Coagulation Assays

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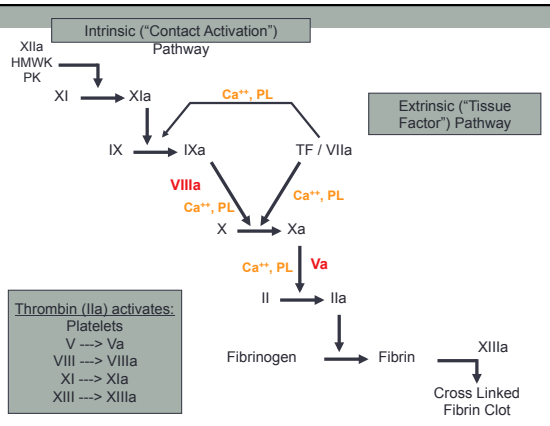
Scientific Advisory Board	RTI International

Presentation includes discussion of the following off-label use of a drug or medical device:
None

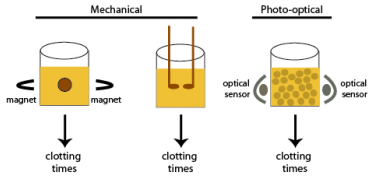
Overview

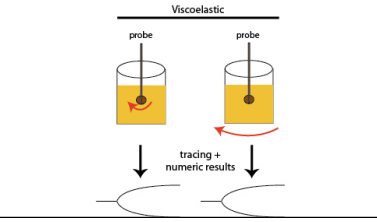
- The Basics
  - The TT (TCT)
  - The PT
  - The aPTT
  - The Mixing Study
  - One-stage and chromogenic factor assays
- Global Assays
  - PFA-100
  - TEG/ROTEM
  - Thrombin generation



Screening Tests of Coagulation

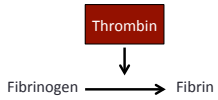
- Blood is anticoagulated in a calcium sequestering agent (citrate)
- Plasma is separated by centrifugation
- Coagulation is initiated by adding an activating agent (usually with calcium and phospholipid)
- Time to clot formation is measured in seconds
- Routine coagulation tests:
  - Prothrombin Time (PT)
  - Activated Partial Thromboplastin Time (aPTT)
  - Thrombin Time (TT) [= Thrombin clotting time (TCT)]





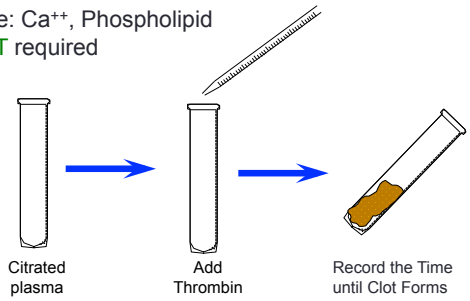
THE THROMBIN TIME (TT) OR (TCT)

What is measured in the thrombin time?



Performing a Thrombin Time

Note: Ca^{++}, Phospholipid NOT required



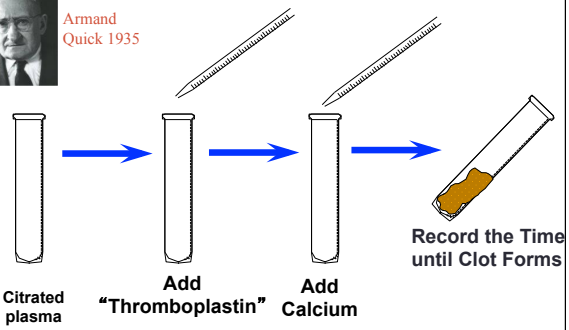
What Causes a Prolonged TT?

1. Low fibrinogen ('hypofibrinogenemia')
2. Abnormal fibrinogen ('dysfibrinogenemia')
   - inherited
   - acquired (severe liver disease)
3. Inhibitor of added thrombin
   - direct inhibitor; e.g. argatroban, dabigatran
   - indirect inhibitor; heparin
4. Something that interferes with fibrin polymerization
   - paraproteinemia
   - very high levels of fibrin degradation products
   - very high level of fibrinogen

THE PROTHROMBIN TIME (PT)

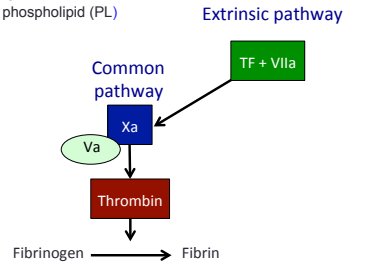
Performing the Prothrombin Time

Armand Quick 1935



The Prothrombin Time (PT)

