

## Quality Coagulation Samples



## Begins With The Sample



- Phlebotomy
  - CLSI Guidelines
  - Needle size 19-21 gauge
  - Venous draw
  - Anticoagulant – 3.2% Na Citrate
  - Draw order
  - Immediate mix of tube – 3-4 times full inversions
  - 90% tube fill – under fill prolongs PT/PTT
  - Ratio 1:9 – Anticoagulant to plasma

## Sample Transportation



- Shaking, vibration, agitation of sample
- Transport to reference lab via courier
- Transport via pneumatic tube system
  - Activates platelets
  - Increases risk of hemolysis

## Time



- PT stable unspun, capped at room temperature for 24 hours
- PTT (non-heparinized) and all other assays processed and performed within 4 hours
- PTT (heparinized) 1 hour to centrifuge and assay (on cells)
- Process/aliquot/freeze if cannot be performed in time frame

## Temperature



- Increase temperature degrades V and VIII
- Decreased Temperature activates VII

## Centrifugation



- Platelet poor plasma <10,000/ul
- Approx. 1500g for 15 minutes at room temp
- Swing-out bucket rotor centrifuge
  - Recommended to minimize remixing of plasma and platelets
- Stat-Spin assay within 10 minutes
  - Platelets resuspend with time
- Validate centrifuge & platelet counts every 6 months

## Frozen Aliquots

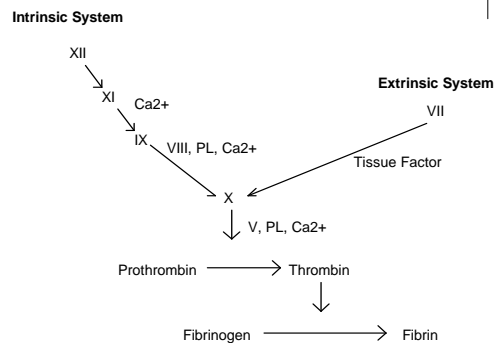
- Double spun
  - Aliquot spun primary tube, respin and aliquot again.
  - Avoid buffy coat and platelet button
- Freeze non-frost free
  - -20°C stable 2 weeks
  - -70°C stable 6 months
- Thaw rapidly in 37°C water bath
  - No longer than 10 minutes
  - Mix

## Why Platelet Poor Plasma?

- Platelets are a source of phospholipid
- Reagents are phospholipid
- Can shorten results
- PF4 can neutralize heparin thus shorten PTT
- Platelets release PF4 in cold and when frozen
- Critical that frozen samples be platelet poor

## Abnormal PT/PTT Mixing Studies

## Start with the Basics



## Prothrombin Time/INR

- Extrinsic System/Common Pathway
- Warfarin (Coumadin) therapy
- Prolonged test?
  - Mixing study (1:1) Correction
  - Possible deficiency of VII
    - Early vitamin K deficiency
    - Early liver disease

## Partial Thromboplastin Time (PTT)

- Intrinsic System/Common Pathway
- Heparin Therapy
- Prolonged PTT?
  - Mixing study correction
  - Possible deficiency VIII, IX, XI, XII

## Prolongation of PT/PTT

Common Pathway Deficiency  
X, V, II

## Other Things To Think About

What is the Fibrinogen Level?

What is the Thrombin Time?

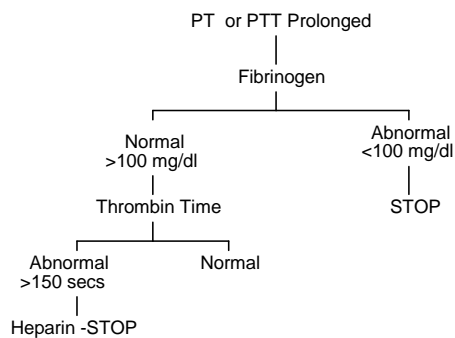
## Fibrinogen

- Fibrinogen → Fibrin Clot
- Quantitative Method
- Concentration of Functional Level of Fibrinogen
- Dilution of Plasma and Concentrated Thrombin
- Referenced Against a Standard Curve
- Reported in mg/dl
- Low levels or dysfunctional Fibrinogen reason for prolongation of PT/PTT.

