Laboratory Tests of Hemostasis (Part 1)

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Disclosures

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  - Amgen
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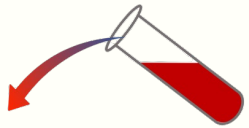
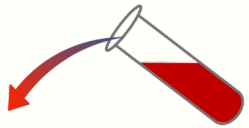
  - Janssen Scientific Affairs
  - Sobi/Dova Pharmaceuticals
  - Luzsana (HengruiUSA) Biotechnology
  - Sanofi



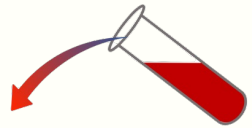
Learning Objectives:
Part 1: Laboratory Tests of Hemostasis

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



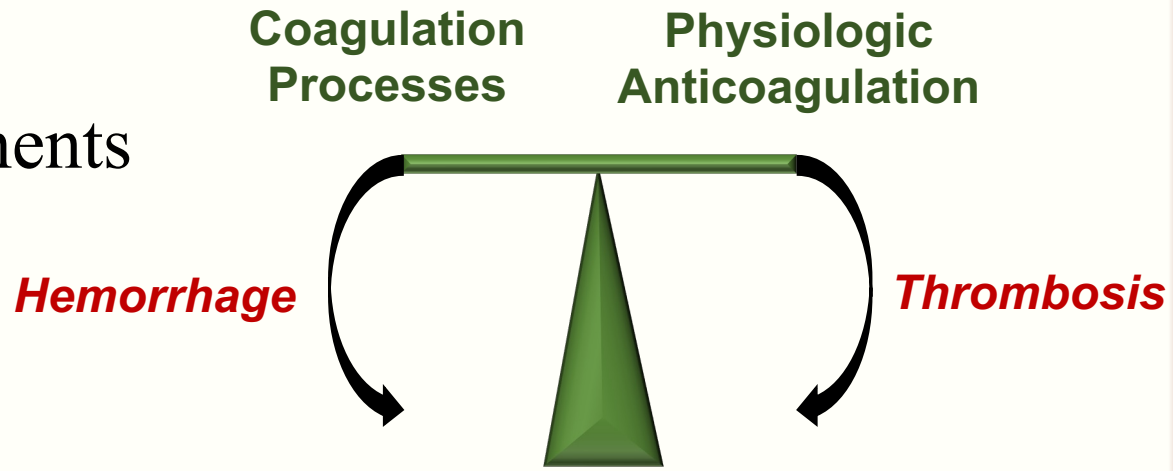
Material To Cover

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:*
   - *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

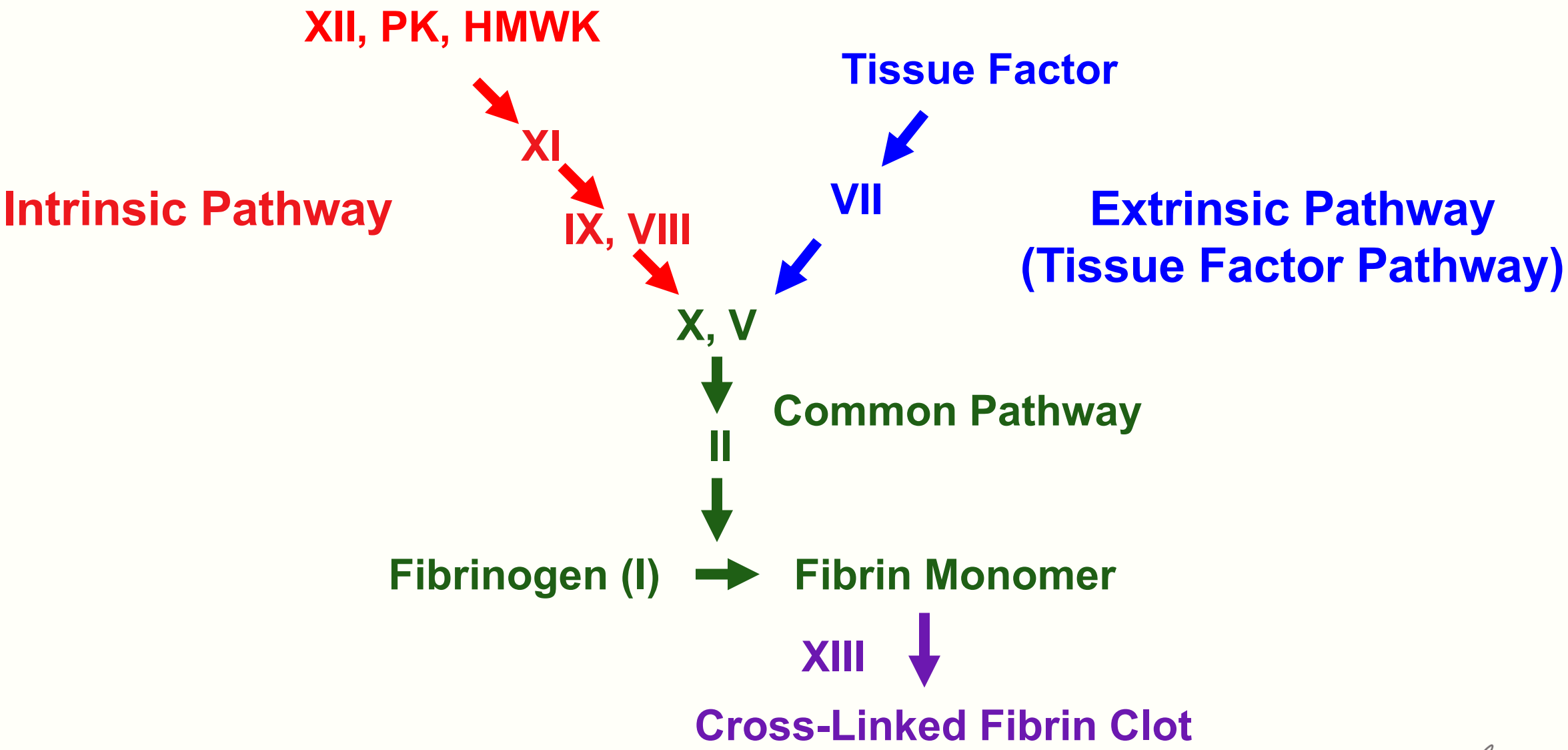
- Hemostasis is the balance between bleeding and clotting and involves both cellular and soluble enzymatic components of the blood and vasculature.



