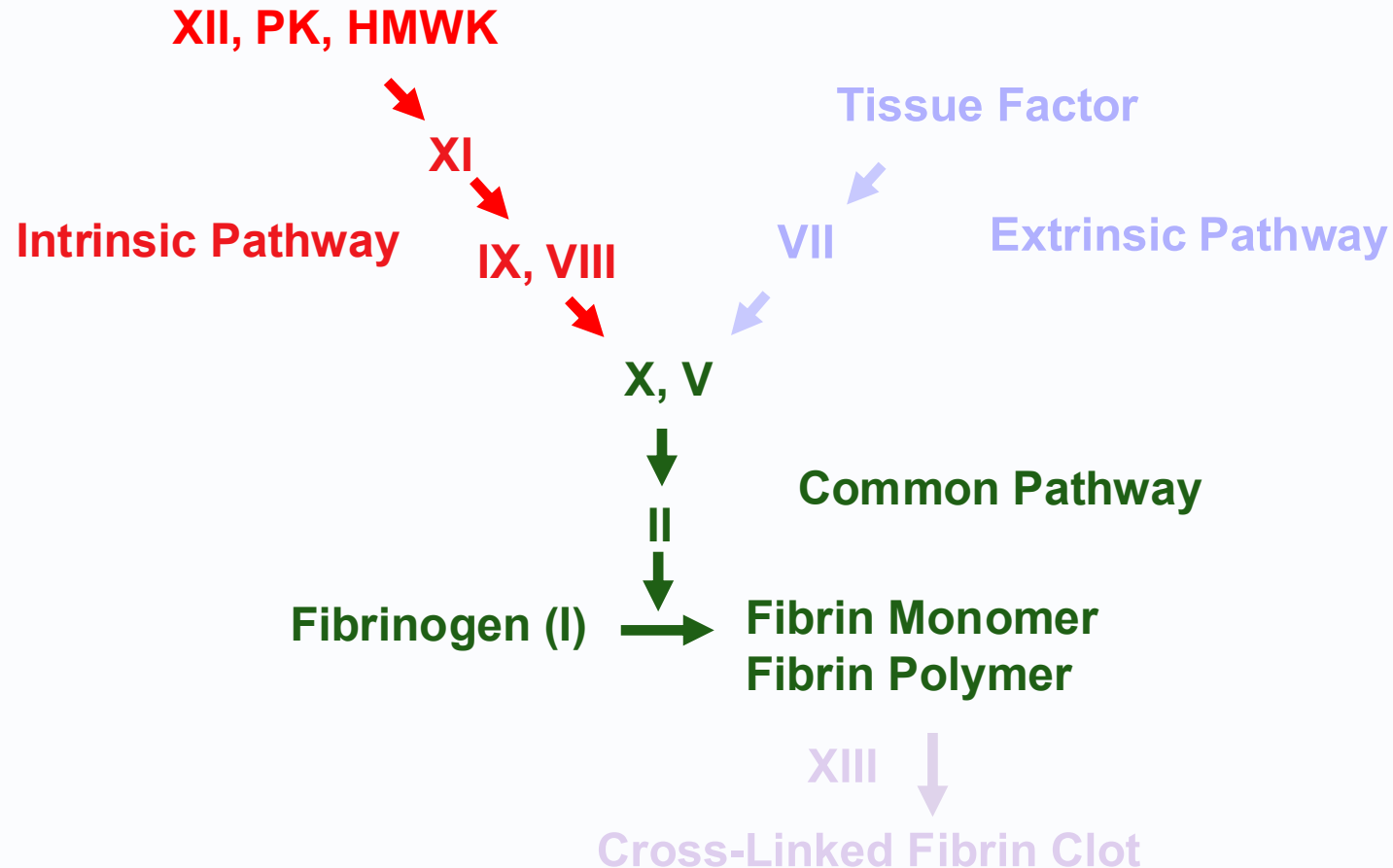
➤ Results:

- Reported in Seconds and INR.

INR (International Normalized Ratio)

- For many years, the prothrombin time was essential to titrate warfarin dosing.
 - Target was 2-3 X normal for optimal balance of efficacy and safety.
 - The reagents for the assay were derived from animal (mostly rabbit) brains.
 - The reagents were not well standardized, leading to inconsistency of results.
- The **INR= International Normalized Ratio** was developed to standardize results, accounting for variation in thromboplastin reagents.
 - Introduced in 1983. (WHO Expert Committee on Biologic Standardization. 33rd Report. Technical Report Series No 687. World Health Organization. 1983.)
- The **INR= International Normalized Ratio**
 - (patient PT/mean normal PT)^{ISI}
 - **ISI= International Sensitivity Index**
- INR validated for warfarin titration, but in practice it is also used in other settings.
 - Dorgalaleh, A, Favaloro, EJ, Bahraini, M, Rad, F. Standardization of Prothrombin Time/International Normalized Ratio (PT/INR). Int. J. Lab Hematol. 2021; 43: 21- 28. <https://doi.org/10.1111/ijlh.13349>

Activated Partial Thromboplastin Time (aPTT)



➤ Measures:

- Intrinsic and Common Pathways
- Endpoint is Fibrin Polymer

➤ Major Uses:

- Hemostasis Screening
- Monitoring unfractionated heparin therapy.
- (Anti-Factor Xa assay is replacing the aPTT for heparin monitoring).

➤ Results:

- Reported in Seconds

APTT: Monitoring UFH Therapy

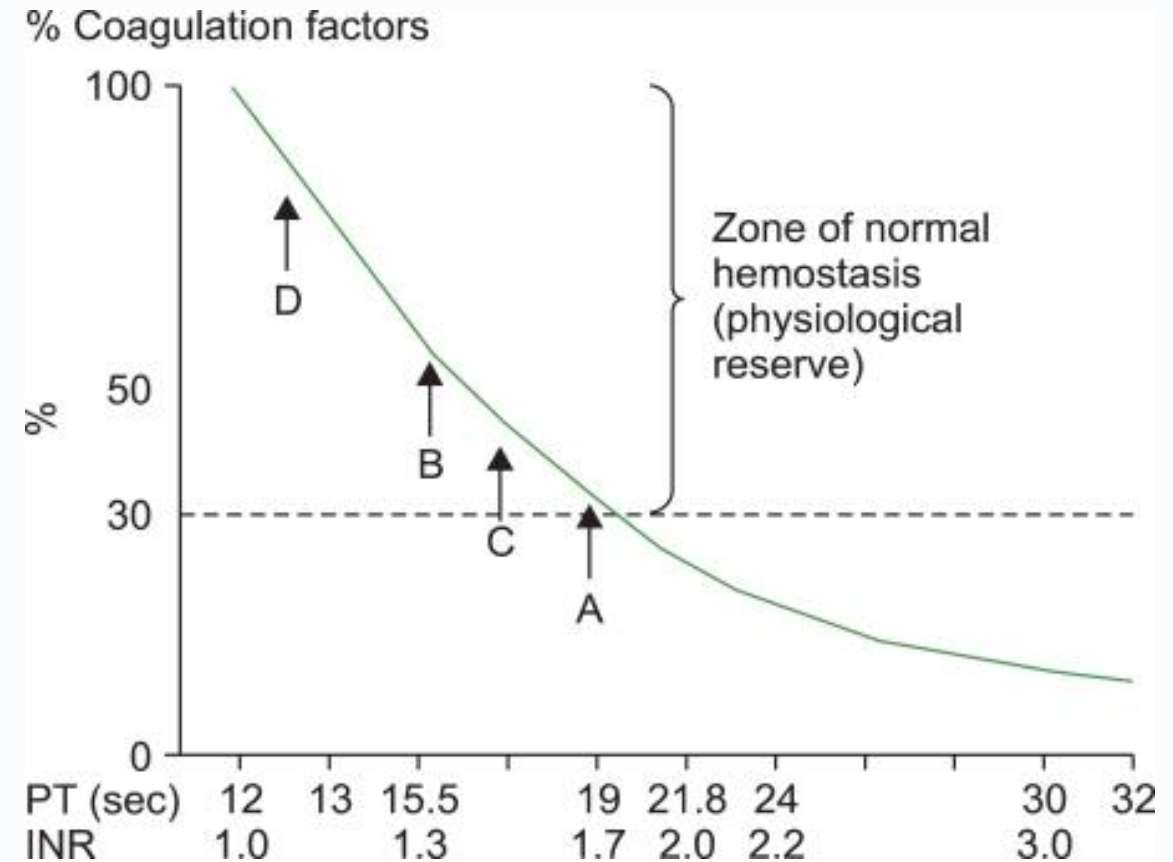
- **APTT reagents vary in their sensitivity to UFH**
 - Laboratories establish reagent-specific therapeutic range.
 - Reagent standardization has not been successful.
- **APTT response to heparin may be exaggerated by:**
 - Conditions that elevate the baseline APTT:
 - Concomitant warfarin therapy
 - Lupus anticoagulant
 - Liver disease
- **APTT response to heparin may be blunted by:**
 - Conditions that shorten the baseline APTT:
 - Cause of *in vitro* drug “resistance”
 - Elevated Factor VIII (inflammation, cancer, etc.)
 - Antithrombin deficiency
- **Alternative: Chromogenic anti Xa assay**