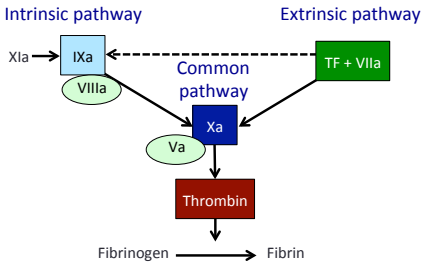
Thromboplastin (reagent) provides tissue factor (TF) and phospholipid (PL)



What Causes a Prolonged PT?

- Anything that prolongs the TT
  - but the PT is *much* less sensitive than the TT to the presence of heparin, abnormal fibrinogen, and FDPs
- Anything that lowers levels or inhibits the common pathway factors
  - Factor X
  - Factor V
  - Factor II (prothrombin)
  - Fibrinogen
- And also low levels of FVII
  - Congenital
  - Acquired
    - Deficiency of Vitamin K
    - Vitamin K inhibitors
    - DIC
    - Liver disease

PT Performed with Dilute Thromboplastin *Can* Detect Hemophilia

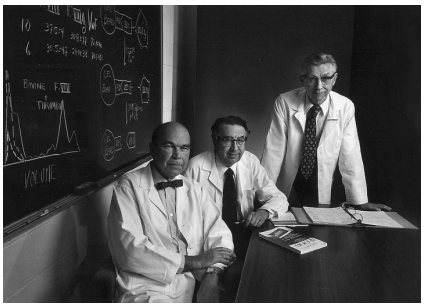


Relationship Between PT and INR

- INR = International Standardized ratio
- Developed as a reporting tool to allow PT measurements from different laboratories to be comparable.
- The ratio of the patient's PT divided by the mean normal PT—raised to an exponent called the ISI (which is dependent on the thromboplastin source)
  - The ISI is calculated for each new batch of thromboplastin.
- Normal value for INR is ≈1.
- Technically, INR was developed to be used exclusively for monitoring warfarin, but other scoring systems have since incorporated the INR (e.g. Child-Pugh cirrhosis score)

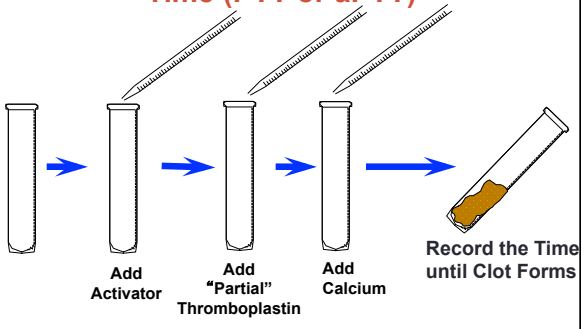
THE ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT)

1953: Invention of the (a)PTT



Drs. Langdell, Wagner and Brinkhous 1977

The (activated) Partial Thromboplastin Time (PTT or aPTT)

