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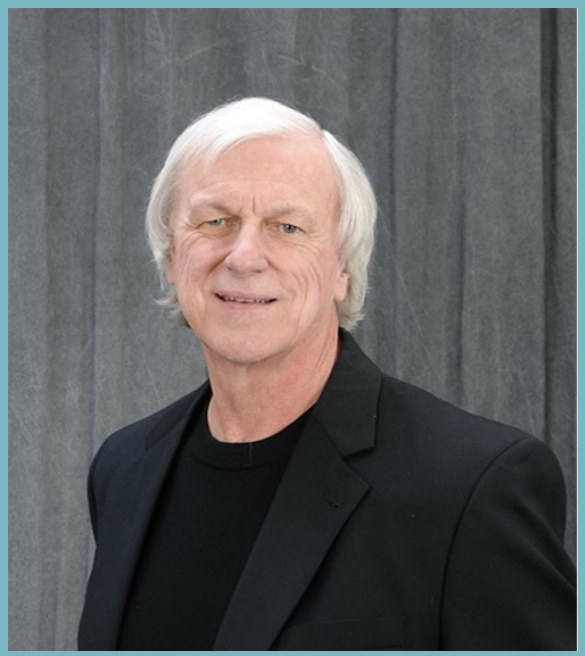
Improving Acute Care with Coagulation Mixing Studies



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Coagulation Mixing Studies Learning Objectives

At the conclusion of this webinar, the participant…

1. Prepares a stepwise PTT and PT mixing study protocol
2. Indicates the clinical purposes for PTT mixing studies
3. Explains why the mixing study is an acute care assay
4. Correlates mixing study results with coagulation test results

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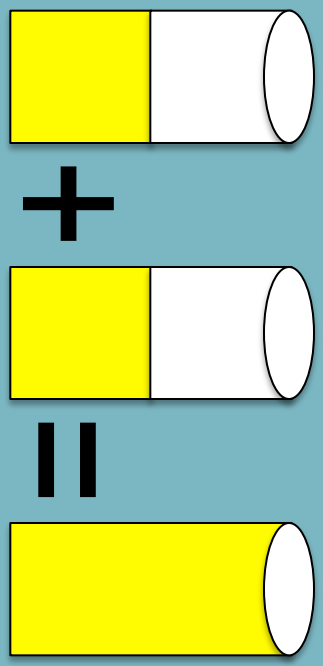
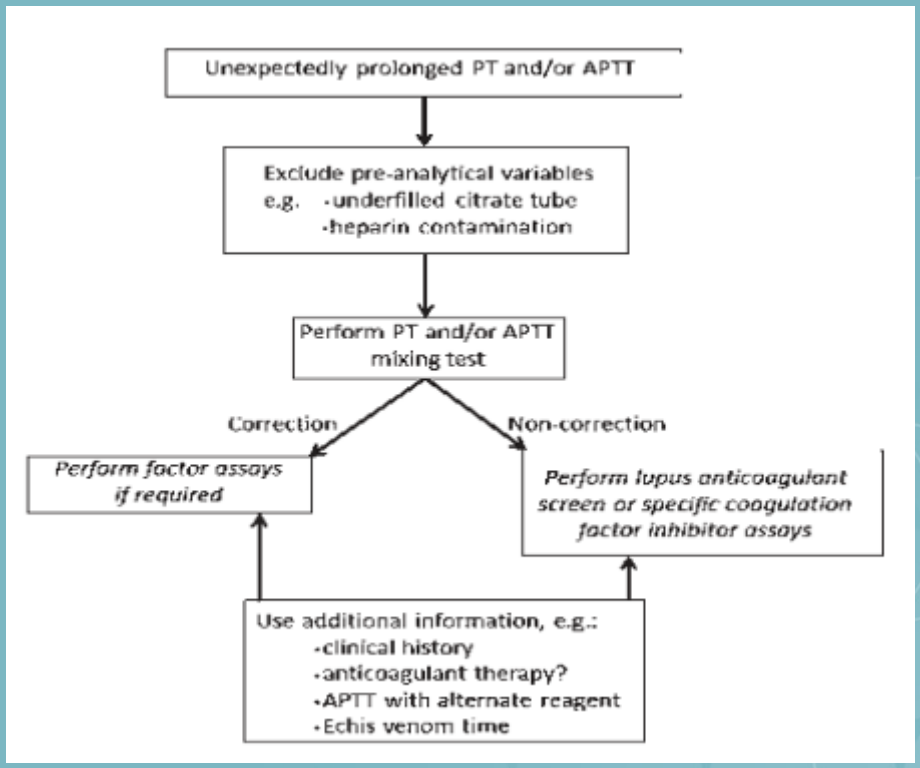
Mixing Study
A First-line Investigation to…
differentiate a coagulation deficiency from an inhibitor





Kershaw GK, Orellana D. Mixing tests: diagnostic aides in the investigation of prolonged prothrombin times and activated partial thromboplastin times. Semin Thrombos Hemost 2013;39:283–90.

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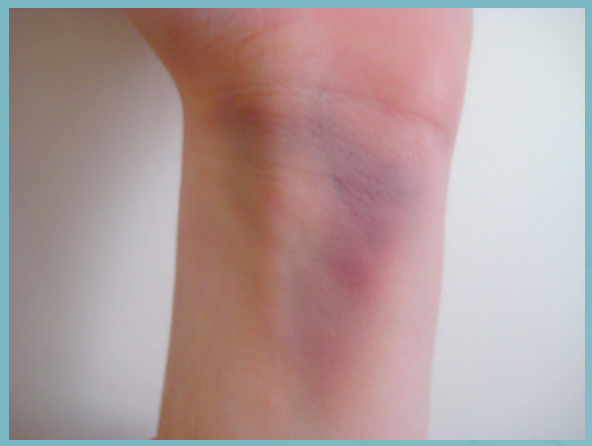
Case: 32-yo Female Pre-op Screen

Six weeks post-partum

Easy bruising, frequent nosebleeds, menorrhagia



Pre-op Screen
32-yo Female, 6 Weeks Post-partum

Assay	Patient	RI
HGB	11.8 g/dL	12–15 g/dL
PT	12.4 s	9.8–12.6 s
PTT (APTT)	42.5 s	25–35 s
PLT count	310,000/µL	250–450,000/µL
Fibrinogen	320 mg/dL	220–498 mg/dL
Isolated, prolonged PTT response? 1:1 PTT mix		