The PT/aPTT Reflect *In Vitro* Coagulation, Not *In Vivo* Coagulation.

- An elevated aPTT does not necessarily mean an increased risk of bleeding.
- Deficiencies of factors not associated with bleeding (i.e. Contact Factors: Factor XII, Prekallikrein, High Molecular Weight Kininogen), prolong aPTT to same extent, or more, as clinically relevant factors.
- Lupus Anticoagulant may prolong the aPTT and is associated with thrombosis.

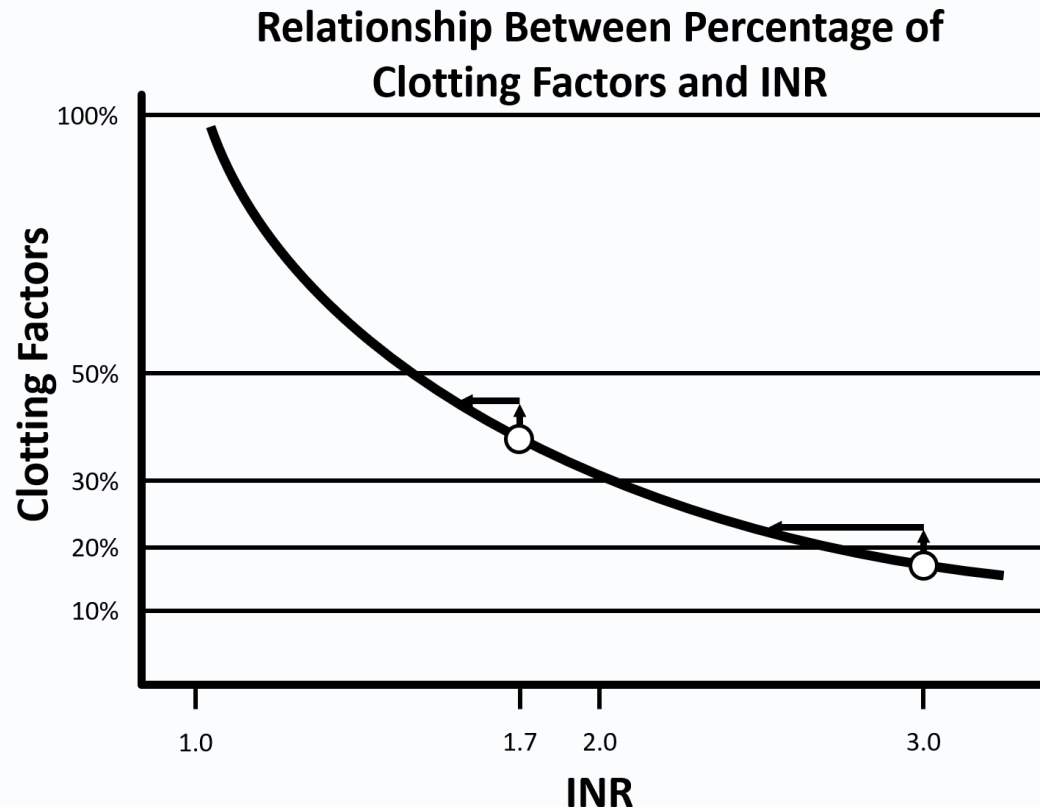
Use of PT/aPTT To Screen For Factor Deficiencies

- Assays are valuable as screening tests for factor deficiencies.
- In general, only when factor levels are under 50% are the PT/aPTT prolonged.
 - Falay et al. J Clin Lab Anal. 2018;32:e22415.



Dzik WH. Component therapy before bedside procedures.
2nd ed. Baltimore, MD: AABB Press, 2005.

Use of PT/INR for Warfarin Titration or Factor Deficiencies



- As an approximate rule of thumb:
- INR 2.0: Vit K dependent factors ~ 30%
- INR 3.0: Vit K dependent factors ~ 20%

- <https://www.aliem.com/inr-reduction-ffp/>
- Adapted from Dzik W. Reversal of drug-induced anticoagulation: old solutions and new problems. Transfusion. 2012;52(s1):45S-55S. doi: 10.1111/j.1537-2995.2012.03690.x. PMID: 22578371.

Preanalytical Considerations (I)

➤ Contamination:

- Blood drawn through a heparinized central venous catheter.
- Blood drawn through a central venous catheter, with "stale" blood.
- Only use blood from venipuncture.

➤ Specimen Collection

- 3.2% sodium citrate
- 9:1 volume of blood to anticoagulant

Preanalytical Considerations (II)

- **Polycythemia/Hemoconcentration:** Plasma volume per unit blood is reduced, relative citrate excess, leaving insufficient calcium available on recalcification and producing artifactual prolongation.
 - In a direct patient study of hematocrits 55–72%, non-adjusted PT and aPTT results were significantly and exponentially prolonged with rising hematocrit, correctable by adjusting citrate volume.
 - Marlar RA, et al. Am J Clin Pathol. 2006 Sep;126(3):400-5. doi: 10.1309/RRQKT2JEYV33D19D. PMID: 16880137.
- **Severe Anemia.** Sample is relatively under-anticoagulated (plasma-to-citrate ratio increased), but the measured effect on PT/aPTT is generally not clinically significant.
 - Siegel JE, et al. Am J Clin Pathol. 1998 Jul;110(1):106-10. doi: 10.1093/ajcp/110.1.106. PMID: 9661929.
- **Specimen Stability**
 - Assays become unreliable in non-refrigerated whole blood.
 - Assays also become unreliable with time in refrigerated whole blood, although with takes longer.
 - Platelet Poor Plasma more stable.
 - If assays cannot be done “promptly,” best to freeze platelet poor plasma.
 - Separate from cells and frozen at -80⁰ C

Factor Assays:

- PT or aPTT are adopted by mixing diluted patient's plasma (or reference plasma for standard curve) with known specific factor deficient plasma and performing a PT or aPTT on the mix.

