PrecisionBioLogic

Improving Acute Care with Coagulation Mixing Studies

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Coagulation Mixing Studies Learning Objectives

The participant…

1. Defines the method and clinical applications for a PTT mixing study.
2. Lists the steps to perform a PTT mixing study.
3. Explains why the mixing study is an acute care assay.
4. Correlates mixing study results with patient coagulation testing.

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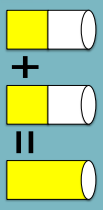
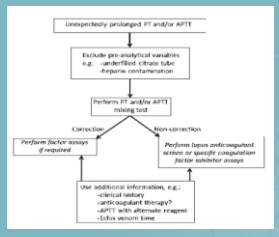
Mixing Study An Acute Care Investigation Purpose: *differentiate a coagulation deficiency from an inhibitor*





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, vaginal bleeding

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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		

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Rule Out Heparin, Dabigatran

Assay	Patient	RI
TT	14 s	<21 s

- R/O unfractionated heparin (UFH) and dabigatran
  - Outpatient—consider dabigatran
  - Inpatient—unrecorded UFH flush of vascular catheter
- If dabigatran, discontinue testing, allow to clear
- If UFH, treat w/ 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)

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PTT Mixing Study: Cheap and Basic

- Start within 2 h to avoid specimen degradation
  - Factors V (FV) and VIII (FVIII) deteriorate
  - Platelet factors released: PF4, FV
  - Ensure patient plasma is platelet-poor, < 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% above NP PTT
  - Factor deficiency
- No correction: 1:1 mix is >10% above NP PTT
  - Non-specific inhibitor, usually LA
  - Specific inhibitor (anti-FVIII), usually requires 37°C incubation

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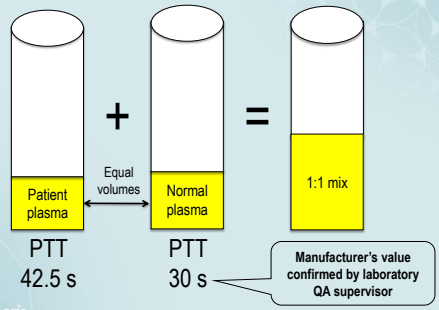
PTT Mixing Study



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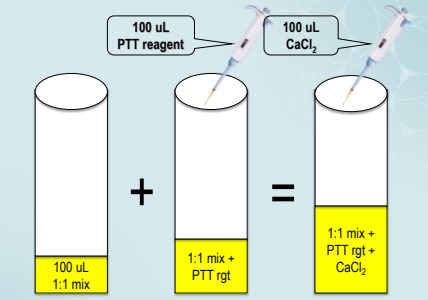

PTT Mixing Study Using 10% Rule

PTT
≤33 s: Correction
>33 s: No correction

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1:1 PTT Mix with Incubation

- PTT of immediate mix ≤10% above NP
  - Correction: factor deficiency? But first…
  - Incubate 1:1 mix 1–2 h and repeat
- Corrected after incubated mix = factor deficiency
- No correction: PTT remains >10% above 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

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1:1 PTT Mix with Incubation

- Reflex to incubation if unincubated mix corrects
- Must also incubate normal control plasma
- Compare mix PTT to incubated normal control PTT
- May also detect temperature-dependent LA
  - ~15% of LAs are temperature-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

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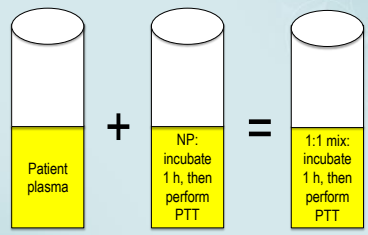
Incubated 1:1 PTT Mix



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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: both immediate *and* incubated mix PTTs correct, suspect factor deficiency, arrange for factor assays and von Willebrand disease profile			

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Factor Assay Results
32-yoFemale, 6 Weeks Post-partum

Assay	Result	RI	Comment
Factor VIII	32%	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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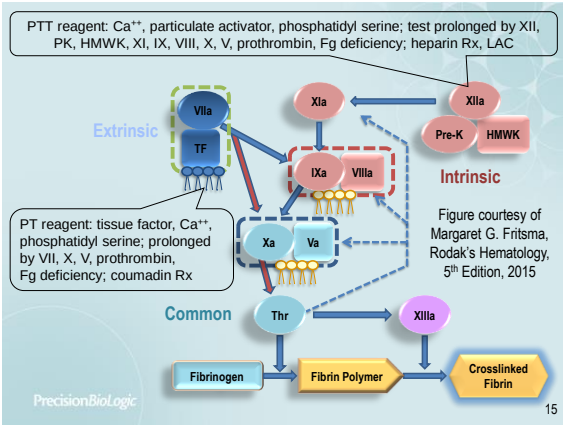

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PT and PTT Test Results in Inherited Coagulopathies

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

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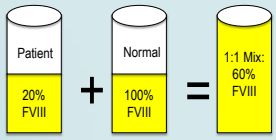
PTT Mix: Why Does This Work?