## Thrombin Time

- Qualitative Test
- Fibrinogen → Fibrin Clot
- Straight Plasma and Dilution of Thrombin
- Prolonged TT
  - Low Fibrinogen Level
  - Dysfunctional Fibrinogen
  - Interfering Substances (Heparin)
  - High levels of FDP

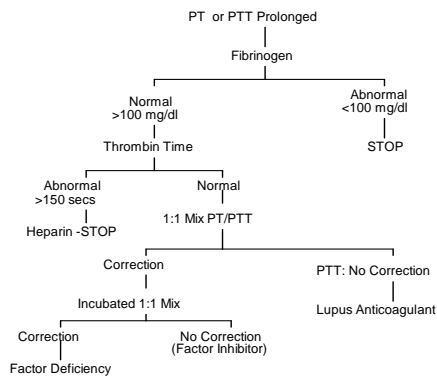
## Algorithm For Prolonged PT/PTT



## Heparinase

- Commercial Reagent
- Neutralizes Heparin in Sample
- Example:
  - PTT = 60 secs
  - HPTT = 30 secs (Heparin)
  - HPTT = 55 secs (No Heparin)

## 1:1 Mixing Studies



## Mixing Studies (PTT/PT)

- Mix 1 part patient (unknown) to 1 part normal plasma (100% factor level)
- At least ~50% factor is present in the mixture, corrects PT or PTT in factor deficiency
- Correction – incubated 1:1 mix to rule out time and temperature factor inhibitor.

## Incubated Mixing Studies

- Tube 1 = patient plasma
- Tube 2 = Normal pooled plasma
- Tube 3 = 1:1 Mix patient + normal pooled plasma
- Incubate all tubes at 37 degrees for 1 hour
- After incubation make a 1:1 mix with tube 1 and tube 2 (control mixer)
- Perform PT/PTT on both mixes

## Incubated Mix

- Example 1:
  - Original PTT = 65 seconds
  - 1:1 mix PTT = 30 seconds
  - Incubated Mix PTT = 47 seconds
  - Control Mix PTT = 38 seconds
  - Inhibitor Present

## Incubated Mix

- Example 2:
  - Original PTT = 65 seconds
  - 1:1 mix PTT = 30 seconds
  - Incubated 1:1 mix PTT = 33
  - Control Mix = 33
  - No inhibitor

## Factor Deficiency

- Defined as factor activity less than the lower limit of normal, <50%
  - Normal Range = 50-150%
- Cannot always be detected by the prolongation of the PT/PTT
  - Depends on the responsiveness/sensitivity of the reagent.
- Causes:
  - Congenital
  - Acquired
    - Liver disease, consumption, drugs

## Reagent Sensitivity

- Begin with a known standard, ~100% factor
- Dilutions of known standard with deficient plasma
- Perform PT/PTT on each dilution
- PT/PTT become abnormal is the % factor sensitivity of the reagent

## Things To Keep In Mind When Performing Mixing Studies

- Source of normal plasma
  - Pooled normal plasma (n ≥ 20)
  - Do not use patients with normal coagulation tests
  - Frozen not lyophilized
  - Platelet poor
  - Know % factor activities
- Reagent Sensitivity
- Establishment of Normal Range
  - Minimum of 20 – 30 Normal Healthy Individuals
  - Do Not Use Patients With Normal Coagulation Tests
  - Equal Mix of Males and Females  
(No Hormones or Hormone Contraceptives)
  - Platelet Poor

## Patient History

- Family History (Bleeding/Clotting)
- Patient History (Bleeding/Clotting)
- Patient Medications
- Patient Age