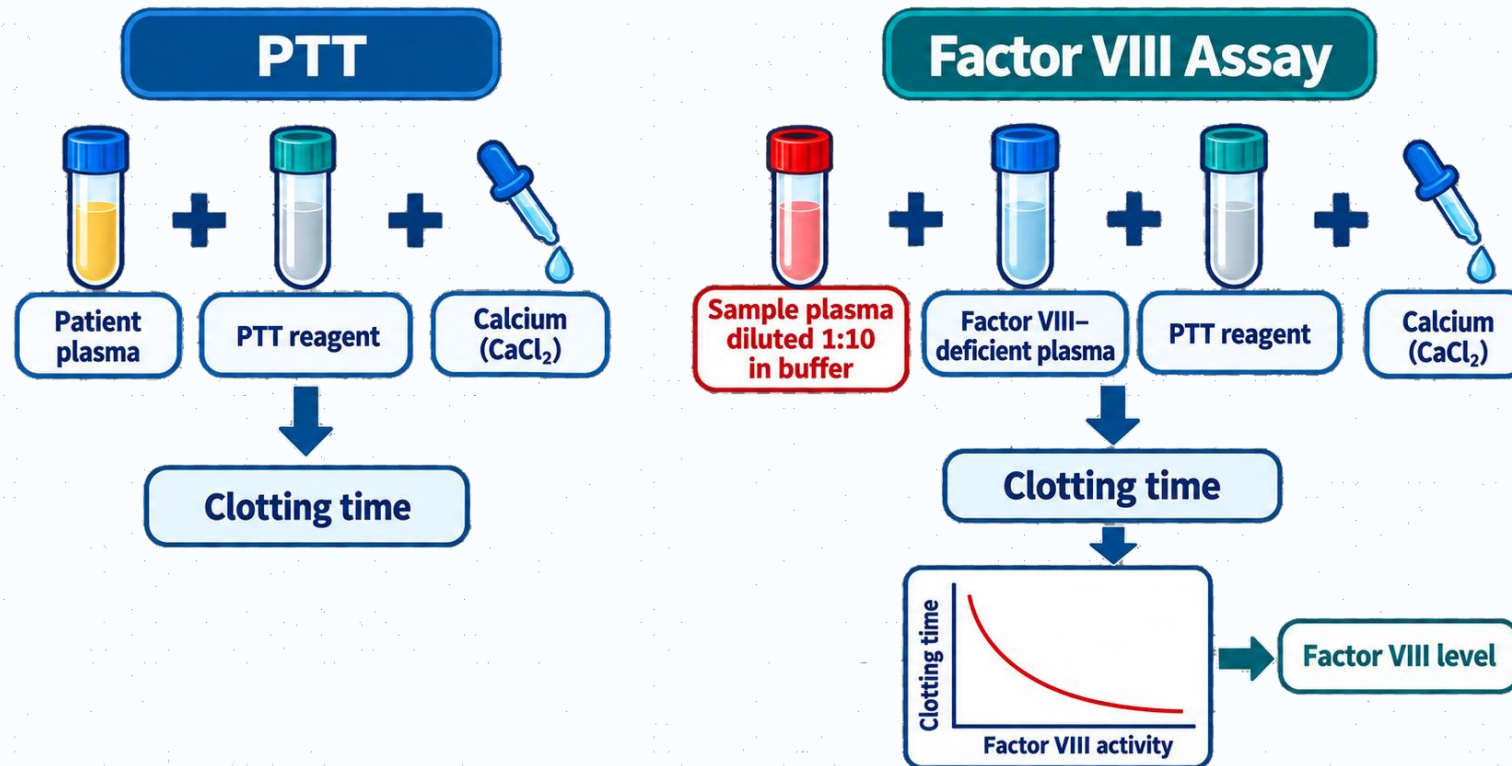


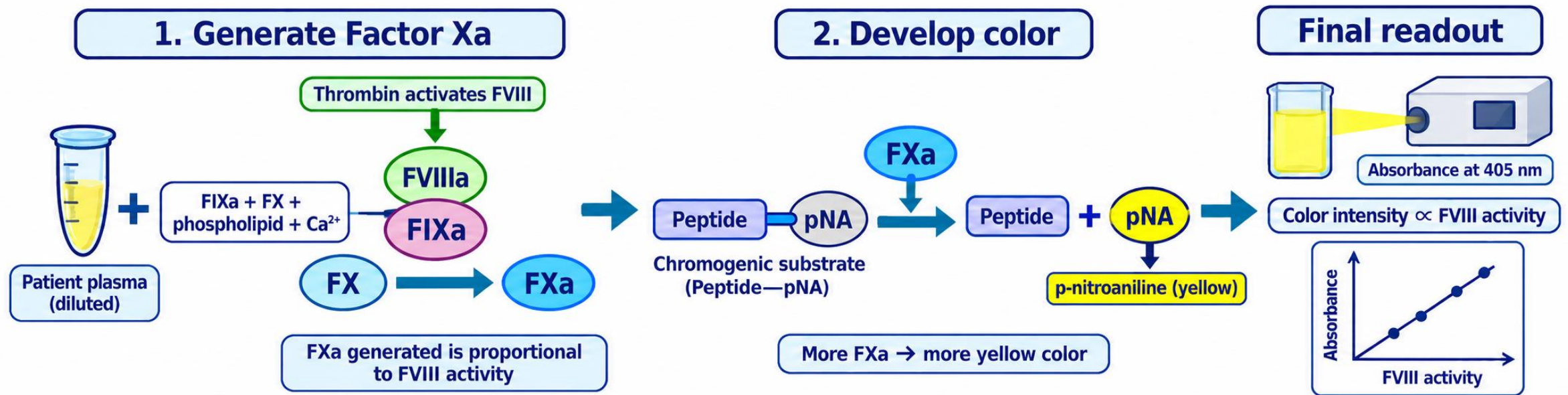
Image created with
AI assistance.

Interferent	Mechanism (Of falsely low one-stage factor activity)	References
Lupus anticoagulant (high titer)	Nonspecific phospholipid-directed inhibitor prolongs the aPTT-based clot endpoint, (chromogenic FVIII is spared.)	[1-2]
DOACs – direct FXa inhibitors (rivaroxaban, apixaban, edoxaban)	Inhibit FXa in the clotting cascade, prolonging the clot time so activity reads low; unlike LA, also lower chromogenic (FXa-based) results.	[3-4]
DOAC – direct thrombin inhibitor (dabigatran)	Inhibits thrombin generation at the assay endpoint, prolonging clot formation and producing falsely low clot-based (and chromogenic IIa-based) factor activity.	[1, 3]
Unfractionated heparin	Antithrombin-mediated inhibition of thrombin/FXa acts as a nonspecific inhibitor across FVIII/FIX/FXI/FXII; flagged by prolonged thrombin time with normal reptilase time.	[1, 5]
Low-molecular-weight heparin	Predominantly anti-FXa activity modestly prolongs the aPTT in a dose-dependent way, lowering clot-based factor activities (usually minimal at trough).	[6]
Protamine sulfate	Directly prolongs PT/aPTT and decreases one-stage FVIII and FIX activity, with weak apparent inhibitor effect at high concentrations ($\geq 200 \mu\text{g/mL}$).	[7]
High-titer single-factor inhibitor (e.g., FVIII autoantibody)	Marked aPTT prolongation causes "pseudo-deficiency" of the other intrinsic factors (FIX/FXI/FXII), which normalize on higher dilution or chromogenic assay.	[1, 8]
Wrong anticoagulant tube (EDTA/heparin plasma; serum)	EDTA/heparin behave like inhibitors (uncorrecting mixing study); serum lacks consumed factors – both give spuriously low FVIII/FIX/FXI/FXII.	[5]
Underfilled citrate tube (over-citration)	Excess citrate relative to plasma chelates added calcium, prolonging clot time and falsely lowering FVIII, FIX, and VWF.	[5]
Sample clotting/activation (partial in-tube clotting)	Consumption of labile factors during clotting lowers measured FVIII/FIX activity (in contrast, cold/contact activation can falsely raise FVIII).	[3, 5]
High hematocrit	Reduced plasma-to-citrate ratio over-citrates the plasma, prolonging clot times and falsely lowering factor activities.	[1, 5]
Hemolysis, lipemia, icterus, paraproteins/rheumatoid factor	Optical/matrix interference with clot detection (and, for paraproteins, impaired fibrin polymerization) distorts and can falsely lower clot-based factor results.	[1, 9]