- Primary Hemostasis/Cellular Components
  - Vascular endothelial cells
  - Platelets

- Secondary Hemostasis/Fluid Phase Components
  - Coagulation proteins:

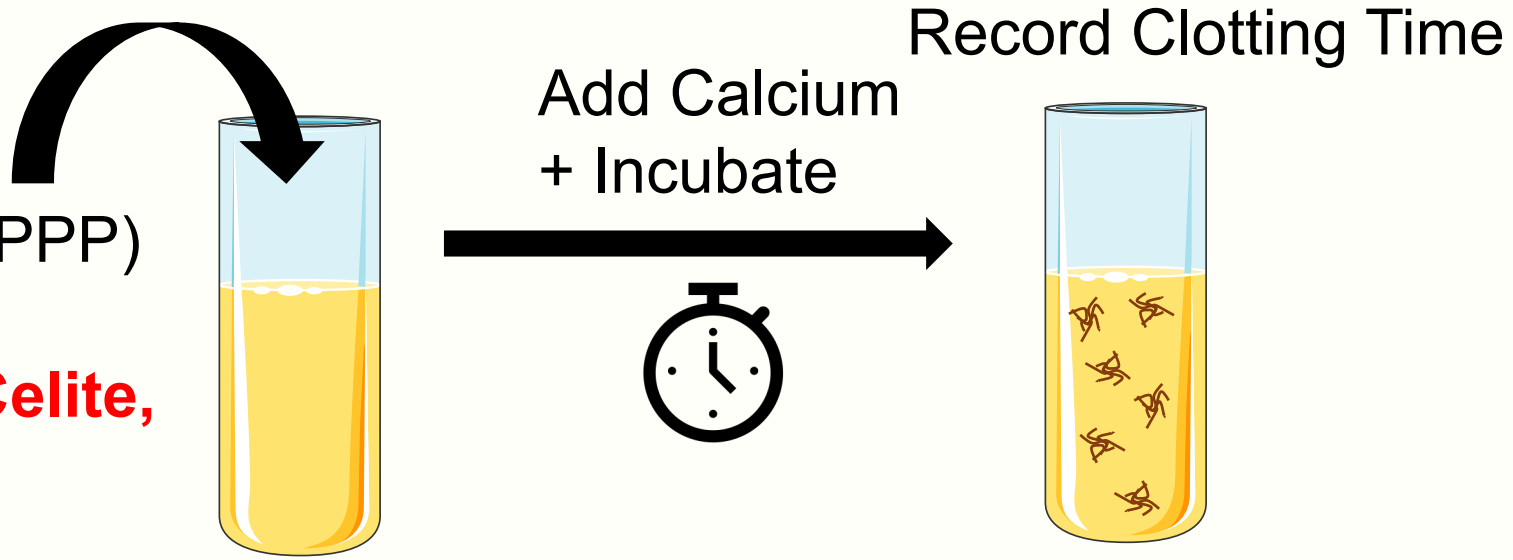
Contemporary Representation of the Coagulation Cascade



Prothrombin Time & activated Partial Thromboplastin Time

- Functional Assays: Clot-Based Assays.
- Good screening assays.
- Based on a functioning coagulation cascade,
- Resulting in fibrin clot.
- Subject to exogenous and intrinsic interferences.

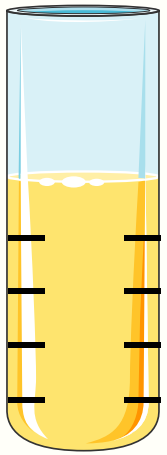
- Platelet Poor Plasma (PPP)
- Phospholipid
- **aPTT: Activator (i.e. Celite, Silica)**
- **PT: Tissue Factor**



There Are Two Ways to Initiate Coagulation System *in Vitro*

Intrinsic Pathway:

Initiated by Negatively Charged Surface



XII, PK, HMWK

↓

XIIa

(APTT)

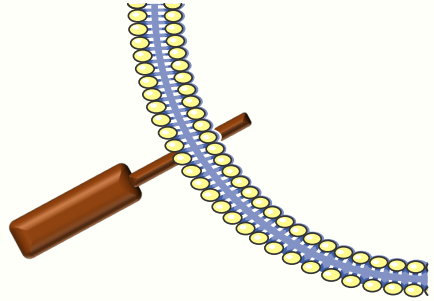
Extrinsic Pathway:

Initiated by addition of Tissue Thromboplastin (Tissue Factor and phospholipid)

TF:VII

↓

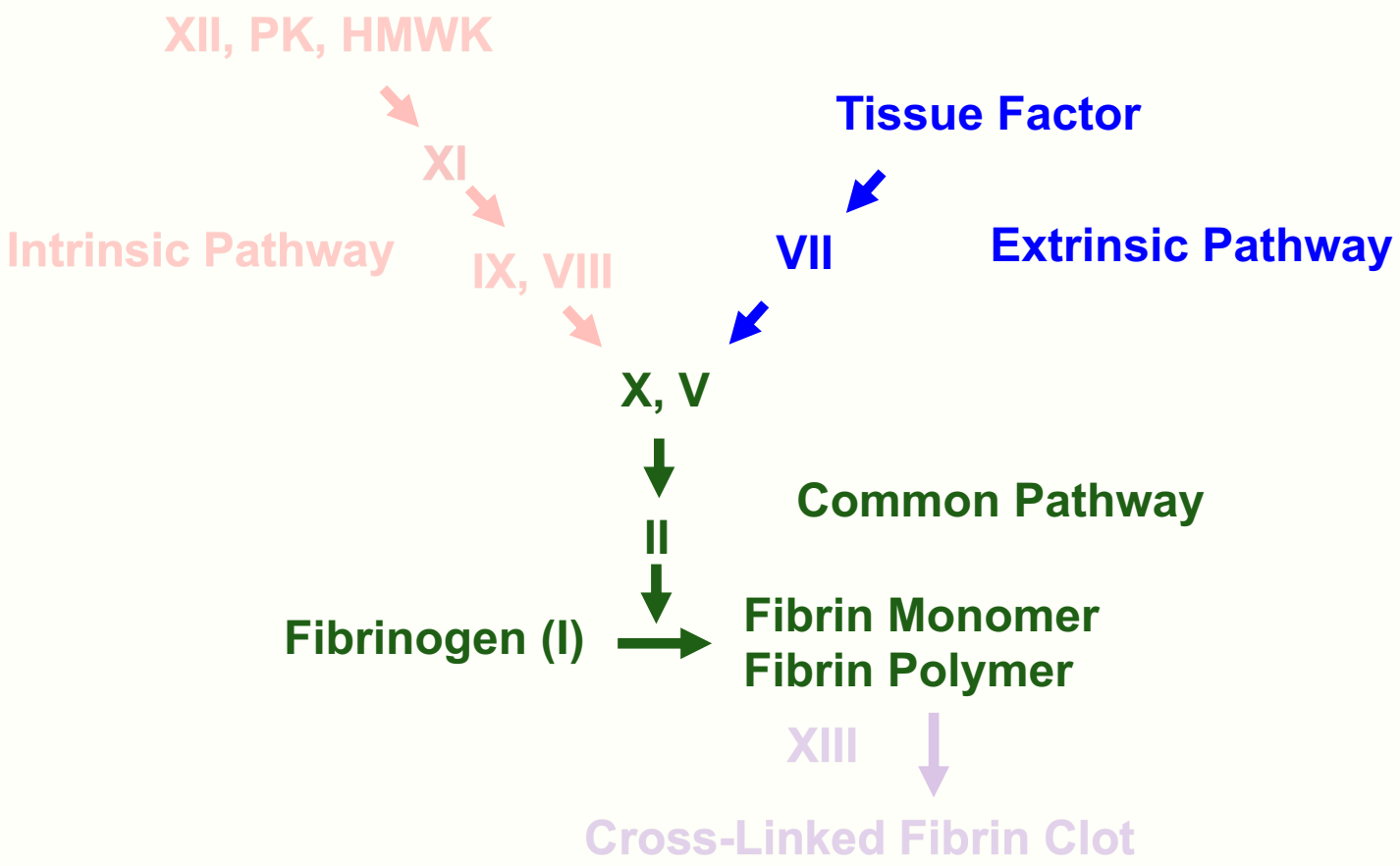
VIIa



TF bound to cell surface

(PT)