Rule Out Heparin, Dabigatran

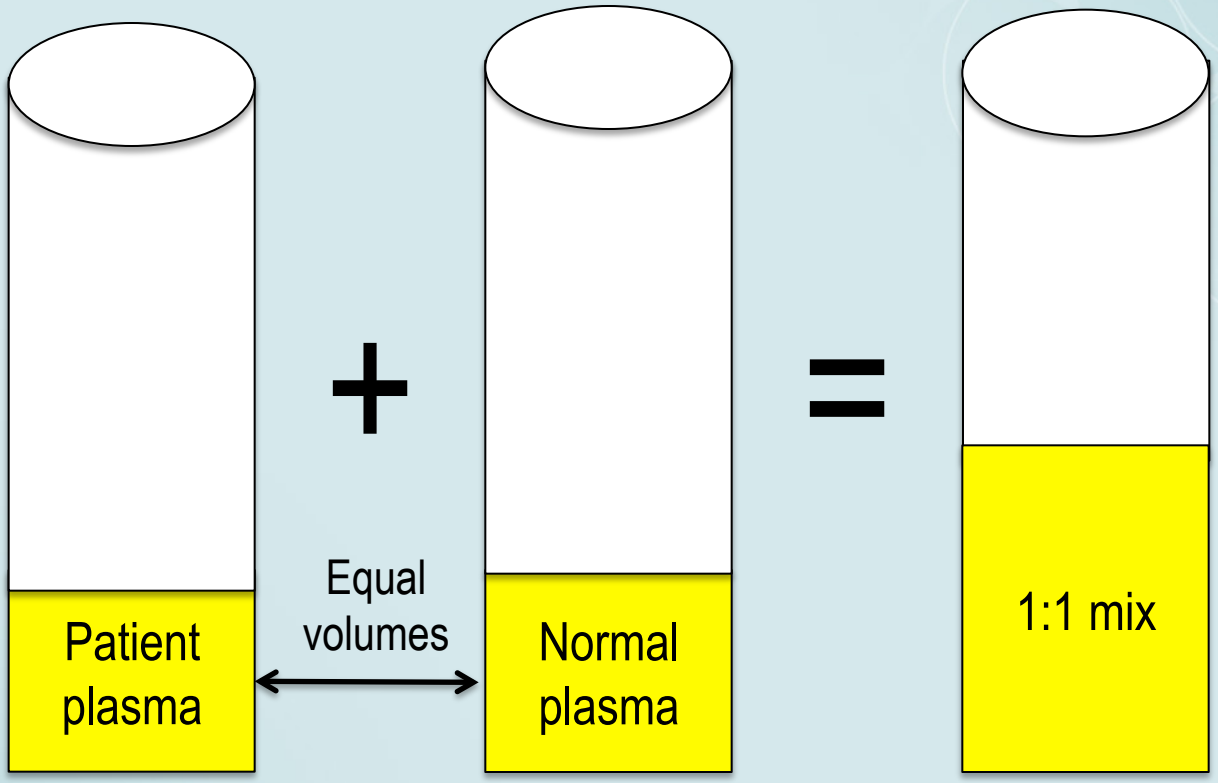
Assay	Patient	RI
TT	14 s	<21 s

- R/O dabigatran and unfractionated heparin (UFH)
  - Outpatient—consider dabigatran
  - Inpatient—unrecorded UFH flush of vascular catheter
- If dabigatran, discontinue, cancel order
- If UFH, use Hepsorb (polybrene) or Hepzyme, proceed
- If no UFH, perform 1:1 PTT mix to differentiate factor deficiency from factor-specific inhibitor or “non-specific inhibitor” lupus anticoagulant (LA)

PTT Mixing Study: Cheap and Basic

- Start within 2 h to avoid specimen degradation
  - Factors V (FV) and VIII (FVIII) are labile
  - Platelet factors (mostly FV) released to plasma
- Ensure pt plasma is platelet-poor (free), <10,000/uL
- Mix plasma 1:1 with pooled normal plasma (NP) and perform immediate PTT on mixture
- PTT of 1:1 mix corrects to ≤10% longer than NP PTT
  - Factor deficiency
- No correction: 1:1 mix is >10% longer than NP PTT
  - Non-specific inhibitor, usually LA
  - Specific inhibitor (anti-FVIII) may be present, usually requires 37C incubation

PTT Mixing Study



PTT
42.5 s

PTT
30 s

Manufacturer's value confirmed by QA staff

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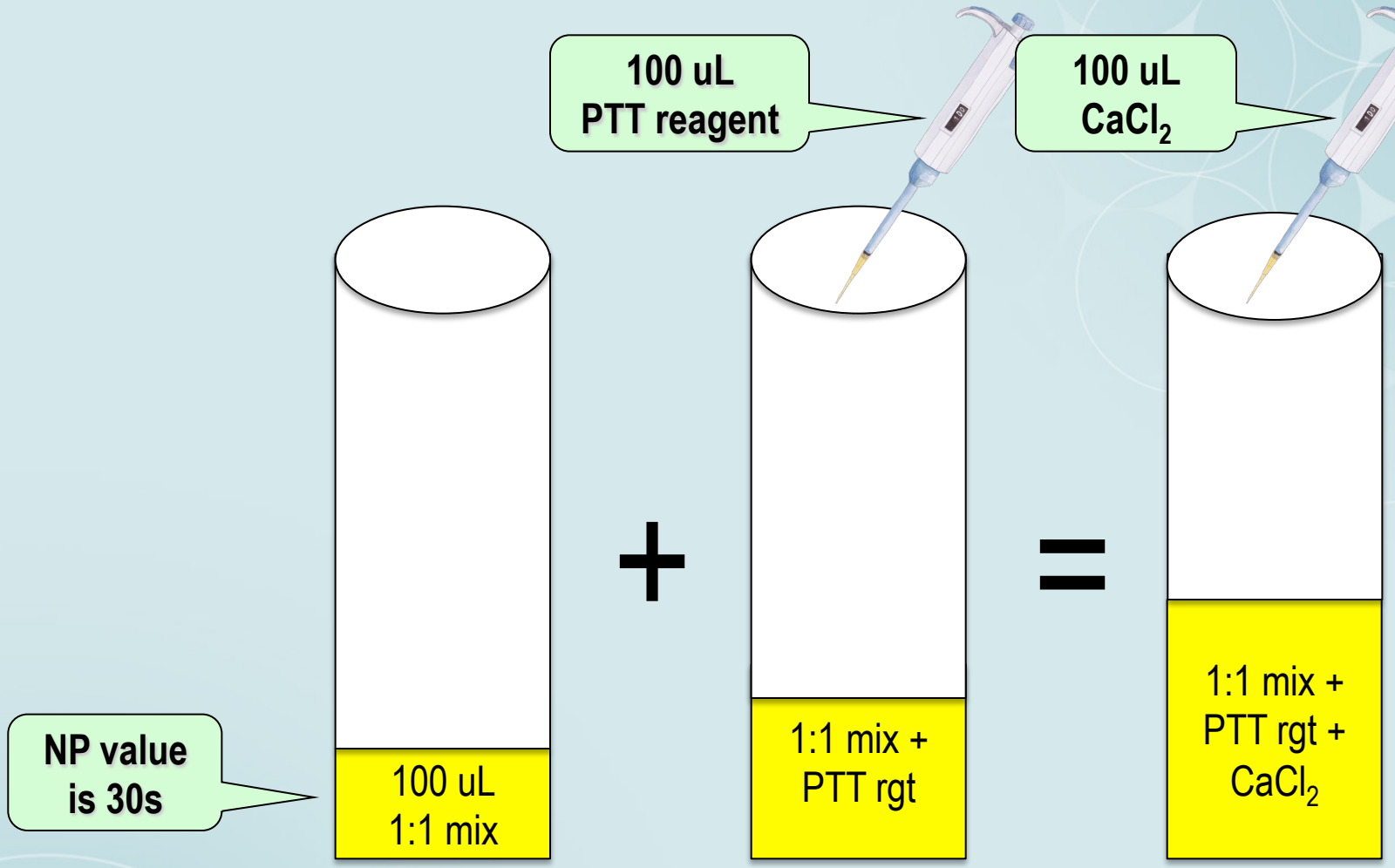