References for Table

1. Quality laboratory issues in bleeding disorders. *Haemophilia : The Official Journal of the World Federation of Hemophilia*. 2016. Adcock DM, Mammen J, Nair SC, de Lima Montalvão SA.
2. Chromogenic factor VIII activity assay. *American Journal of Hematology*. 2014. Moser KA, Adcock Funk DM.
3. Shifting landscape of hemophilia therapy: Implications for current clinical laboratory coagulation assays. *American Journal of Hematology*. 2018. Al-Samkari H, Croteau SE.
4. Clinical utility and impact of the use of the chromogenic vs one-stage factor activity assays in haemophilia A and B. *European Journal of Haematology*. 2020. Marlar RA, Strandberg K, Shima M, Adcock DM.
5. Preanalytical issues that may cause misdiagnosis in haemophilia and von Willebrand disease. *Haemophilia : The Official Journal of the World Federation of Hemophilia*. 2018. Favaloro EJ, Lippi G.
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7. Confounding Effect of Therapeutic Protamine and Heparin Levels on Routine and Special Coagulation Testing. *Blood Coagulation & Fibrinolysis : An International Journal in Haemostasis and Thrombosis*. 2020. Khandelwal A, Phua CW, Chaudhry HR, et al.
8. Retrospective Examination of Coagulation Parameters in 33 Patients With Autoimmune Coagulation Factor Deficiencies in Japan: A Single-Center Analysis. *Thrombosis Research*. 2022. Osaki T, Souri M, Ogawa Y, et al.
9. Guidelines on the laboratory aspects of assays used in haemostasis and thrombosis. *British Journal of Haematology*. 2020. Baker P, Platton S, Gibson C, et al.

Chromogenic Factor VIII Assay



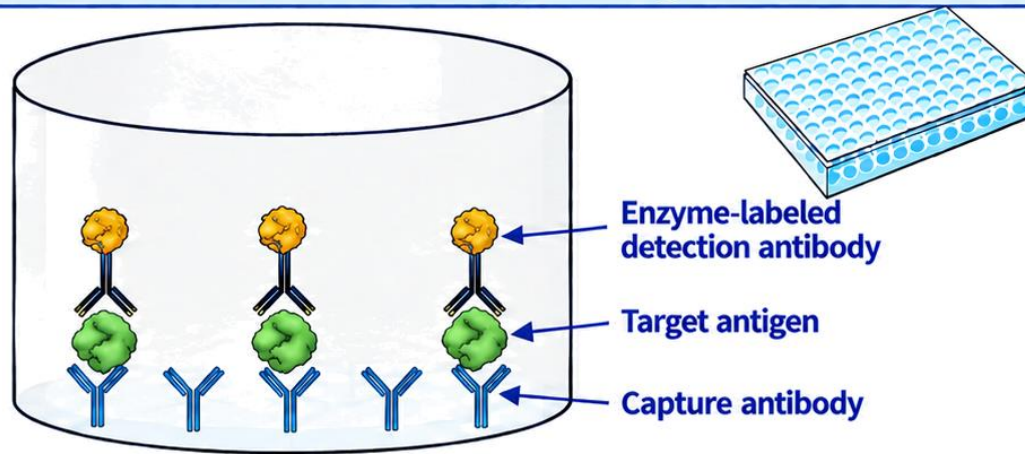
- The chromogenic FVIII assay readout is based on Factor Xa (FXa) generation, measured indirectly through cleavage of an FXa-specific chromogenic substrate.
- The assay does not measure FVIII directly; it exploits FVIII's physiologic role as the cofactor that accelerates FIXa-mediated activation of FX to FXa.
- It is largely protected from heparin and direct thrombin inhibitors (and lupus anticoagulant), but direct FXa inhibitors (rivaroxaban, apixaban, edoxaban) inhibit the FXa endpoint itself and cause falsely low chromogenic FVIII activity.
 - Moser, K.A. and (Adcock) Funk, D.M. (2014), Chromogenic factor VIII activity assay. Am.J. Hematol., 89: 781-784. <https://doi.org/10.1002/ajh.23723>

Antigen Assays

- Measures the amount of protein antigen present rather than function.
- Measured Immunologically
 - Latex Induced Agglutination Assays (LIA)
 - Enzyme-Linked Immunosorbent Assay (ELISA)

Sandwich ELISA Assay

1. Form the antibody-antigen sandwich



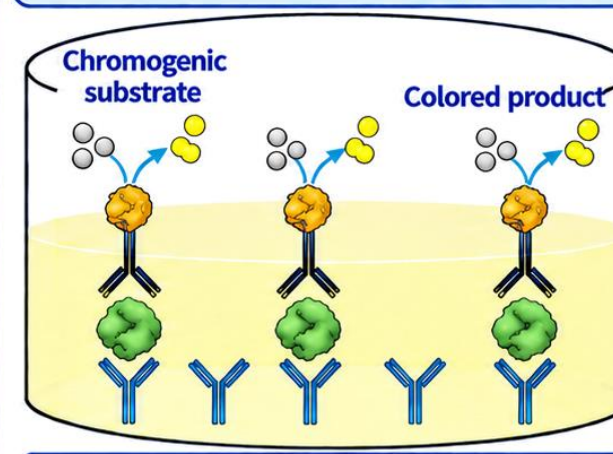
Add sample

Wash

Add detection antibody

Wash

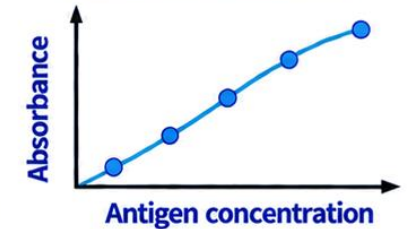
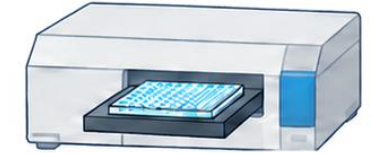
2. Develop and measure color



Enzyme converts substrate to colored product

Color intensity $\propto$ antigen concentration

Measure absorbance



- First antibody captures antigen to surface.
- Second antibody, labelled with enzyme, binds to immobilized antigen.
- Substrate cleaved by conjugated enzyme
- Color development a function of enzyme capture
- Spectrophotometric quantification