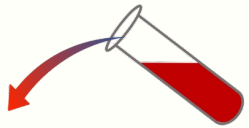
Prothrombin Time



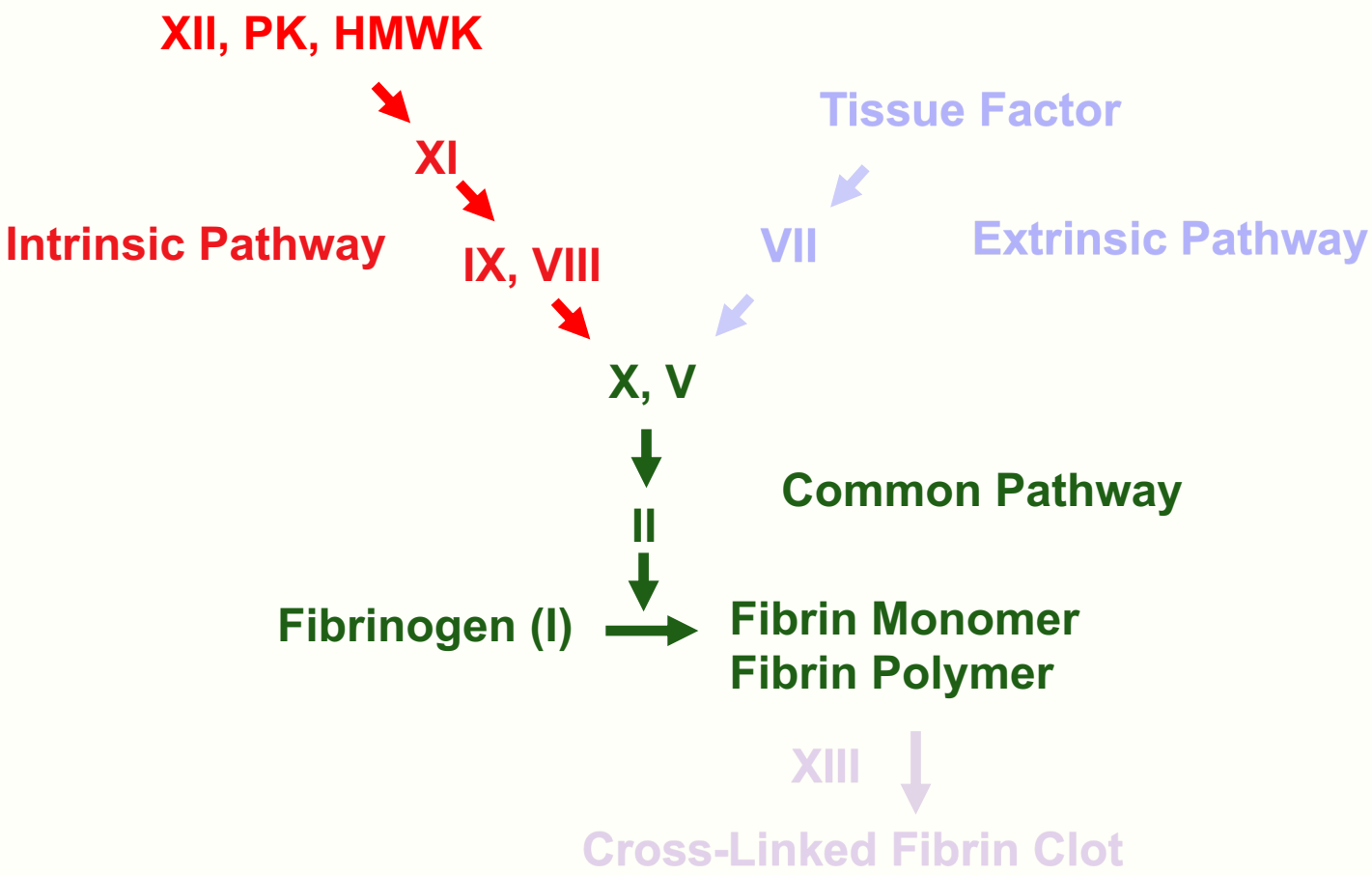
- **Measures:**
  - Extrinsic and Common Pathways
  - Endpoint is Fibrin Polymer
- **Major Uses:**
  - Hemostasis Screening
  - Monitoring Warfarin Anticoagulation
- **Results:**
  - Reported in Seconds and INR.

INR (International Normalized Ratio)

- **INR= International Normalized Ratio**
  - **(patient PT/mean normal PT)^{ISI}**
  - **ISI= International Sensitivity Index**
- Developed to standardize result reporting, accounting for variation in thromboplastin reagents.
- INR validated for warfarin titration, but practically 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



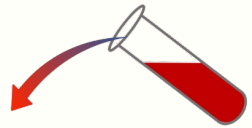
Activated Partial Thromboplastin Time (aPTT)



- **Measures:**
  - Intrinsic and Common Pathways
  - Endpoint is Fibrin Polymer
- **Major Uses:**
  - Hemostasis Screening
  - Monitoring unfractionated heparin therapy.
- **Results:**
  - Reported in Seconds

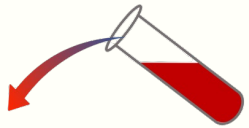
APTT: Monitoring UFH Therapy

- **APTT reagents are variably sensitive 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
    - 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.
- But still widely used due to low cost, use for coagulation screening, near universal access, and use for anticoagulant monitoring.
- PT does not reflect the activation of Factor IX by the TF:VIIa complex. i.e. no TFPI present in the test.

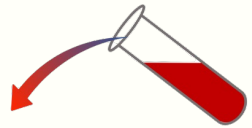


Preanalytical Considerations

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

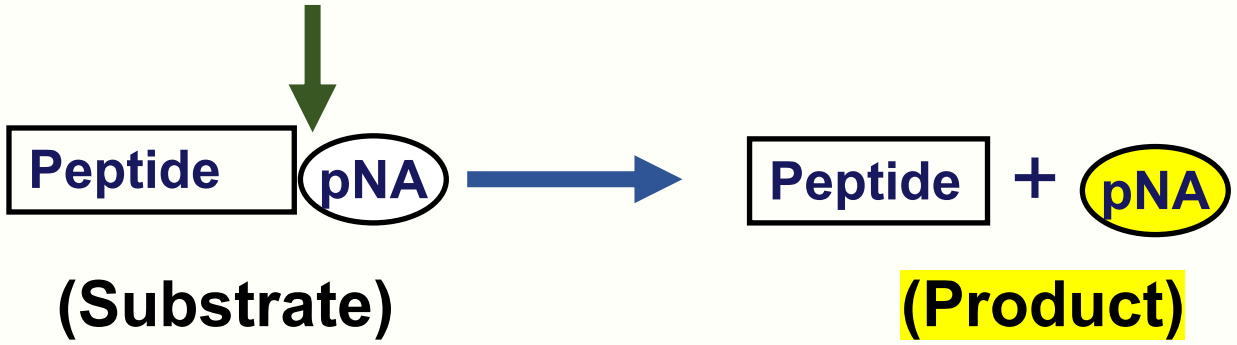
- Specimen Collection
  - 3.2% sodium citrate
  - 9:1 volume of blood to anticoagulant
  - Hct <25% or >50% may affect results. (Citrate:plasma ratio)