PTT Mixing Study Using 10% Rule

PTT

≤33 s: Correction

>33 s: No correction

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1:1 PTT Mix with Incubation

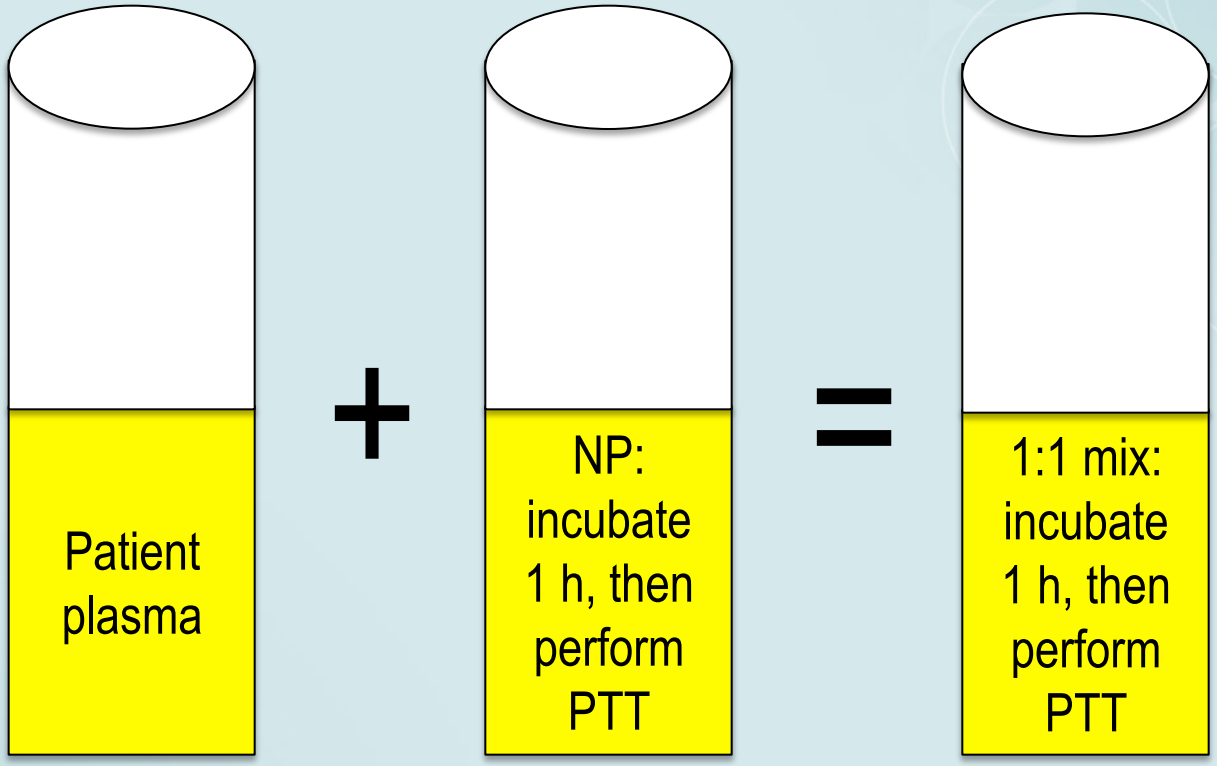
- PTT of immediate mix ≤10% longer than NP
  - Correction: factor deficiency? But first…
  - Incubate 1:1 mix, 37C, 1–2 h and repeat
- Correction after 37C mix = factor deficiency
- Incubated PTT remains >10% longer than NP
  - Specific inhibitor such as anti-FVIII
    - IgG_4: Temp dependent, may require incubation
    - However, some FVIII neutralization within 10 m
    - May detect in immediate mix

1:1 PTT Mix After 37C Incubation

- Only when unincubated mix corrects
- Must also incubate normal control plasma
- Compare mix PTT to incubated NP PTT
- May also detect temp-dependent LA
  - ~15% of LAs are temp-dependent

Thom J, Ivey L, Eikelboom J. Normal plasma mixing studies in the laboratory diagnosis of lupus anticoagulant. J Thromb Haemost 2003;1:2689–91

37C Incubated 1:1 PTT Mix



PTT
42.5 s

Incubated PTT 35 s

PTT of Mix
≤38.5 s: Correction
>38.5 s: No correction

Still using 10% prolongation as correction cutoff

Mixing Study Result
32-yo Female, 6 Weeks Post-partum

Assay	Result	RI	Comment
PTT	42.5 s	25–35 s	Confirms previous PTT
PTT/control 1:1 mix immediate	32.1 s	Control 30 s	Commercial platelet-free control plasma (NP)
PTT/control 1:1 mix 1 h at 37°C	37.3 s	Control 35 s	Incubate both 1:1 mix and NP
Conclusion: immediate *and* incubated mix PTTs correct, suspect factor deficiency, arrange for factor assays and von Willebrand disease workup			