## Case Study #1

- 4 day old male with tonsil plate bleed.
- Possible Family History of Great Grandfather with Hemophilia
- ↑PTT = 69 sec
- Fibrinogen = 200 mg/dl
- Thrombin Time = 16 sec
- 1:1 Mix PTT = 30 sec.
- ↓VIII = <1%
- IX = 67%
- VWFA = 108%
- VWAG = 114%

## Case Study #2

- 21 year old male with Congenital Heart Disease
- No History of Bleeding
- ↑PT = 17 sec
- Fibrinogen = 235
- Thrombin Time = 18
- 1:1 Mix PT = 11 sec
- ↓VII = 18%

## Case Study #3

- 69 year old male with GI Bleed
- Bleed as child with Tonsillectomy
- Platelet Count = 196,000
- ↑PTT = 56 sec
- Fibrinogen = 402 mg/dl
- Thrombin Time = 17 sec
- 1:1 Mix PTT = 30 sec
- VIII = 115%
- VonWillebrands Studies/Bleeding Time – Normal
- IX = 101%
- ↓XI = <1%

### Case Study #4

- 9 day old Female with Intracranial Bleed and Sepsis
- Family History of Great Aunt with Hemophilia?
- Platelet Count = 662,000
- $\uparrow$ PT = 63 sec
- $\uparrow$ PTT = >150 sec
- Fibrinogen = 353 mg/dl
- Thrombin Time = 19 sec
- 1:1 Mix PT = 15
- 1:1 Mix PTT = 36
- X = 57%
- $\downarrow$ V = <1%

### Case Study #5

- 44 year old male with upper GI Bleed.
- Abdominal pain for 3 months/Swelling of Groin
- Possible History of Factor Deficiency
- $\downarrow$ Platelet Count = 72,000
- $\uparrow$ PTT = >150 sec
- Fibrinogen = 300 mg/dl
- Thrombin Time = 18 sec
- 1:1 Mix PTT = 32
- VIII = 120%
- IX = 90%
- XI = 86%
- $\downarrow$ XII = <1%

### Case Study #6

- 81 year old female with Retroperitoneal Bleed
- Platelet Count = 345,000
- $\uparrow$ PTT = 72 sec
- $\uparrow$ Fibrinogen = 714 mg/dl
- Thrombin Time = 15
- 1:1 Mix PTT = 36
- Incubated 1:1 Mix PTT = 51/39
- $\downarrow$ VIII = <1%
- $\uparrow$ VIII Inhibitor = 4.2 BU

### Case Study #7

- 9 month old male with Scalp hematoma and other bruising.
- No Family History
- Platelet Count = 393,000
- $\uparrow$ PT = 15 sec
- $\uparrow$ PTT = 61 sec
- Fibrinogen = 235 mg/dl
- Thrombin Time = 17 sec
- 1:1 Mix PT = 12 sec
- 1:1 Mix PTT = 31 sec
- VonWillebrands Studies/Bleeding Time = Normal
- X = 140%
- $\downarrow$ V = 19%
- $\downarrow$ VIII = 8%
- IX = 77%

### Case Study #8

- 3 year old male pre-op Tonsillectomy
- No History/No Family History
- $\uparrow$ PTT = 44
- Fibrinogen = 292 mg/dl
- Thrombin Time = 18 sec
- $\uparrow$ 1:1 Mix PTT = 36 sec
- Platelet Neutralization Procedure (PNP) = 40/47 , 15%

### Case Study #9

- 61 year old male with Recent Stroke
- DVT 20 years ago
- Placed on Coumadin
- $\uparrow$ PT = 90 sec INR = 9.5
- $\uparrow$ PTT = 89 sec
- $\uparrow$ Fibrinogen = 582
- Thrombin Time = 20 sec
- $\uparrow$ 1:1 Mix PTT = 59 sec
- Platelet Neutralization Procedure (PNP) = 35/78, 55%