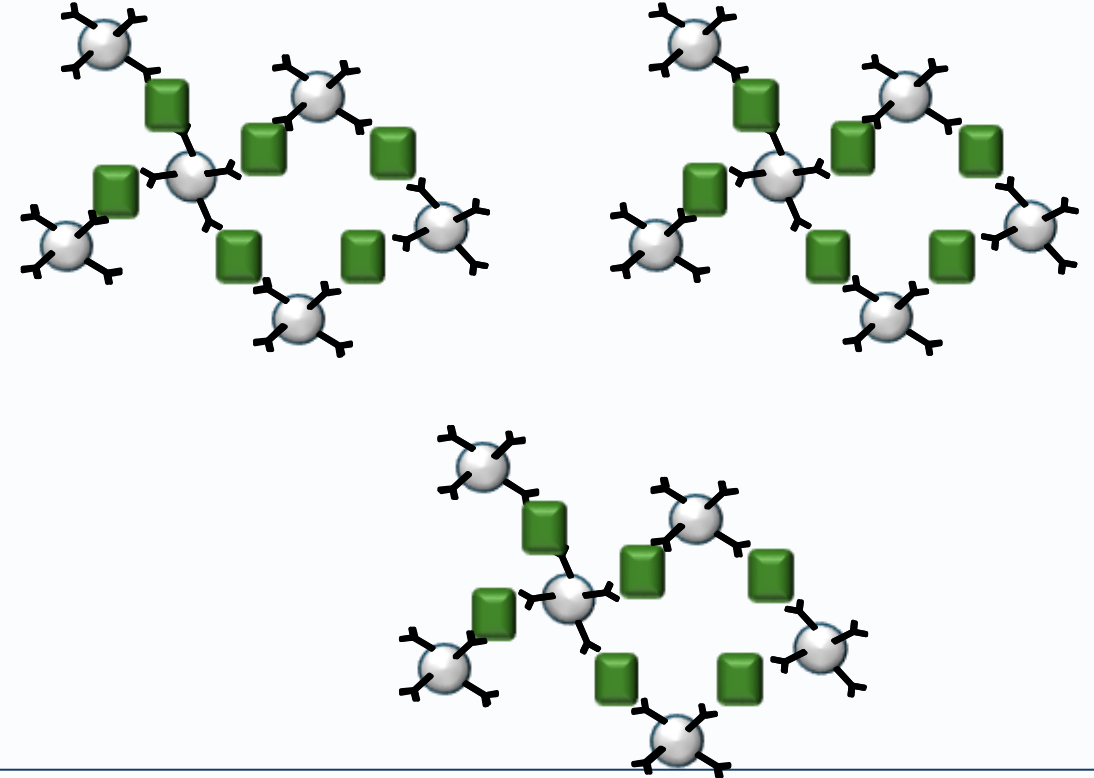
Latex Agglutination

- Antibody coated latex beads
- Agglutination in presence of antigen
- Agglutination is measured optically

Absence/Low Level of Antigen



High Level of Antigen



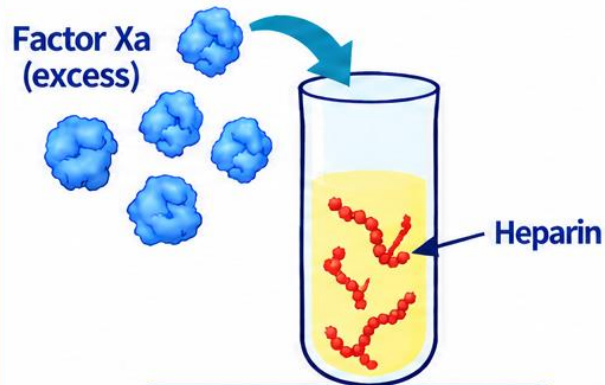
Other Tests

- Anti-Factor Xa Chromogenic Assay
- Thrombin Time
- Fibrinogen Assay
- D-Dimer
- Thromboelastography (TEG) and Thromboelastometry (ROTEM)

Anti-Factor Xa Chromogenic Assay: Monitoring Unfractionated Heparin and Low Molecular Weight Heparin

Anti-Factor Xa Chromogenic Assay

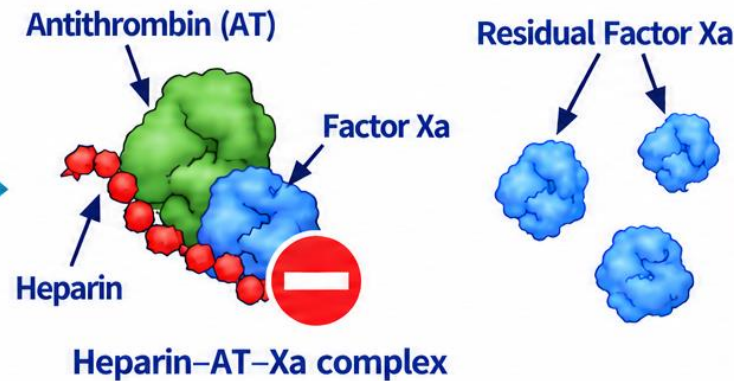
1. Add excess Factor Xa



Patient plasma containing heparin and endogenous antithrombin

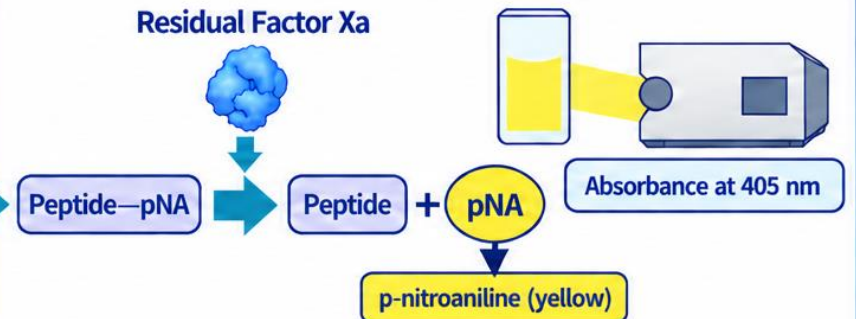
A fixed excess of Factor Xa is added

2. Heparin-antithrombin inhibits Factor Xa



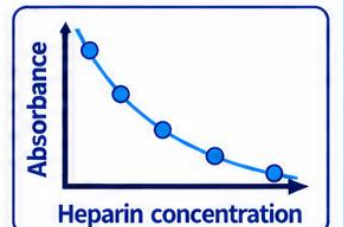
Factor Xa inhibited is proportional to heparin concentration

3. Develop and measure color



Color intensity $\propto$ residual Factor Xa

More heparin $\rightarrow$ less Factor Xa
 $\rightarrow$ less yellow color



*Some, but not all, anti-Xa assay kits add exogenous antithrombin.