Factor Assay Results
32-yo Female, 6 Weeks Post-partum

Assay	Result	RI	Comment
Factor VIII	39%	50–150%	VWD?
Factor IX	92%		
Factor XI	131%		
Factor XII	113%		XII, HMWK & PK deficiency not associated with bleeding
HMWK	ND	65–135%	
PK			

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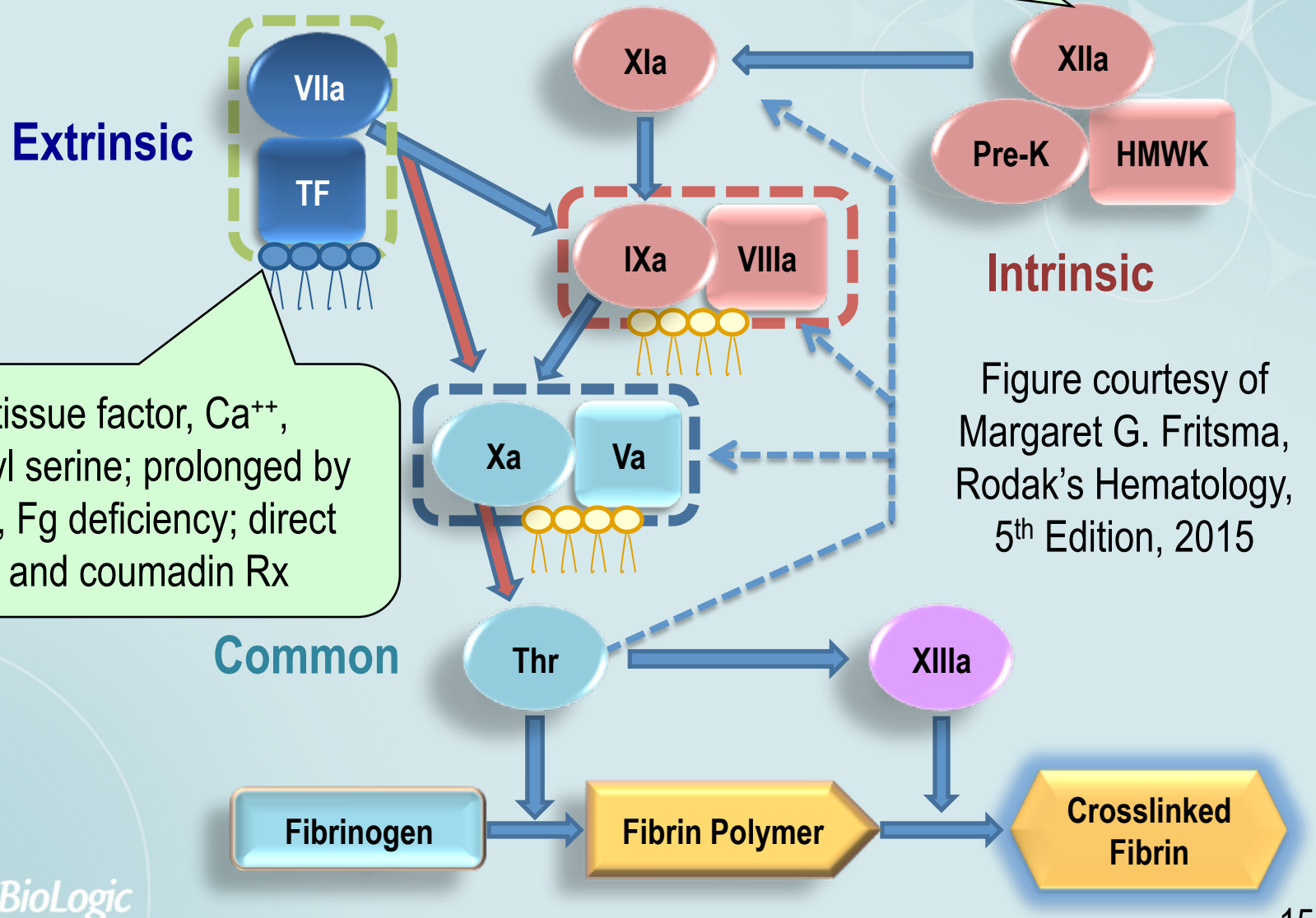
PTT rgt: Ca^{++}, activator, phosphatidyl serine; prolonged by XII, PK, HMWK, XI, IX, VIII, X, V, II, Fg deficiency; heparin, DTIs, LA



PT rgt: tissue factor, Ca^{++}, phosphatidyl serine; prolonged by VII, X, V, II, Fg deficiency; direct anti-Xa and coumadin Rx

Figure courtesy of Margaret G. Fritsma, Rodak's Hematology, 5th Edition, 2015

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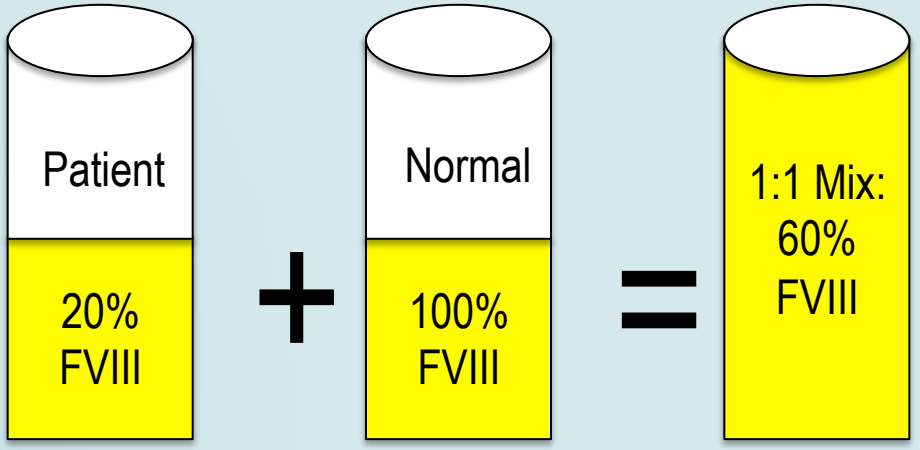
PT and PTT Test Results in Inherited Coagulopathies

PT	PTT	Single Factor Deficiency
Long	Normal	VII
Long	Long	X, V, II, and fibrinogen[1]
Normal	Long	VIII, IX, XI[2]

[1]PT & PTT prolonged when fibrinogen is <100 mg/dL, perform fibrinogen assay
[2]Contact factor deficiencies XII (1–3% prevalence), prekallikrein (PK, Fletcher), or high molecular weight kininogen (HMWK, Fitzgerald) also prolong PTT results, but no bleeding

PTT Mix: Why Does This Work?