- Specimen Stability
  - Assays become unreliable in non-refrigerated whole blood.
  - Assays also become unreliable with time in refrigerated whole blood, although longer time.
  - Platelet Poor Plasma more stable.
  - If assays not to be done “promptly,” best to freeze platelet poor plasma.
    - Separated from cells and frozen at -80^{0}C



Functional Assay: Chromogenic Assay

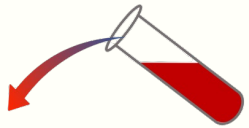
Enzyme of interest cleaves substrate



- Color develops.
- Change in absorbance over time, correlates with enzyme activity.
- Quantify spectrophotometrically.
- Discreet measure of a specific enzyme activity.
- Can also measure inhibition of the enzyme (i.e. anti-Xa)
- Affected by *fewer* preanalytical variables.

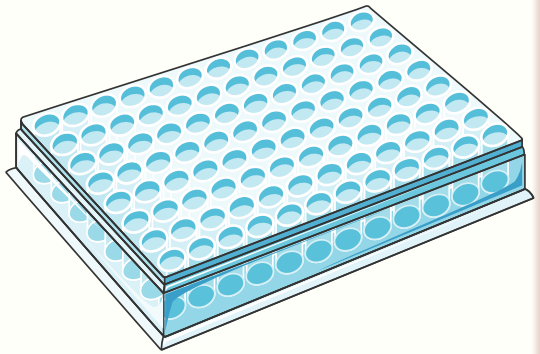
Antigen Assays

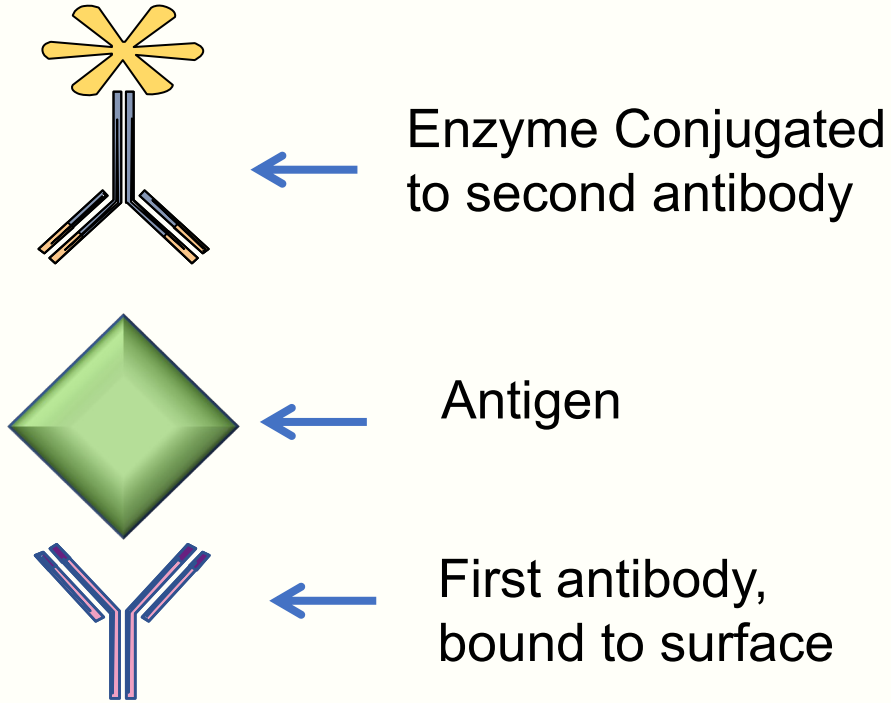
- Measured Immunologically
- Measures the amount of protein antigen present rather than function.
- Latex Induced Agglutination Assays (LIA)
- Enzyme-Linked Immunosorbent Assay (ELISA)