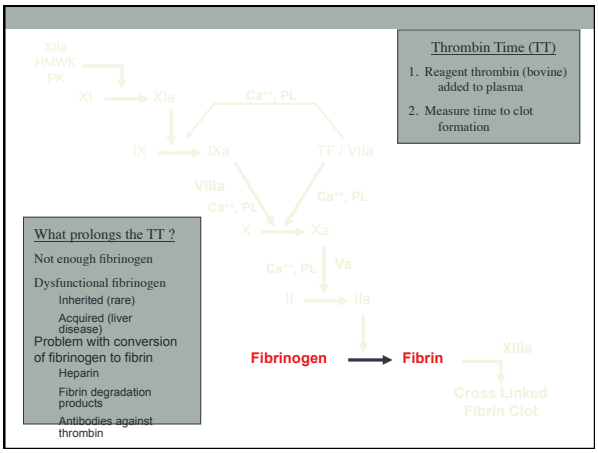


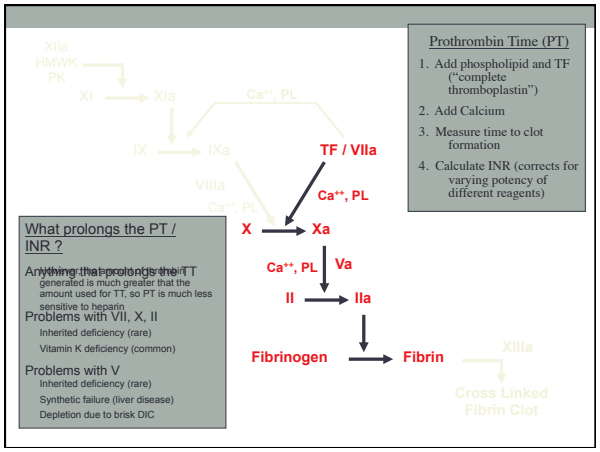
Definitions

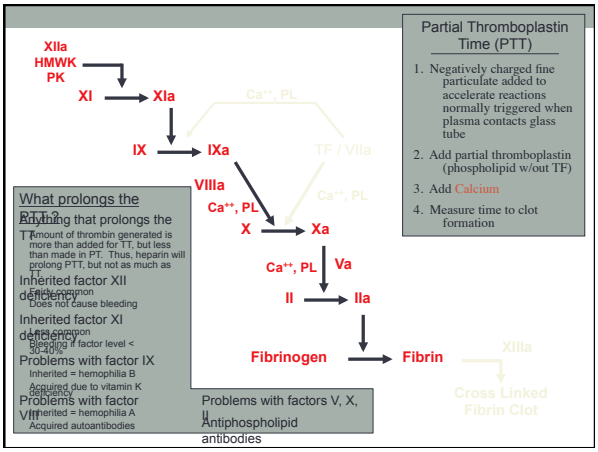
- The "activator" in the aPTT
  - Provides negative charges to activate the contact system
  - Things that can do this include kaolin, celite, and silica
- What's missing in the "Partial thromboplastin" that's present in a complete thromboplastin?
  - The partial thromboplastin is missing tissue factor.
  - It DOES have phospholipid

What Causes a Prolonged aPTT?

- Problems with factors in the common pathway
  - Factor II, V, X
  - Fibrinogen
- Problems with the contact factors
  - Factor XII, HMWK, PK
- Problems with
  - Factor XI—Hemophilia C
  - Factor IX—Hemophilia B
  - Factor VIII—Hemophilia A
- Warfarin in excess, or severe vitamin K deficiency (due to their effects on IX, X, and II)
- Heparin (unfractionated)
- Lupus Inhibitor (AKA Lupus anticoagulant)





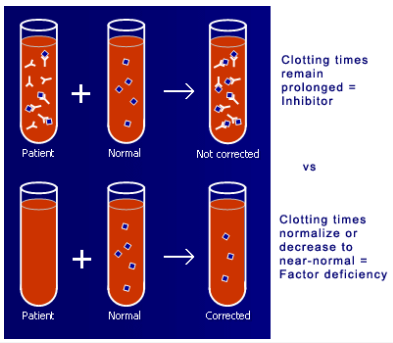


THE MIXING STUDY

The Mixing Study

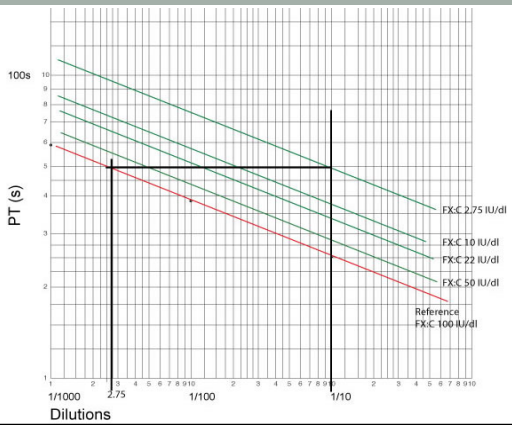
- Helps us differentiate between the two broad causes of a prolonged aPTT
- When there's a prolonged aPTT, the broad differential diagnosis is
  - Factor deficiency (something's missing)
  - Inhibitor (something's interfering)
- Take the patient's plasma and mix it 1:1 with normal plasma—then repeat the aPTT

Interpreting the Mixing Study



Factor Assays

- A method based on the PT is used to measure levels of factors V, VII, X and II.
- The assay relies upon correction of the PT when the test plasma is added to plasma deficient in the factor to be measured
- Perform a PT on the mix (at various dilutions)—then figure out where on the reference curve the patient falls—derive the FVIII activity level from the PT