- 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%, PTT 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

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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		

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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
- Acquired FVIII inhibitor with severe bleeding
  - "Acquired hemophilia"
- Lupus anticoagulant (LA)

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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?		

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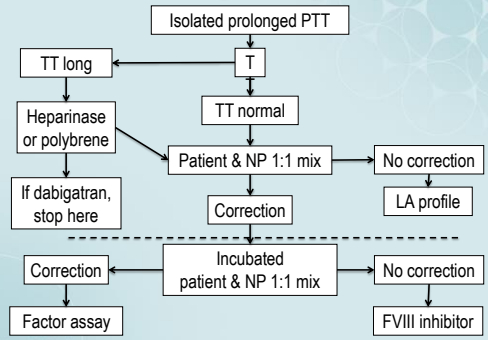
Acute Care Mixing Study Algorithm



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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
  - Must be platelet-poor, <10,000/uL patient and NP

Mostly

- Heparinase or polybrene neutralize only ≤1 unit/mL UFH
- Anti-FVIIIs may generate immediate neutralization
- 15% of LAs require incubation
- Weak LAs may be missed in 1:1 mix: ask for consult
  - Select a more LA-sensitive PTT reagent or prepare 4:1 mix

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The "LA Cofactor Effect"

- Screen PTT 48 s, RI 25–35; 1:1 mix *prolongs* to 54 s
- LA "cofactor" effect thought to be prothrombin combining with LA
- LA potentiates clotting via annexin V, mix abrogates 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

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Normal Plasma Source?

- Home brew: ~20 normal plasmas, male ≅ female
  - Ensure plasma is platelet-poor; < 10,000/uL; PTT ≅ MRI
  - Ensure NP has ~100% of all factors
  - Elevated FVIII causes false negative results
  - Screen for LA, specific factor inhibitors. HBV, HCV, HIV
  - Aliquot and freeze
- Or purchase commercial plasma
  - GMP & frozen meets all criteria
  - Lyophilized acceptable when validated
    - 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.

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What Limit Defines Correction?

No Consensus; Fritsma Factor Quick Question Results

- Limits based on fixed PTT value
  - 1:1 mix within RI upper limit (95% or 99% CI, 39%)
  - 1:1 mix within RI upper limit + 5 seconds (8%)
- Limits based on NP PTT value
  - 1:1 mix within NP PTT value + 5 seconds (14%)
  - 1:1 mix within NP PTT + 10% (same as ratio 1:1, 32%)
- Limit formula using patient, NP, and 1:1 mix
  - Must incubate patient sample, NP, and 1:1 mix
  - Chang's % deviation; Rosner index
- Other (7%): combination of RI and Rosner
  - Dedicated RI for mix

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1:1 Mix Limit 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 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

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

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

Inhibitor	≥ 11
Correction	< 11

Rosner index 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

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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		

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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.

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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?		

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59-yo Male Former Hockey Player Clinical Consult

- Consult: if he is well, go on to procedure
- Prior thrombotic events (VTE)
  - Perform mix using 4:1 patient plasma 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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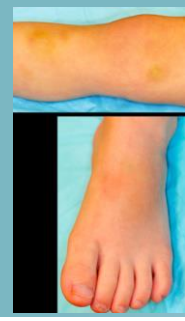
2-yo Hemophilic Boy



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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		

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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	
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			

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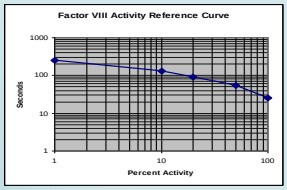
Factor VIII Assay

- Dilute plasma 1:10
- Add factor VIII-depleted reagent plasma 1:1
- Add PTT reagent, incubate 3 minutes
- Add $CaCl_2$, record interval to clot formation
- Compare result in seconds to calibration curve



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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

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

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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?		

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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
- Vitamin K deficiency: des-carboxy II, VII, and X
- Anti-Xa direct oral anticoagulants
  - Rivaroxaban, apixaban, edoxaban

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Isolated Prolonged PTT: Summary

- Random benign prolongation, 95% CI
- Lupus anticoagulant: Prevalence of 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
  - Postpartum, malignancy
  - Autoimmune disorders, > 60-yo

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

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Develop Mixing Study Reliability

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

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Perform Mixing Studies Locally

- Unexpected isolated prolonged PTT or PT may require immediate action
- Local results may immediately direct therapy
- Delayed specimen may deteriorate
- Forward results to ref lab to direct follow-up

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BLATE (Bottom Line at the End)

1. Define the method and clinical applications for a mixing study.
2. List the steps to perform a mixing study
3. Explain why the mixing study is a first-line assay.
4. Correlate mixing study results with patient coagulation testing.

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