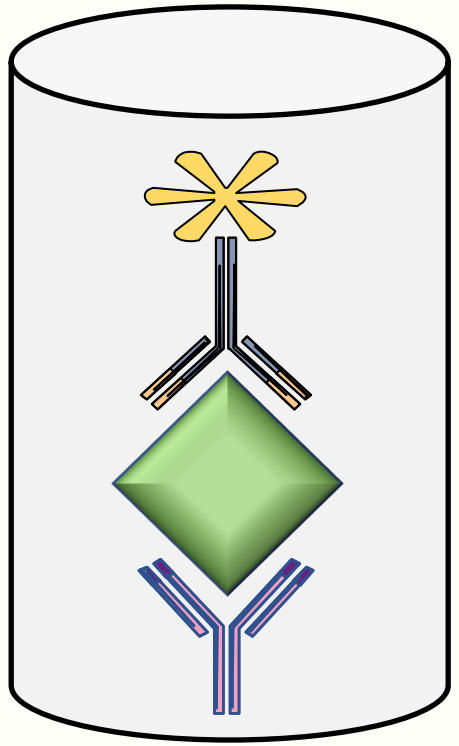


Sandwich ELISA

- 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

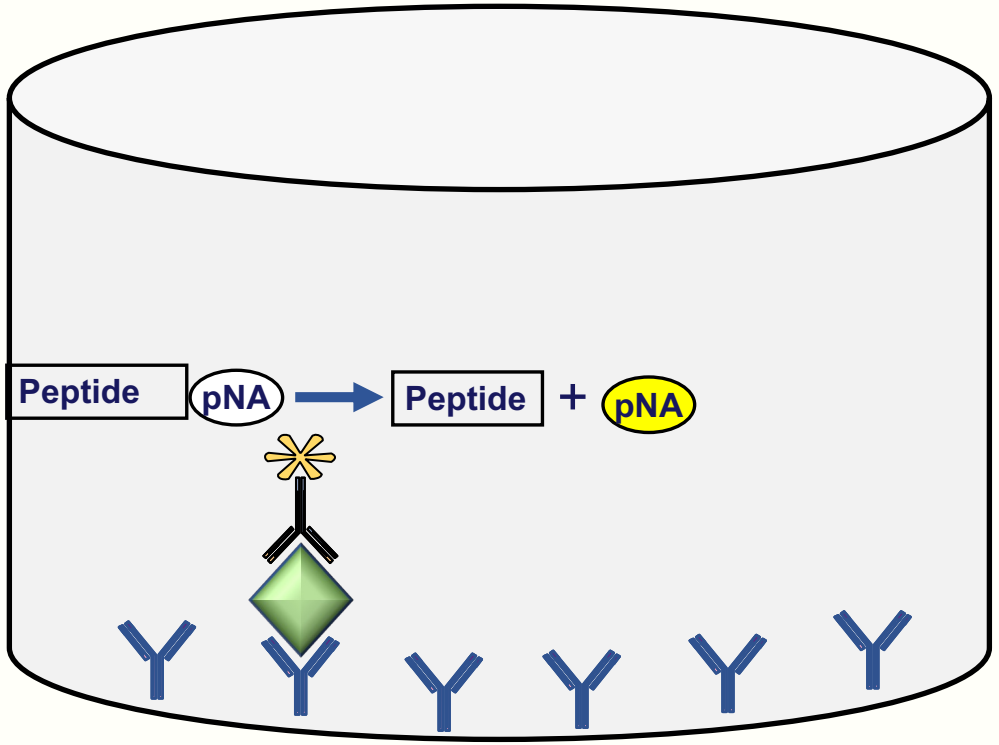




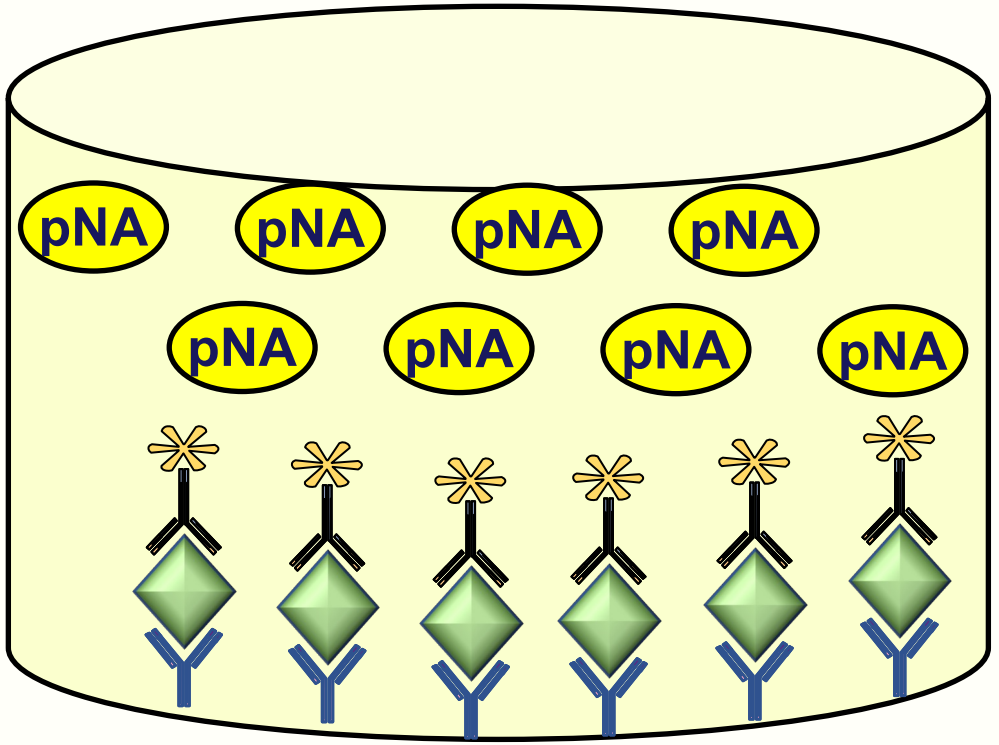


Sandwich ELISA

Low Level of Antigen



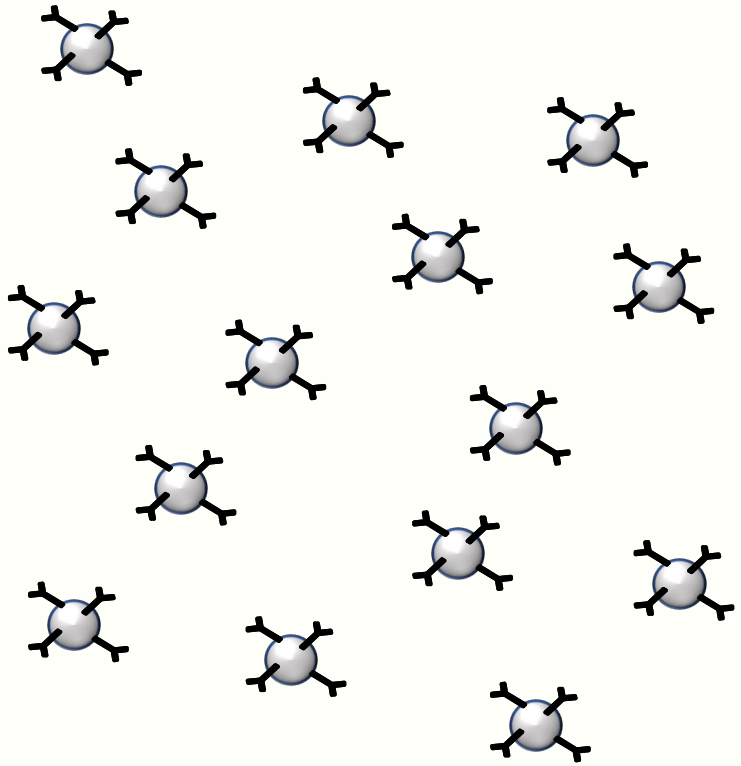
High Level of Antigen



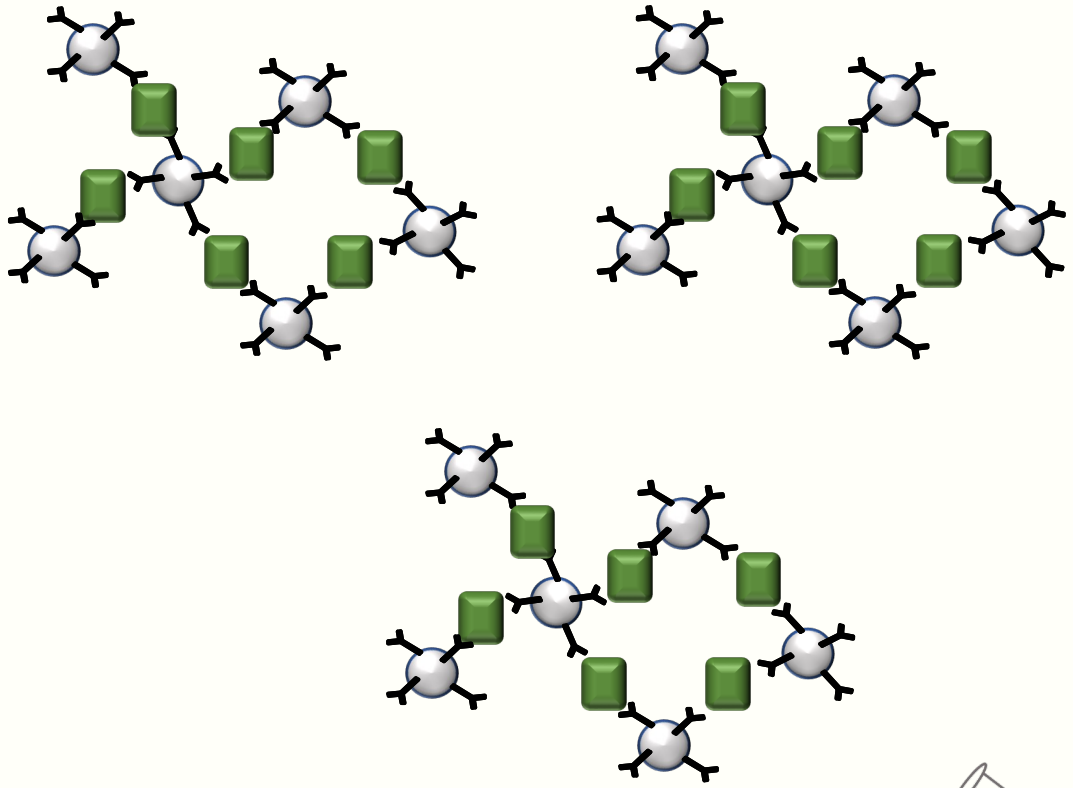
Latex Agglutination

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

Absence/Low Level of Antigen

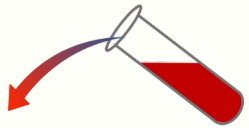


High Level of Antigen



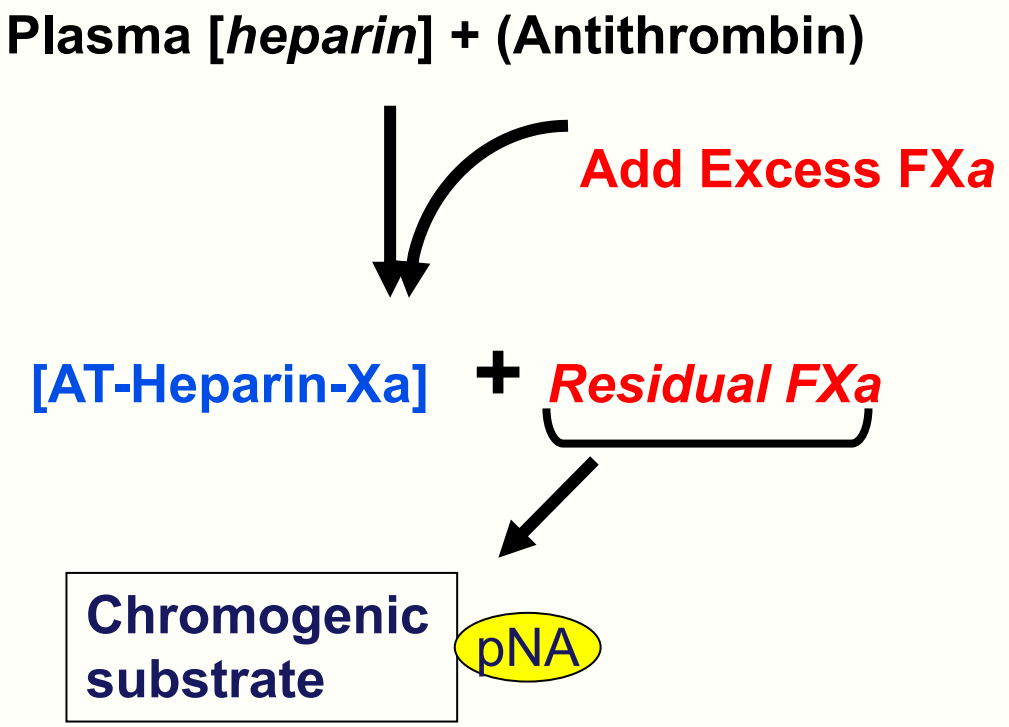
Other Tests

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