FVIII Bioassays

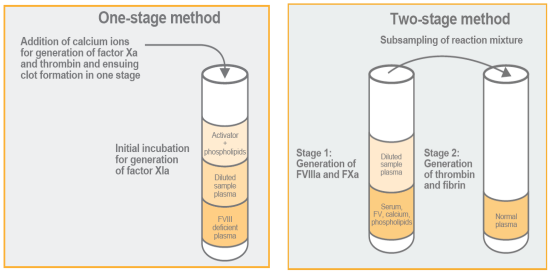
1- stage assay

- Clot based
- Shorter incubation time (<2 min)
- Sensitive to pre-activation of FVIII
- Based on aPTT
- Need FVIII-deficient reagent plasma with surplus of other clotting factors
- Easy to automate
- High CV (up to 40%), especially at lower FVIII levels

2 – stage assay

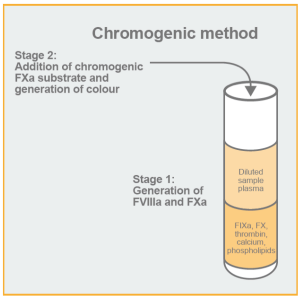
- Clot based
- Longer incubation time (up to 12 min)
- Does not require FVIII-deficient plasma
- Not sensitive to pre-activation of FVIII
- Difficult to automate
- Lower CV

Overview of 1-Stage and 2-Stage FVIII Assays



Chromogenic FVIII Assay: A Reasonable Compromise?

- Reference method recommended by the ISTH to measure FVIII activity in plasma and FVIII concentrates
- Not clot based
- Does not require FVIII-deficient plasma
- High dilution of patient sample minimizes interferences
- Low CV (2.4-7.4%)
- Can be easily automated

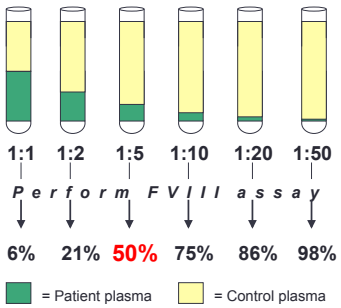


"Non-Linear Assay: Inhibitory Pattern"

Dilution	FVIII	FIX
1:5	*	1.8
1:10	*	18.2
1:20	*	50.7
1:40	*	69.3
1:80	*	70.6

- Serial dilutions are performed, and the assays are corrected for the dilution factor.
- An apparent rise in the factor activity level with deeper and deeper dilutions is indicative of the presence of a non-specific inhibitor affecting the aPTT
- If there is a specific inhibitor to that particular factor, then the activity level is low and remains low.
- In this example, there is a FVIII inhibitor that is interfering with the aPTT-based FIX assay. This would be reported as FIX >70.6%--inhibitory pattern noted.