- Hypothetical 20% F VIII level prolongs PTT
  - PTT rgts calibrated to prolong at 30–40% FVIII, IX, XI
- Add NP with established 100% factor level
  - 1:1 mix, average of 100% and 20% = 60% (corrects)
- Hypothetical anti-FVIII or lupus anticoagulant
  - With typical avidity, retains ability to prolong the mix



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Case
52-yo Athletic Female

Pre-op screen for total hip replacement

52-yo Athletic Female
Screen Prior to Hip Replacement Surgery

Test	Result	RI
HGB	14.1 g/dL	12–15 g/dL
PT	11.2 s	9.8–12.6 s
PTT	58 s	25–35 s
PLT	170,000/μL	150–400,000/μL
Fibrinogen	410 mg/dL	220–498 mg/dL
Patient reports no bleeding or bruising, no thrombosis		

Isolated Prolonged PTT: Differential

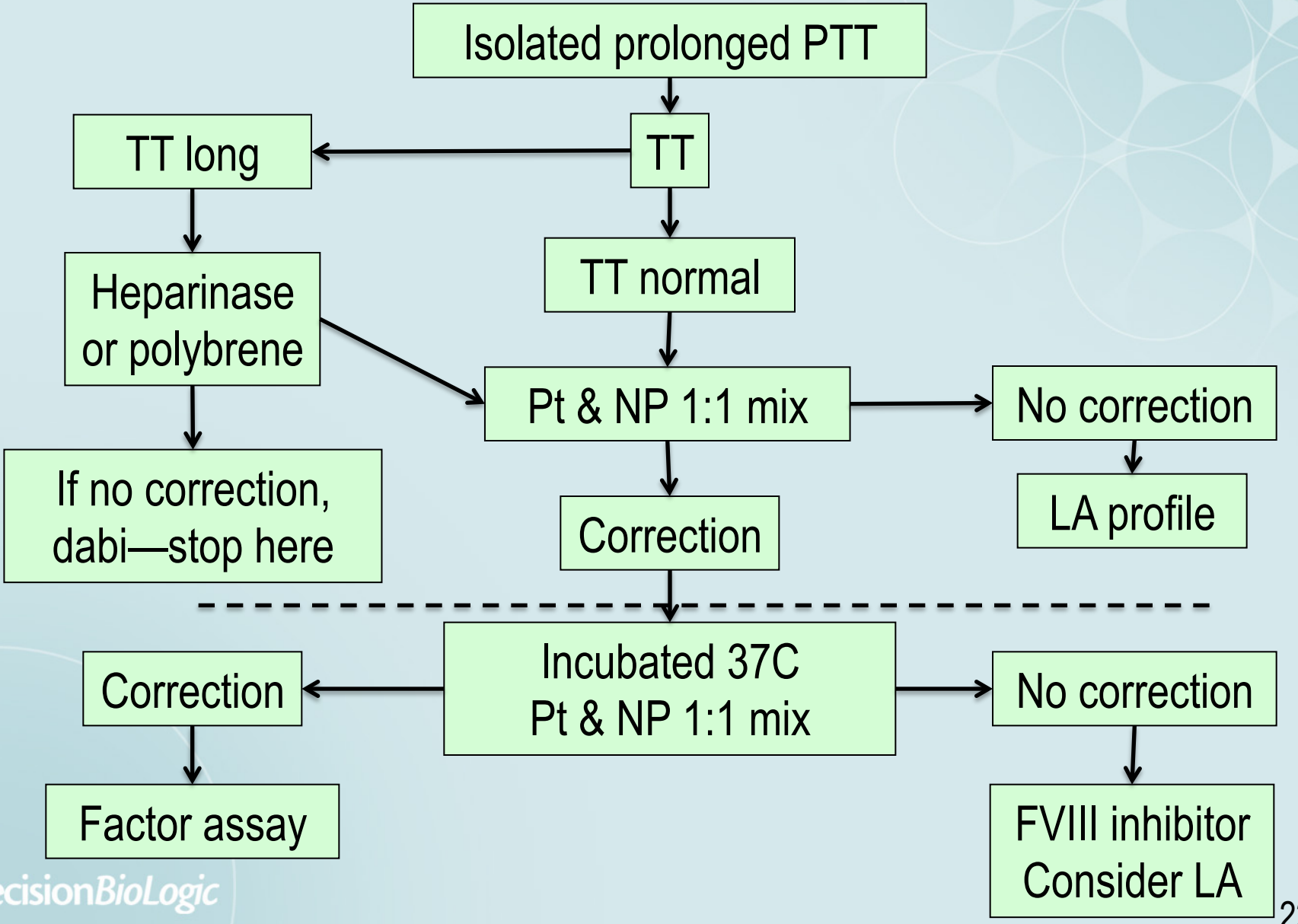
- Could be nothing: 5% of normals exceed limit
- Preanalytical variable: green or lavender-closure tube, hemolysis, lipemia, clotted specimen
- Outpatient: dabigatran
- Inpatient: unreported UFH

- Congenital single factor deficiency: VIII, IX, or XI, hemophilia A, B, or C with bleeding, VWD
- Congenital FXII, PK, or HMWK without bleeding
- FVIII inhibitor (acquired hemophilia) with bleeding
- Lupus anticoagulant (LA)

52-yo Female PTT Mixing Study

Test	Result	Comment
TT	17 s	RI: < 21 s, rules out dabigatran
PTT	58 s	RI: 25–35 s
PTT NP	28 s	Correction if ≤ 30.8 s (10%)
1:1 mix	35 s	25% over NP = no correction
What is the next step?		