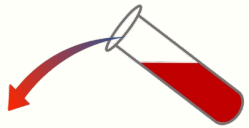
Anti-Xa Assay: Monitoring Unfractionated Heparin and Low Molecular Weight Heparin

- Specifically determines *anticoagulant activity* of LMWH and UFH by measuring ability of heparin-bound antithrombin to inhibit F Xa
- More specific than aPTT since it measures inhibition of a **single enzyme**
- Major advantage is **lack** of biologic interference
  - Eikelboom JW. Thromb Haemost 2006;96:547-52.
  - Francis JL. Pharmacotherapy 2004;24:108S-19S.



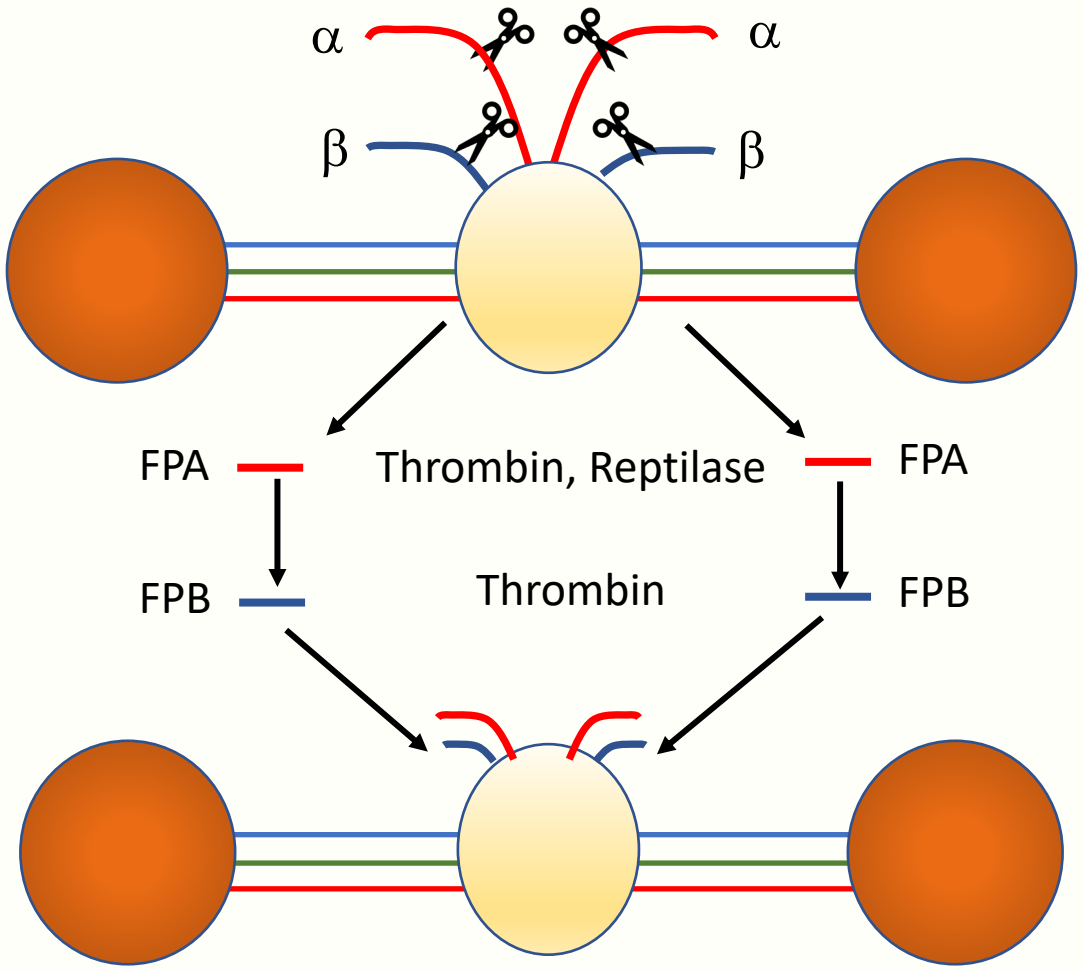
*Color development is **Inversely** proportional to the anticoagulant concentration in the plasma sample*