The Bethesda Assay

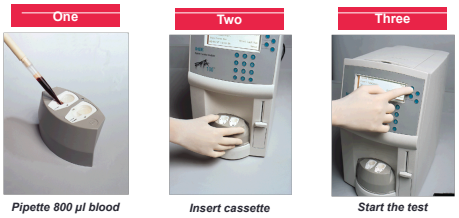


Reciprocal of dilution at which 50% of normal FVIII activity is observed

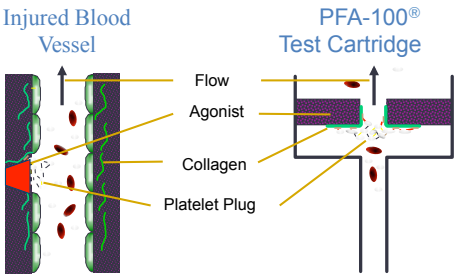
1 / 1/5

Inhibitor Titer = 5 BU

PFA-100™ Test Principle



PFA-100™ Simulates Primary Hemostasis

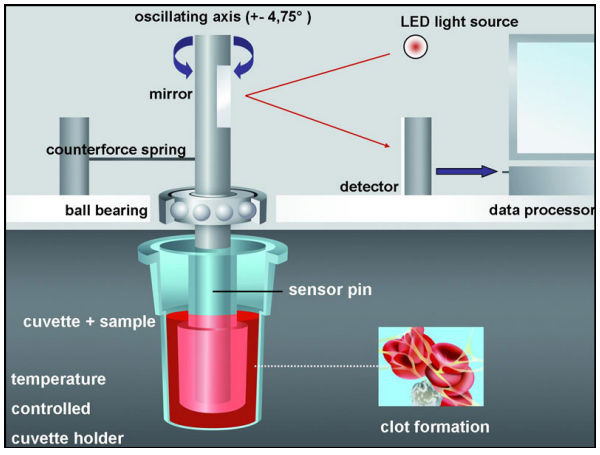


PFA-100™ Closure Times: Interpretation

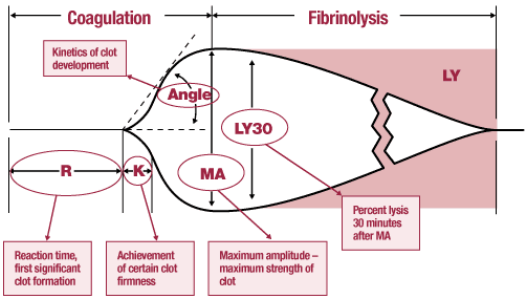
	C-Epi Normal	C-Epi ↑
C-ADP Normal	Excludes: Drug effect Severe thrombocytopenia severe platelet dysfunction Severe VWD	Drug effect (ASA, NSAID) Low Hct Mild thrombocytopenia Mild platelet dysfunction Mild VWD
C-ADP ↑	Rare event	Drug effect Very low Hct Severe thrombocytopenia Severe platelet dysfunction Severe VWD

Viscoelastic Hemostasis Assays

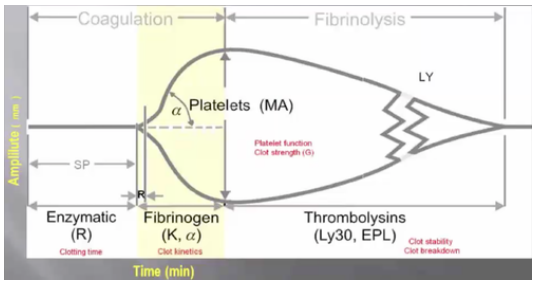
TEG ROTEM



TEG Parameters

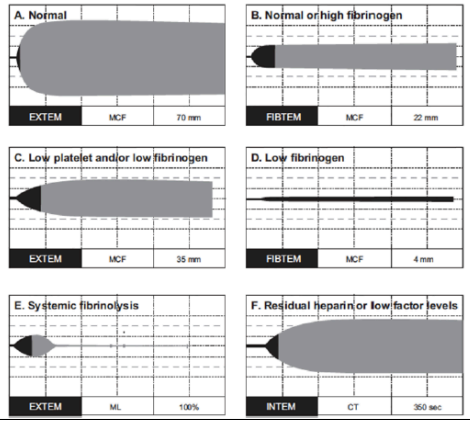


TEG Phases Related to Hemostatic Processes

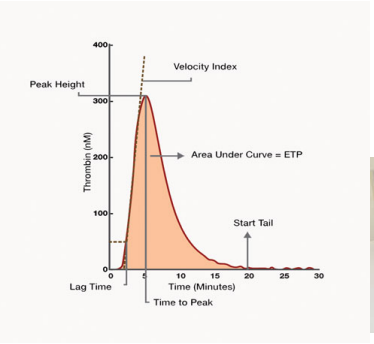


Assays Used in Rotational Thromboelastometry (ROTEM)

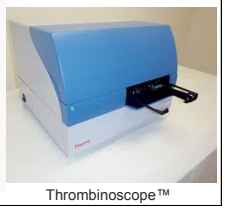
Assay	Activator/Inhibitor	Information Provided
INTEM	Contact activation	Fast assessment of clot formation, fibrin polymerization, and fibrinolysis via the intrinsic pathway
HEPTEM	Contact activation + heparinase	ROTEM analysis without heparin influence: Specific detection of heparin (compared to INTEM), assessment of clot formation in heparinized patients
EXTEM	Tissue factor activation	Fast assessment of clot formation, fibrin polymerization, and fibrinolysis via the extrinsic pathway
FIBTEM	Tissue factor activation + platelet inhibition	ROTEM analysis without platelets: Qualitative assessment of fibrinogen status
APTEM	Tissue factor activation + aprotinin	In vitro fibrinolysis inhibition: Fast detection of lysis when compared with EXTEM
NATEM	Recalcification only = classical TEM (thromboelastometry)	Very sensitive assessment of the equilibrium of coagulation activation or inhibition



Thrombin Generation Curve Using Calibrated Automated Thrombography (CAT) Assay (Platelet-Free Plasma)



Assay triggered by low concentration TF (1 pM) with added phospholipid (4μM)



Thrombinoscope™