Acute Care Mixing Study Algorithm



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Mixing Study Considerations

- Preanalytical variables
  - Anti-Xa rivaroxaban, apixaban, edoxaban prolong PT, PTT
  - Dabigatran and UFH prolong PTT
  - Clotted, hemolyzed, lipemic specimen
  - Underfilled tube, wrong anticoagulant
  - PT & NP must be platelet-poor (free), <10,000/uL
  - Cfg at 2500 g/10 m or double-spin
- Heparinase/polybrene neutralize ≤ 1 unit/mL UFH
- Anti-FVIIIs may generate immediate neutralization
- Weak LAs may be missed in 1:1 mix: ask for consult
  - Select a more LA-sensitive PTT reagent or request 4:1 mix
  - 15% of LAs require incubation

Not so much

Mostly

The “LA Cofactor Effect”

- Initial PTT 48 s, RI 25–35; 1:1 mix *prolongs* to 54 s
- LA “cofactor” effect may be prothrombin binds LA
- Or maybe LA potentiates clotting via annexin V?
- Mix reverses potentiation?

- Magrath M. Lupus cofactor phenomenon. Letter J Clin Pathol 1990,42:264.
- Rand JH, Wu XX, Andree HA, et al. Antiphospholipid antibodies accelerate plasma coagulation by inhibiting annexin-V binding to phospholipids: a "lupus procoagulant" phenomenon. Blood. 1998;92:1652–60.
- Clyne LP. Plasma requirement for expression of lupus-like anticoagulant. Folia Haematologica int Ma Klin Morphol Blutforsch 1986;113:841

Normal Plasma Source?

- Home brew: ~20 normal plasmas, male ≅ female
  - Ensure plasma is platelet-poor; < 10,000/uL
  - Ensure NP has ~100% of all factors; PTT ≅ MRI
    - For instance, elevated FVIII causes false negatives
  - Screen for LA, specific factor inhibitors. HBV, HCV, HIV
  - Aliquot and freeze
- Or purchase commercial plasma
  - GMP meets all criteria
  - Frozen meets all criteria
  - Lyophilized acceptable when validated in house
    - Processed with stabilizers

Clinical and Laboratory Standards Institute. One-stage prothrombin time (PT) test and activated partial thromboplastin time test (APTT) approved guideline—second edition. CLSI Document H47-A2. CLSI, Wayne PA. 2008.

What Limit Defines Correction?

No Consensus; Fritsma Factor Quick Question Results

- **Limit based on fixed PTT value from reference interval**
  - 1:1 mix within RI upper limit (95% or 99% CI, 39%)
  - 1:1 mix within RI upper limit + 5 seconds (8%)
  - 1:1 mix within mean of RI + 2 or 3 SD (0%)
- **Limits based on NP PTT value**
  - 1:1 mix within NP PTT value + 5 seconds (14%)
  - 1:1 mix within NP PTT + 10% (32%)
- **Limit formula using patient, NP, and 1:1 mix**
  - Must incubate patient sample, NP, and 1:1 mix
  - Chang's % deviation; Rosner index
- **Combo of RI and Rosner (dedicated RI for mix, 7%)**

Chang Formula Based on % Correction

$$\%\ \text{Correction} = \frac{\text{Patient PTT} - \text{1:1 mix PTT}}{\text{Patient PTT} - \text{NP PTT}} \times 100$$

$$\%\ \text{Correction} = \frac{42.5 - 32.1 = 10.4}{42.5 - 30 = 12.5} = 0.83 = 83\%$$

Factor Deficiency = ≥ 75%

Inhibitor = < 75%

% Correction Chang formula verified by local laboratory

Chang SH, Tillema V, Scherr D. A "percent correction" formula for evaluation of mixing studies. Am J Clin Pathol 2002;117:62–73.

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Rosner Index Based on Ratio

$$\text{Rosner Index} = \frac{\text{1:1 mix PTT} - \text{NP PTT}}{\text{Patient PTT}} \times 100$$

$$\text{Rosner Index} = \frac{32.1 - 30}{42.5} \times 100 = 4.9$$

Inhibitor	≥ 11
Correction	< 11

Rosner index limit validated by local laboratory

Rosner E, Pauzner R, Lusky A, Modan M, Many A. Detection and quantitative evaluation of lupus circulating anticoagulant activity. Thromb Haemost 1987; 57: 144-147.

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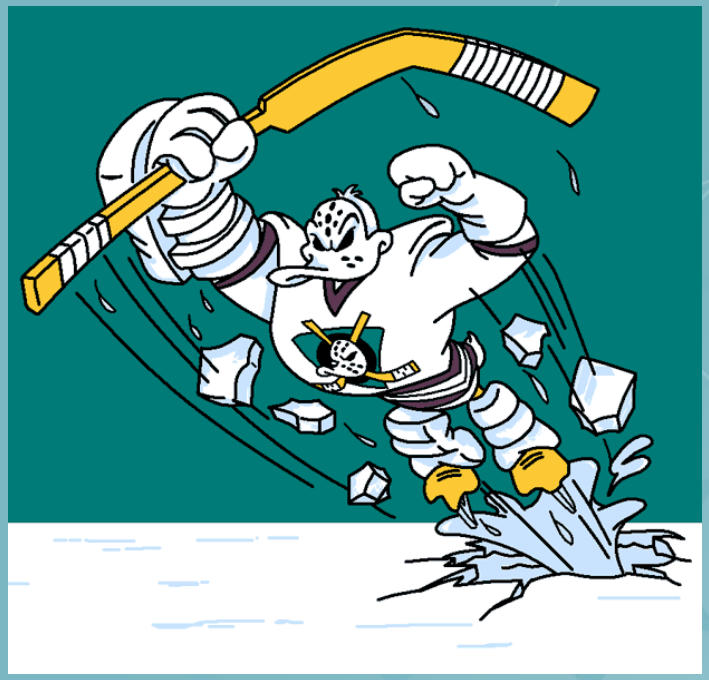
59-yo Male
Former Hockey Player

Total knee replacement preop



59-yo Male Former Hockey Player
Screen Prior to Knee Replacement Surgery

Test	Result	RI
HGB	14.8 g/dL	12–15 g/dL
PT	11.2 s	9.8–12.6 s
PTT	38 s	25–35 s
PLT	310,000/μL	150–400,000/μL
Fibrinogen	390 mg/dL	220–498 mg/dL
Patient reports no bleeding or bruising, no thrombosis		

When to Perform Mixing Study

- Any PTT > RI upper limit
- Any PTT > RI upper limit + 5 seconds
- Any PTT > RI upper limit with consult
  - Is patient bleeding or clotting?
  - Possible “weak” LA: use 4:1 mix
  - Lupus sensitive PTT reagent
  - Factor sensitive PTT reagent

Pengo V, Tripodi A, Reber F, et al. Update of the guidelines for lupus anticoagulant detection. J Thrombos Haemost 2009;7:1737–40.