Anti-Xa Assay Now Adapted to Measure Levels of Anti-Factor Xa DOACs



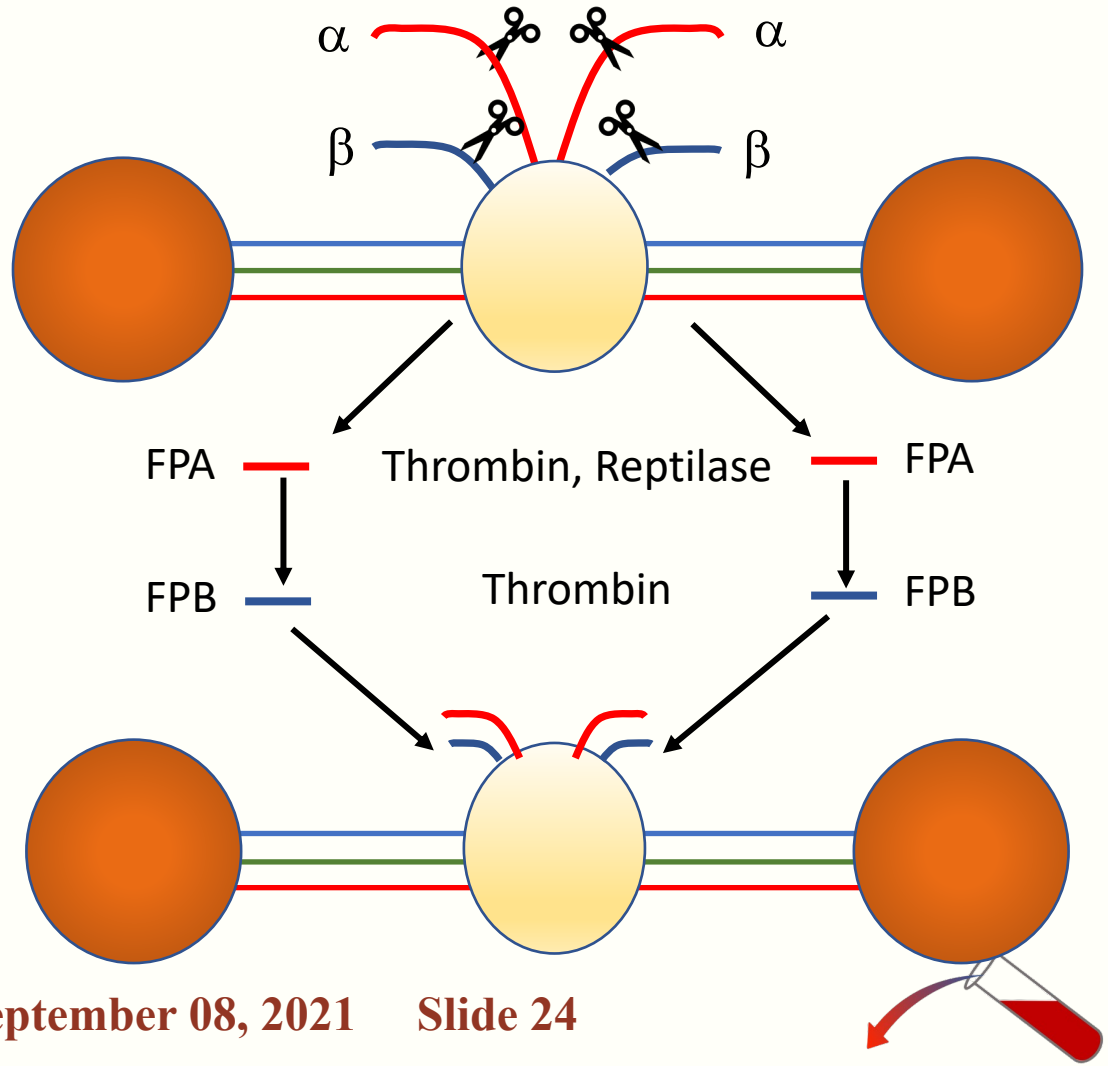
Thrombin Time:
Evaluates the Conversion of Fibrinogen to Fibrin

- Screening test for fibrinogen quantity and quality.
- Thrombin cleavage of Fibrinopeptides A and B, results in fibrin monomer.
- Fibrin monomers polymerize into fibrin clot, as end point.
- Thrombin Time is prolonged by hypofibrinogenemia or dysfibrinogenemia.
  - Need to assess fibrinogen antigen levels.

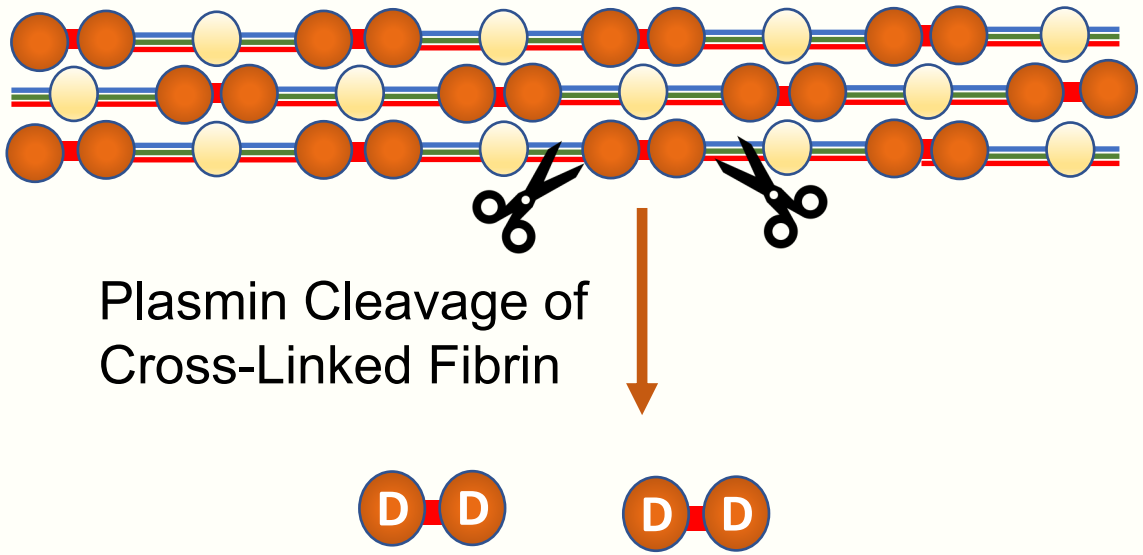


Thrombin and Reptilase Times:

- Reptilase Time often run in parallel.
- Dysfibrinogenemia (follow with Reptilase Time)
  - Thrombin: FPA & FPB
  - Reptilase: FPA
  - Need fibrinogen result for interpretation
- Effect of Heparin:
  - Thrombin Time prolonged
  - Reptilase Time not prolonged
  - Thrombin/Reptilase Times may be used as quick screening test for possible heparin contamination.