Anti-Factor Xa Chromogenic Assay

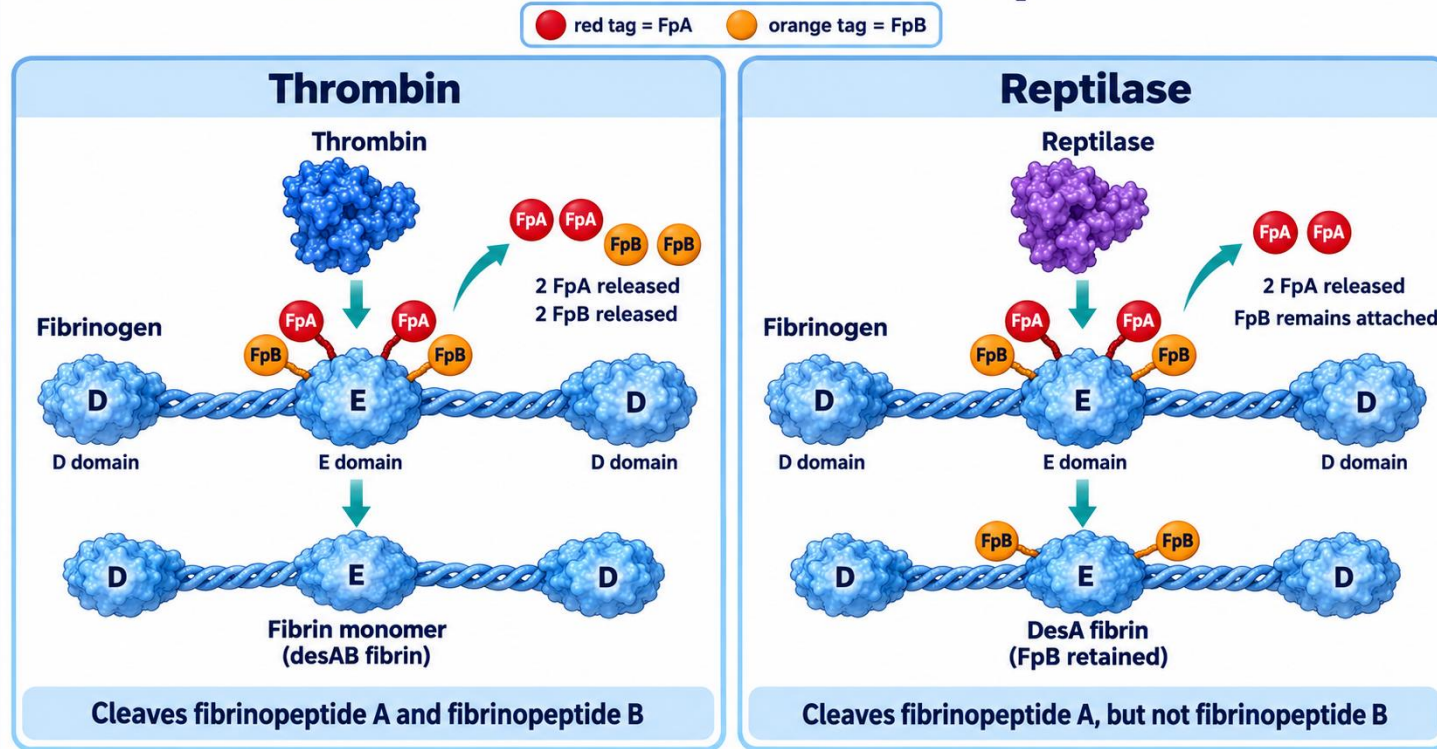
- Specifically determines anticoagulant activity of LMWH and/or UFH by measuring ability of heparin:antithrombin to inhibit F Xa.
- More specific than aPTT since it measures inhibition of a single enzyme.
- Major advantage is lack of biologic interference.
- Assay can be utilized for unfractionated heparin, Low Molecular Weight Heparin, and Anti-Factor Xa DOACs.
 - Eikelboom JW. Thromb Haemost 2006;96:547-52.
 - Francis JL. Pharmacotherapy 2004;24:108S-19S.

Thrombin Time:

Evaluates the Conversion of Fibrinogen to Fibrin

- Screening test for fibrinogen quantity and quality.
- Thrombin cleaves Fibrinopeptides A and B, resulting in fibrin monomer.
- Fibrin monomers spontaneously polymerize into fibrin clot as endpoint.
 - Does not require Factor XIIIa cross-linking.
- Thrombin Time is prolonged by hypofibrinogenemia or dysfibrinogenemia.
 - Need to assess fibrinogen antigen levels.
- Thrombin Time is prolonged by some anticoagulants (i.e. heparin, dabigatran).

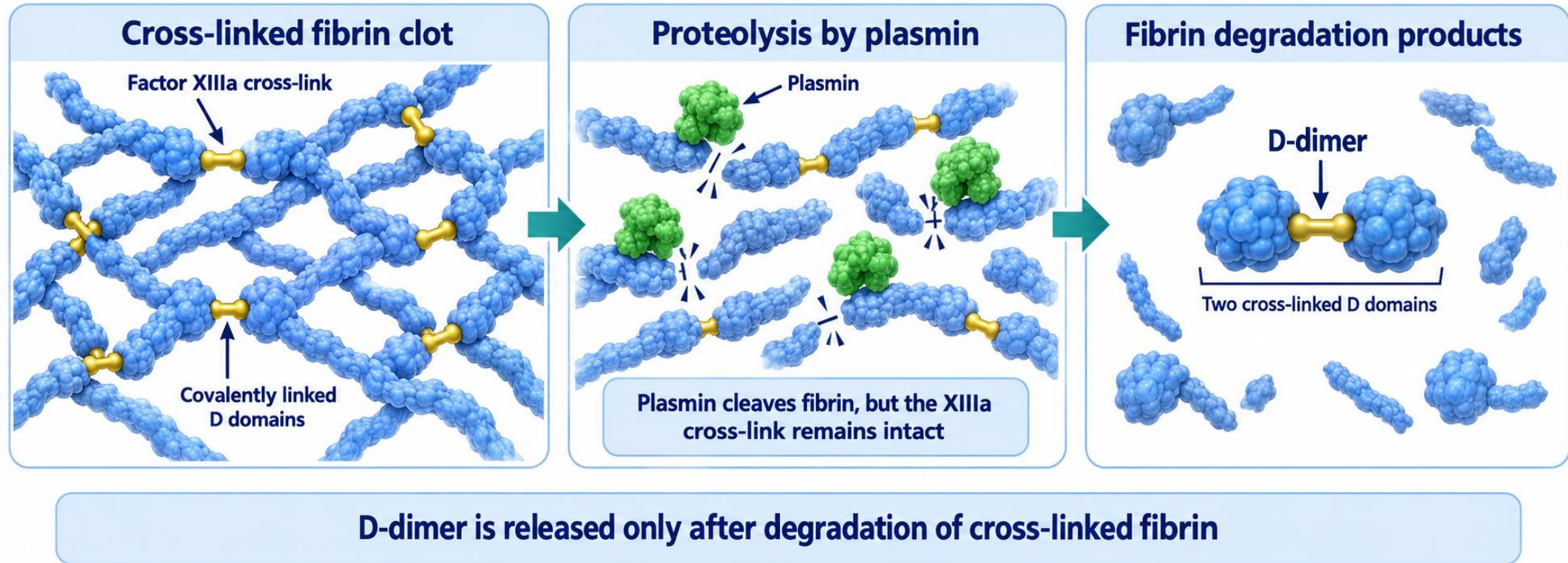
Fibrinogen Cleavage: Thrombin vs Reptilase



- Reptilase-time clot composed initially of desA fibrin and a thrombin-time clot containing desAB fibrin can generate a readable clotting endpoint.
- Thrombin, but not reptilase, is inhibited by heparin:AT complex.
- Therefore, discordance of the two is usually due to heparin contamination.
 - Discordance also seen in some types of dysfibrinogenemia.

D-Dimer:

Plasmin Degradation of Cross-Linked Fibrin



- Presence of D-Dimer indicates activation of both coagulation (thrombin) and fibrinolysis (plasmin).

Utilization of D-Dimer Testing

- Evaluate for DVT/PE
 - Rule out thrombosis in the outpatient setting in individuals with low suspicion for thrombosis.
 - Good negative predictive value.
 - Cut off: <230 ng/ml (Reference Range: <243 ng/ml)
 - Negative Predictive Value 100%
 - The lower the cutoff, the better the NPV.
 - Specificity 49%
- DIC
- Elevated by any process that activates the coagulation system:
 - Cancer
 - Inflammatory conditions
- A positive D-Dimer does not necessarily indicate a thrombosis or DIC is occurring. Other conditions may elevate D-Dimer.
 - Lim W, et al. American Society of Hematology 2018 guidelines for management of venous thromboembolism: diagnosis of venous thromboembolism. Blood Adv. 2018 Nov 27;2(22):3226-3256. doi: 10.1182/bloodadvances.2018024828. PMID: 30482764; PMCID: PMC6258916.
 - Righini M, et al. Age-adjusted D-dimer cutoff levels to rule out pulmonary embolism: the ADJUST-UNLIKELY study. JAMA. 2014;311(11):1117-1124. doi:10.1001/jama.2014.2135. PMID: 24643601. PubMed
 - Kearon C, et al. Diagnosis of pulmonary embolism with D-dimer adjusted to clinical probability. N Engl J Med. 2019;381(22):2125-2134. doi:10.1056/NEJMoa1909159. PMID: 31774957. PubMed