When to Perform Mixing Study Some Practical Considerations

- If you use a value slightly longer than the RI limit and define correction as return to the RI you miss most inhibitors.
- If you perform mixing studies on prolonged PTTs from inpatients, at least 50% will be due to anticoagulant therapy.
- If you call the unit on any prolonged PTT you are likely to get no information.

59-yo Male Former Hockey Player

Test	Result	Comment
TT	17 s	RI: < 21 s, rules out dabigatran
PTT	38 s	RI: 25–35 s
PTT NP	31 s	Correction if < 34.1 s (10%)
1:1 mix	35 s	Correction? No correction?
What is the next step?		

59-yo Male Former Hockey Player Clinical Consult

- Consult: if no thrombosis or bleeding, go no further
- Thrombosis: perform mix using 4:1 patient to NP
- Or choose PTT reagent that is LA-sensitive
- If anatomic bleeding symptoms, test FVIII, FIX, FXI
  - Vitamin K deficiency, renal insufficiency, liver disease, malignancy, VWD

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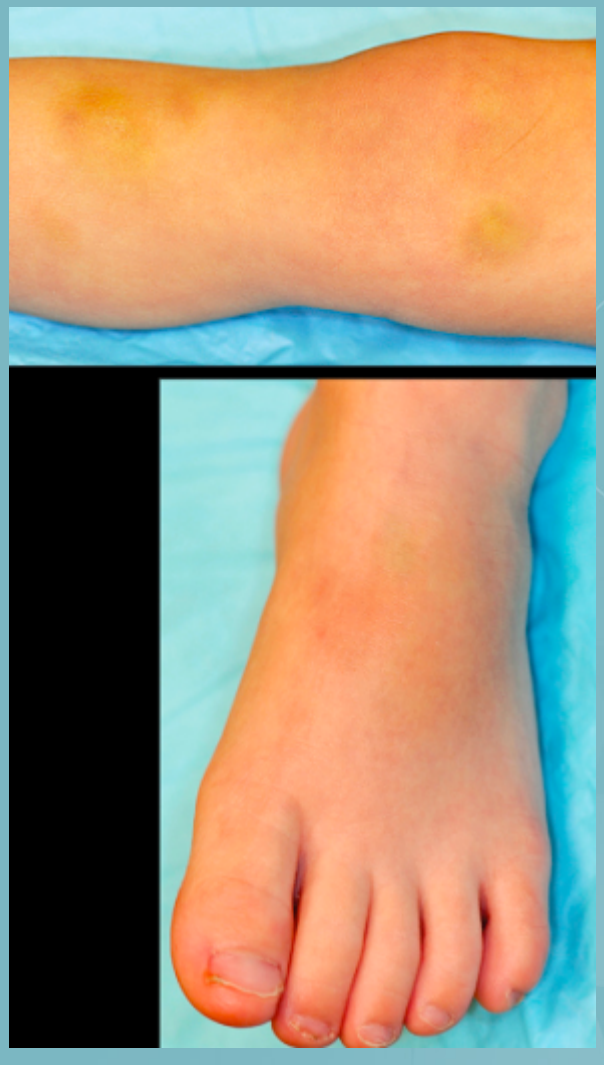
2-yo Hemophilic Boy



2-yo Hemophilic Boy

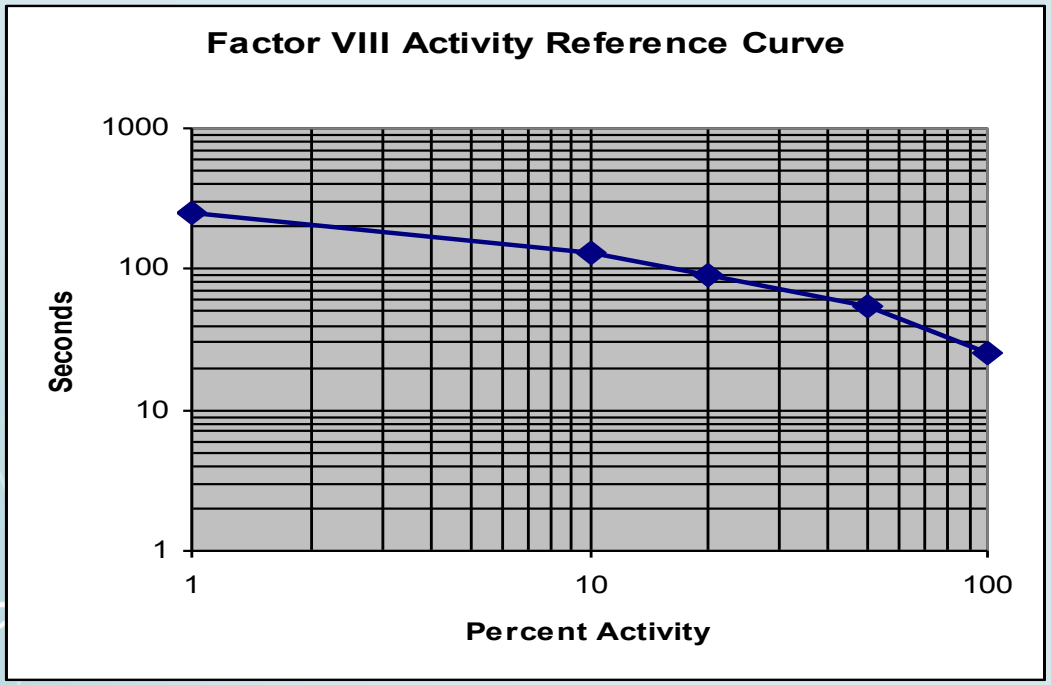
Test	Result	RI
HGB	11.8 g/dL	9.6–15.6 g/dL
PT	11.2 s	9.8–12.6 s
PTT	65 s	25–35 s
PLT	310,000/μL	150–400,000/μL
Fibrinogen	390 mg/dL	220–498 mg/dL
Inflamed, swollen knee and ankle		