D-Dimer:
Degradation Product of Crosslinked Fibrin



Quantitation

LIA TEST

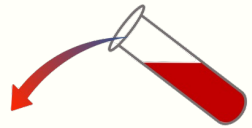
- Immunoassay.
- MoAb to D-dimers linked to microbeads
- Agglutination of beads occurs in the presence of D-dimers
- Agglutination is measured optically

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

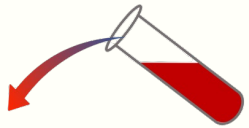
Utilization of D-Dimer Testing

- Evaluate for DVT/PE
  - Rule out thrombosis in the outpatient setting in individuals with low suspicion for thrombosis
    - 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 indicate a thrombosis or DIC is occurring.

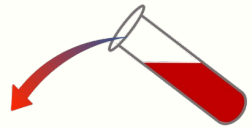


Interpretation of Prolonged PT and/or aPTT Results



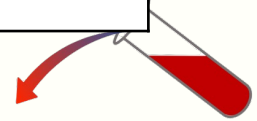
Interpretation of Prolonged PT and/or aPTT Results

- 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 Anticoagulant
  - Lupus Anticoagulant
  - Paraproteins
  - Therapeutic Anticoagulants: UFH, LMWH, Direct Oral Anticoagulants



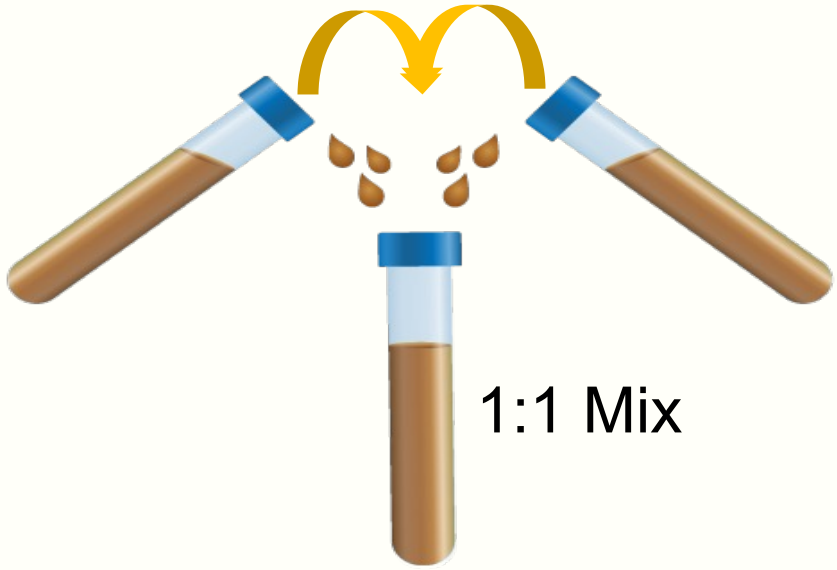
Sensitivity of PT/aPTT to Factor Deficiencies

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**

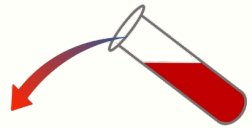
aPTT	Patient	Normal	1:1
Immediate	51”	29”	33”
1 Hour Incubation @ 37°C	52”	29”	32”

- **Lupus Anticoagulant**
- **Anticoagulants**

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

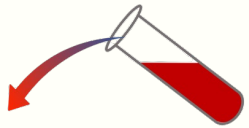
- **Anti-Factor VIII Antibody**

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



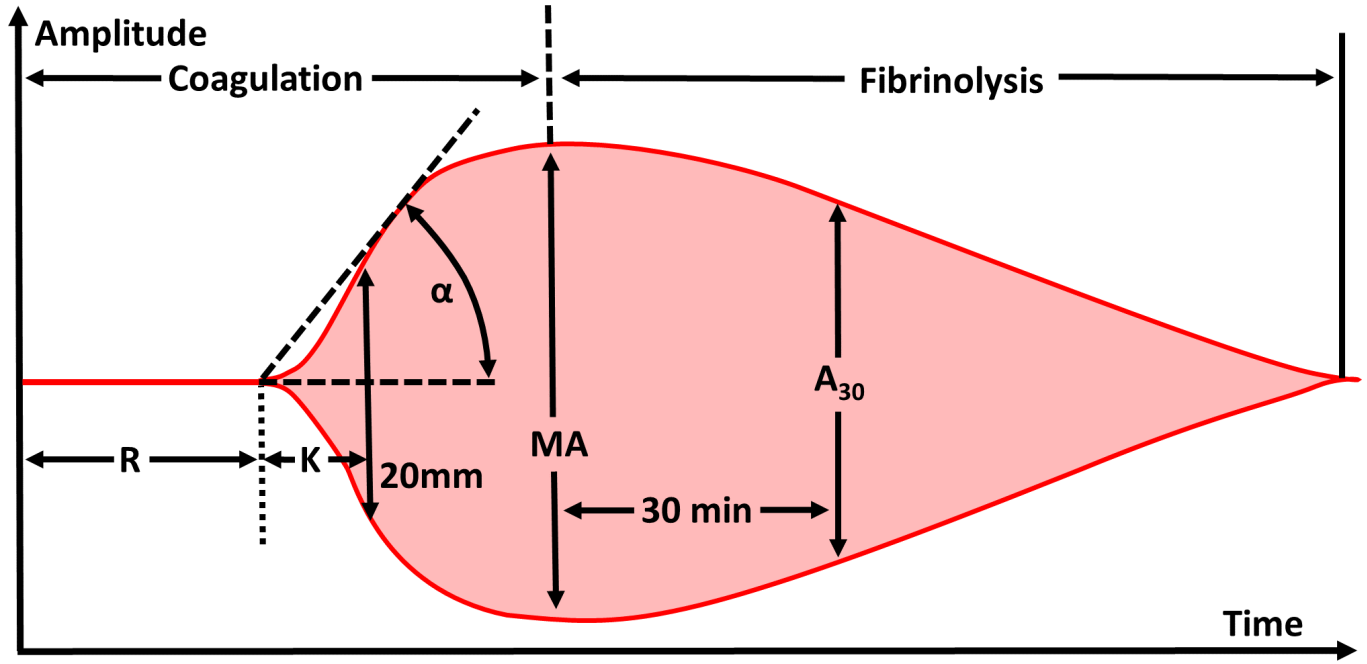
Thromboelastography (TEG) and Thromboelastometry (ROTEM)

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



Thromboelastography (TEG) and Thromboelastometry (ROTEM)

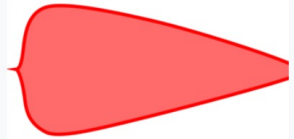
- Global assessment of hemostatic function.
  - Mostly used for Point Of Care testing, in Operating Room, Critical Care.



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.
A_{30}: Amplitude 30 minutes after reaching maximum amplitude.

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 ?