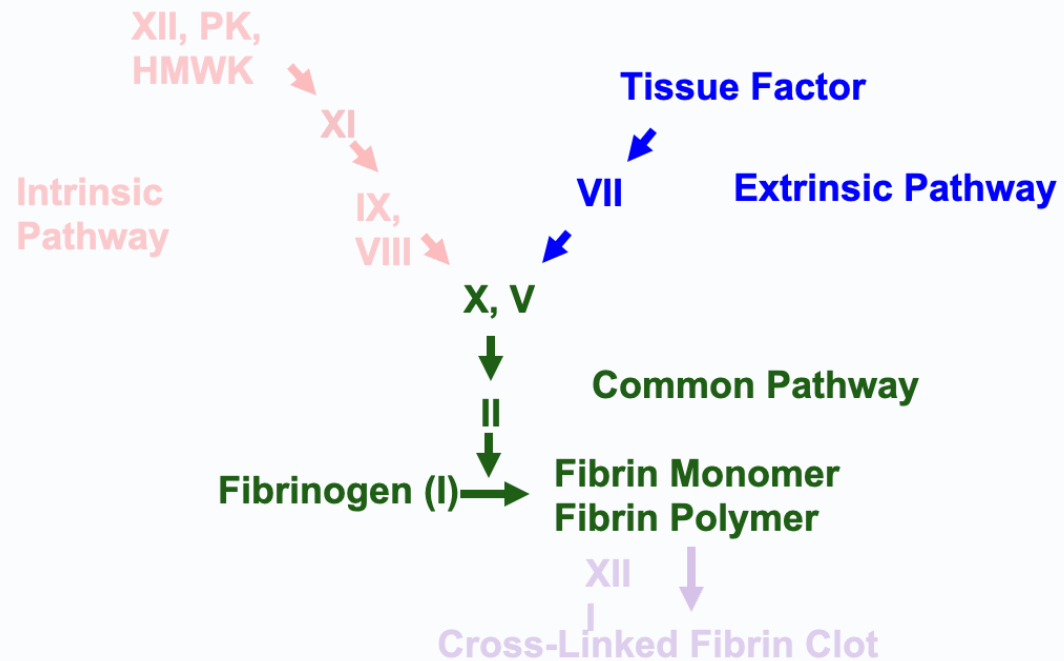


Interpretation of Prolonged PT and/or aPTT Results

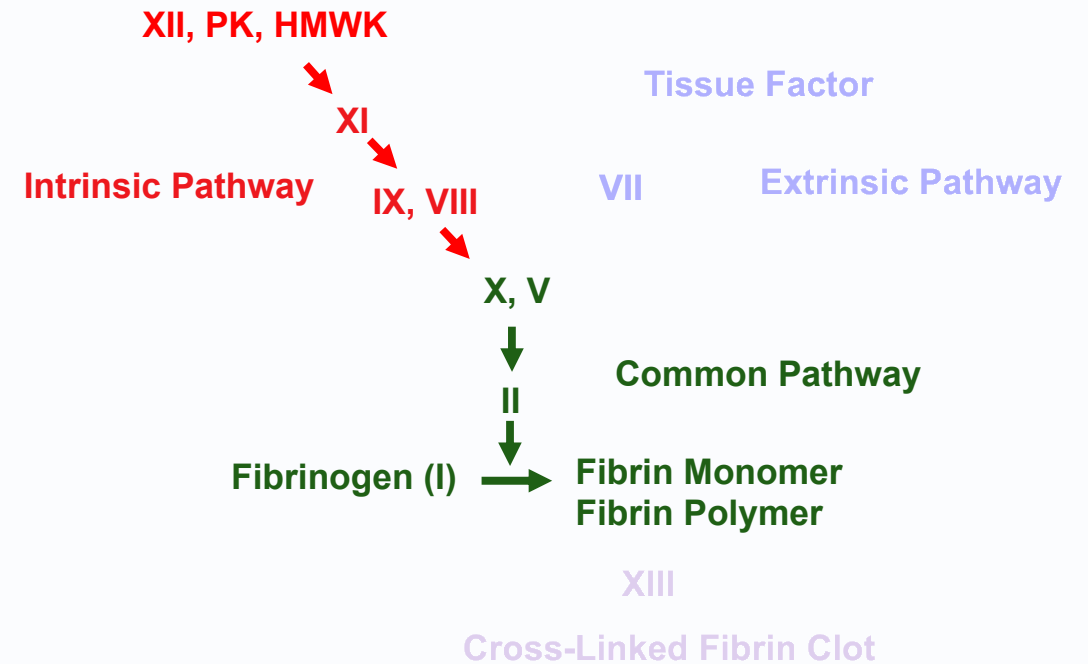
Use of PT and/or aPTT and Interpretation

- Factor Deficiency
 - Single vs multiple deficiencies.
 - In general, factor levels must be under ~40-50% of normal to prolong the test.
 - Factor XIII deficiency does not prolong PT or aPTT.
- Acquired Inhibitors
 - Specific factor inhibitor (i.e. F VIII)
- “Global” Anticoagulants
 - Lupus Anticoagulant
 - Paraproteins
- Therapeutic Anticoagulants: UFH, LMWH, Direct Oral Anticoagulants.

Prothrombin Time



aPTT



Sensitivity of PT/aPTT to Factor Deficiencies

Factor	PT	aPTT
I (Fibrinogen)	Yes	Yes
II (Prothrombin)	Yes	Yes
V	Yes	Yes
VII	Yes	No
VIII	No	Yes
IX	No	Yes
X	Yes	Yes
XI	No	Yes
XII	No	Yes
XIII	No	No
Prekallikrein (PK)	No	Yes
High Molecular Weight Kininogen HMWK)	No	Yes

Prolonged PT/APTT Work-Up: Mixing Studies

- Mix patient and normal plasma 1:1
- Perform aPTT and/or PT immediately and after 1-hour incubation at 37°C
- In presence of an inhibitor, the 1:1 mix “fails to correct”.
- Specific antibodies require time to bind to the antigen target.
- Common inhibitors: heparin, DOACs, Lupus Anticoagulant, dysproteins, paraproteins, Fibrin Split Products (DIC), factor-specific antibodies.



Mixing Studies

➤ **Factor Deficiency**

aPTT	Patient	Normal	1:1
Immediate	51"	29"	33"
1 Hour Incubation @ 37°C	52"	29"	32"