Mixing Study Result
2-yo Hemophilic Boy

Assay	Result	RI	Comment
PTT	65 s	25–35 s	Confirms previous PTT
PTT/control 1:1 mix immediate	33.5 s	Control 30 s	Correction
PTT/control 1:1 mix 1 h at 37°C	47.9 s	Control 35 s	Control is incubated alone and with mix
Conclusion: Anti-FVIII inhibitor			

Factor VIII Assay

- Dilute plasma 1:10, add FVIII-depleted rgt plasma 1:1
- Add PTT reagent, incubate 3 minutes
- Add $CaCl_2$, record interval to clot formation
- Compare result in seconds to dilution curve



Factor VIII Assay Dilutions Parallelism Indicates No Inhibitor

Plasma Dilution	Seconds	Raw Factor VIII Activity	Computed Factor VIII Activity (× dilution)
1:10 “undiluted”	90 s	20%	20%
1:20	104 s	10%	20% (parallel)*
1:40	107 s	5%	20% (parallel)
1:80	110 s	2.5%	20% (parallel)
* <10% difference from undiluted indicates parallelism, no inhibitor			

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FVIII Assay Dilutions non-Parallelism Indicates Inhibitor

Plasma Dilution	Seconds	Raw Factor VIII Activity	Computed Factor VIII Activity (× dilution)*
1:10 “undiluted”	80 s	10%	10%
1:20	93 s	8%	16%
1:40	107 s	5%	20%
1:80	108 s	4%	32%
* >10% difference from undiluted, rising = non-parallel, implies inhibitor			

Kasper CK. Laboratory diagnosis of factor VIII inhibitors. In Kessler C, Garvey MB, Green D, Kasper C, Lusher J. Acquired Hemophilia 2nd Edition. Excerpta Medica 1995

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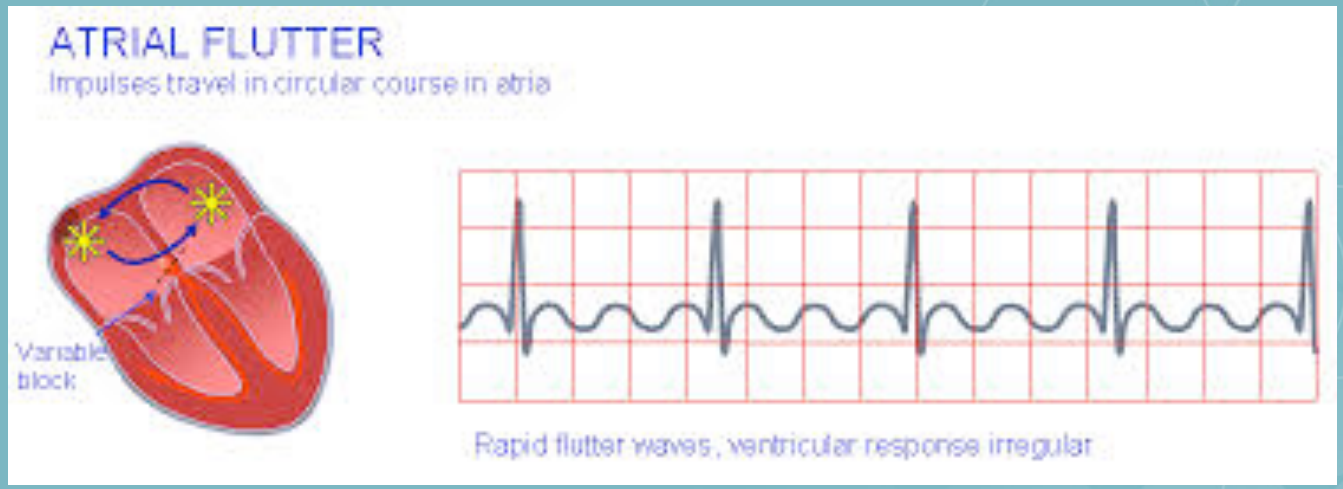
55-YO Male with Atrial Fibrillation



55-yo Male with Atrial Fibrillation

Test	Result	RI
HGB	13.8 g/dL	12–15 g/dL
PT	17.2 s	9.8–12.6 s
PTT	159 s	25–35 s
PLT	310,000/μL	150–400,000/μL
Fibrinogen	20 mg/dL	220–498 mg/dL

55-yo Male with Atrial Fibrillation

Assay	Result	RI
PTT	159 s	25–35 s
TT	> 150 s	< 21 s
PTT/control 1:1 mix immediate	78 s	Control 30 s
PT/control 1:1 mix immediate	15.2 s	Control 12 s
What do you recommend?		

If the PT is Prolonged

- Congenital deficiencies of II, V, VII, or X
  - PT and PTT long: II, V, X
  - PT only: VII, skip mixing and go to factor assay
  - Prevalence: 500,000–1:2,000,000
- Liver disease: PT prolongs before PTT due to des-carboxy II, VII, and X, reduced factor V
- Vit K deficiency: des-carboxy II, VII, and X
- Anti-Xa direct oral anticoagulants
  - Rivaroxaban, apixaban, edoxaban

Isolated Prolonged PTT: Summary

- Random benign prolongation, 95% CI
- Lupus anticoagulant: 1–3%
  - Drug reaction producing transient LA
- Unrecorded heparin, dabigatran, oral anti-Xa
- Known hemophilic who fails FVIII concentrate Rx
- Hemorrhage or ecchymoses signal acquired coagulopathy; vitamin K deficiency, liver disease
- Specific inhibitor, anti-FVIII: post partum, malignancy, autoimmune disorders, > 60 YO

Sahud MA. Factor VIII inhibitors. Laboratory diagnosis of inhibitors Semin Thromb Hemost 2000;26:195–203.

Develop Mixing Study Reliability

- Test PTT reagent sensitivities
  - 30–40% FVIII, FIX, FXI
  - Select Intermediate sensitivity to LA
- NP consistency: ~100% activity for all factors
- Consultation for equivocal patient results
- Employ consistent correction limit

Perform Mixing Studies Locally

- Unexpected isolated prolonged PTT or PT requires immediate action
- Delay results in specimen deterioration
- Perform locally, results may immediately direct therapy
- Forward results to ref lab to direct follow-up

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Summary: Mixing Studies at the Acute Care Facility

The participant…

1. Prepares a stepwise PTT and PT mixing study protocol
2. Indicates the clinical purpose for PTT mixing studies
3. Explains why the mixing study is an acute care assay
4. Correlates mixing study results with coagulation test results

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Thanks for listening!

Questions?