➤ **Lupus Anticoagulant**
➤ **Anticoagulants**

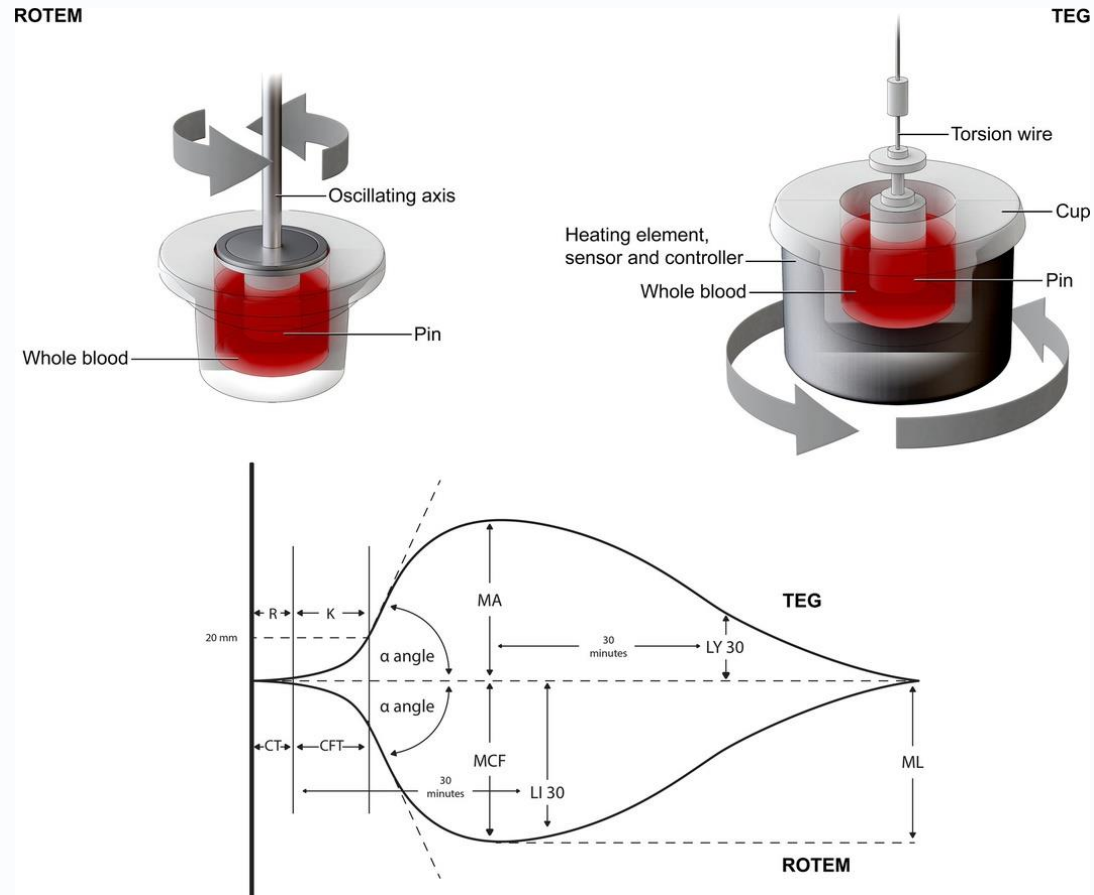
aPTT	Patient	Normal	1:1
Immediate	51"	29"	48"
1 Hour Incubation @ 37°C	52"	29"	50"

➤ **Anti-Factor VIII Antibody**

aPTT	Patient	Normal	1:1
Immediate	51"	29"	33"
1 Hour Incubation @ 37°C	52"	29"	50"

Thromboelastography (TEG) and Thromboelastometry (ROTEM)

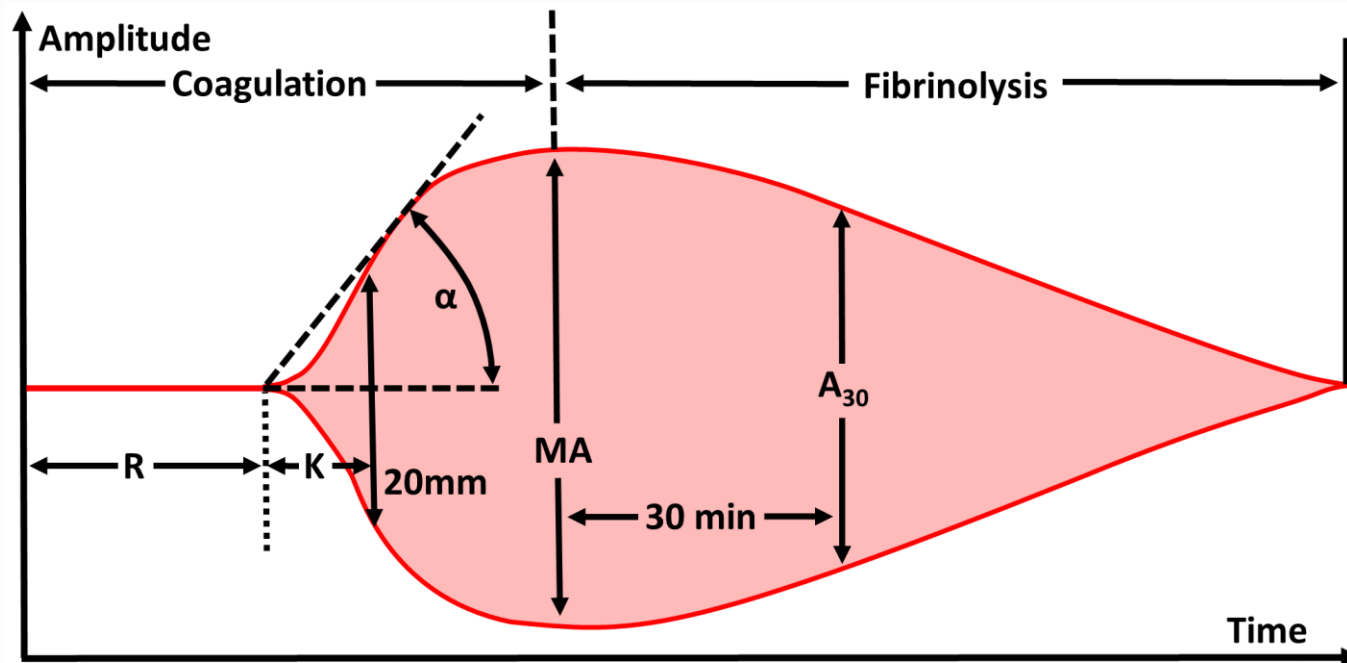
- Global assessment of hemostatic function.
 - Mostly used for Point Of Care testing, in Operating Room, Critical Care.
- TEG and ROTEM measure viscoelastic clot properties in whole blood,
 - Differ chiefly in the mechanical detection system, the activating reagents used, and the nomenclature/reference ranges for their parameters



M. M. Longacre and J. C. Ibla, “TEG and ROTEM: Technology and Clinical Applications, 2026 Update,” *American Journal of Hematology* 100, no. 12 (2025): 2357–2370, <https://doi.org/10.1002/ajh.70074>.

Thromboelastography (TEG) and Thromboelastometry (ROTEM)

- Global assessment of hemostatic function.
 - Mostly used for Point Of Care testing, in Operating Room, Critical Care.



Mikael Häggström, M.D., CC0, via Wikimedia Commons

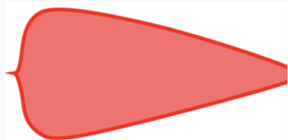
R time: Time to initial clot formation
K time: Time from initial clot formation until reaching 20 mm in amplitude
Alpha angle (α): Angle between the baseline at initial clot formation, and a tangent line that intersects the tracing curve.
Maximum amplitude (MA): Maximum deviation of tracing to baseline.
A₃₀: Amplitude 30 minutes after reaching maximum amplitude.

TEG and ROTEM Interpretation

Clot stage	TEG parameter	ROTEM parameter
Initiation (time to fibrin/2 mm amplitude)	R (reaction time)	CT (clotting time)
Clot kinetics	K, α -angle	CFT (clot formation time), α -angle
Maximum clot strength	MA (maximum amplitude)	MCF (maximum clot firmness)
Fibrinolysis	CL30/CL60, LY30	LI30/LY30, LI60

- Ramiz S, et al. Clinical utility of viscoelastic testing (TEG and ROTEM analyzers) in the management of old and new therapies for hemophilia. *Am J Hematol.* 2019; 94: 249-256. <https://doi.org/10.1002/ajh.25319>
- Mallett, SV. Et al. "Clinical utility of viscoelastic tests of coagulation in patients with liver disease." *Liver International* 33.7 (2013): 961-974

Use of TEG/ROTEM

Condition	Appearance	Main treatment
Normal		
Hemodilution or clotting factor deficiency		Fresh frozen plasma
Fibrinogen deficiency		Cryoprecipitate
Low or dysfunctional platelets		Platelets
Thrombosis		Anticoagulant
Primary fibrinolysis		Antifibrinolytics or tranexamic acid
Secondary fibrinolysis		Treating disseminated intravascular coagulation

Kreitzer et al. Review of Thromboelastography in Neurocritical Care, Neurocritical Care volume 23, pages 427–433 (2015)

Any Questions?

