The Fritsma Factor

YOUR INTERACTIVE HEMOSTASIS RESOURCE

Improving Acute Care Using Coagulation Mixing Studies

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The Fritsma Factor,

Your interactive Hemostasis Resource℠

Sponsored by Precision BioLogic

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Acute Care Mixing Studies
Bottom Line at the Start (BLAST)

The participant…

- Lists the clinical applications for PT and PTT mixing studies.
- Lists the steps to perform a PTT mixing study.
- Reviews the technical demands of mixing studies.
- Explains why the mixing study is an acute care assay.
- Correlates mixing study results with lupus anticoagulant and specific inhibitor testing.

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Mucocutaneous Bleeding
32-yo Female, 2 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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Isolated Prolonged PTT: Differential

- Could be nothing: 5% of normals exceed limit (± 2SD)
- Outpatient: DOAC, esp Pradaxa (dabigatran), a DTI
- 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 or IX inhibitor with severe bleeding
  - Auto-anti-factor VIII, “Acquired hemophilia”
- Lupus anticoagulant (LA, LAC)

But First, Rule Out Heparin, DOACs

Assay	Patient	RI
Thrombin time (TT)	14 s	<21 s
Chromogenic anti-Xa heparin assay	0.0 U/mL	0.4–0.7 U/mL

- Inpatient—unrecorded UFH flush of vascular access device
  - neutralize w/ Hepsorb (polybrene) or Hepzyme, proceed if TT normal
- Outpatient: direct oral anticoagulant (DOAC): discontinue
  - Dabigatran, direct thrombin inhibitor, TT markedly prolonged
  - Direct oral anti-Xa elevates anti-Xa, prolongs PT, may prolong PTT

Direct Oral Anticoagulants (DOACs)

- Oral direct anti-Xa anticoagulants: prolong the PT
  - Rivaroxaban; Xarelto
  - Apixaban: Eliquis
  - Edoxaban; Savaysa
  - Betrixaban; Bevyxxa

Mostly

- Oral direct thrombin inhibitor (DTI): prolong the PTT
  - Dabigatran; Pradaxa

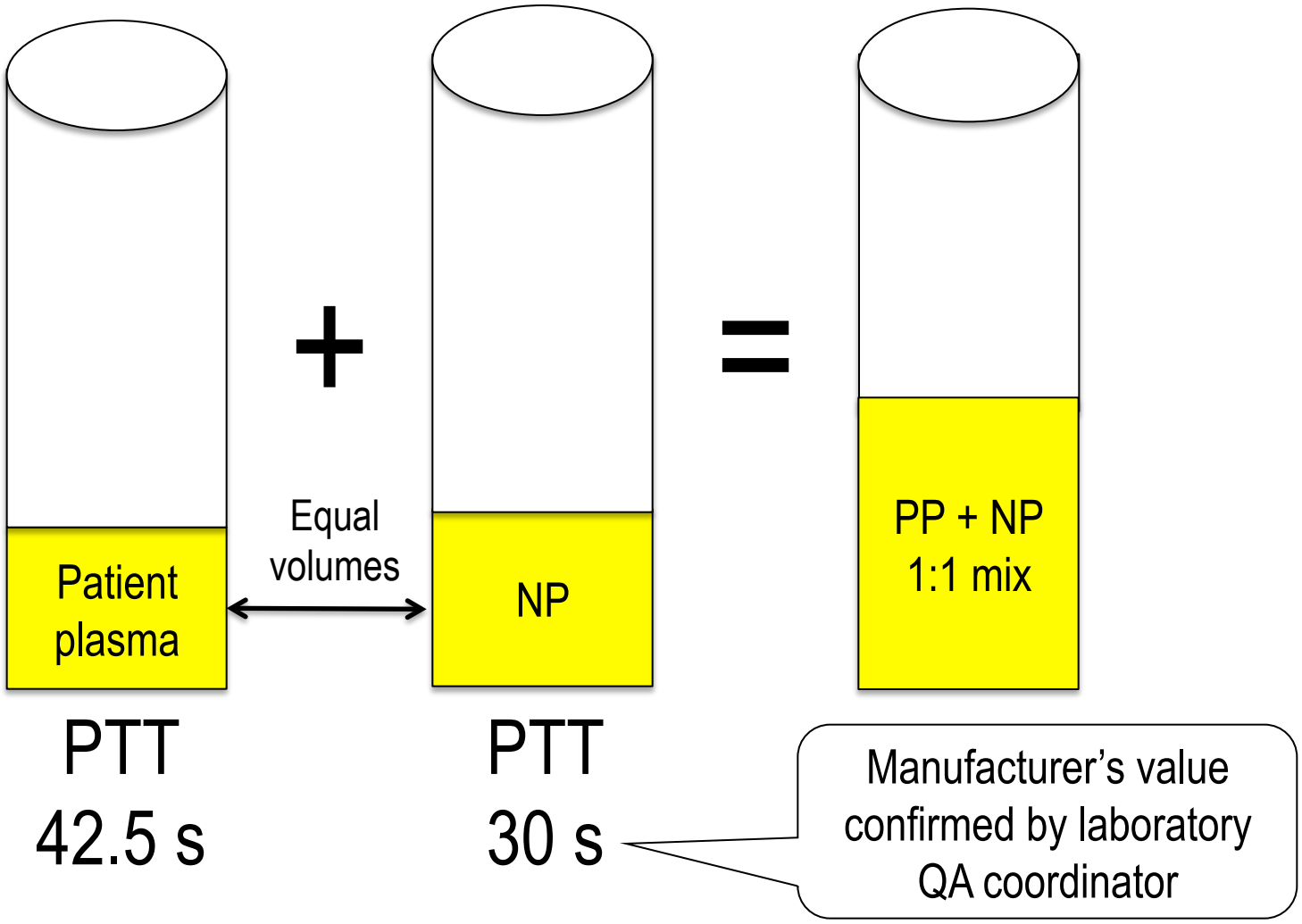
Mostly

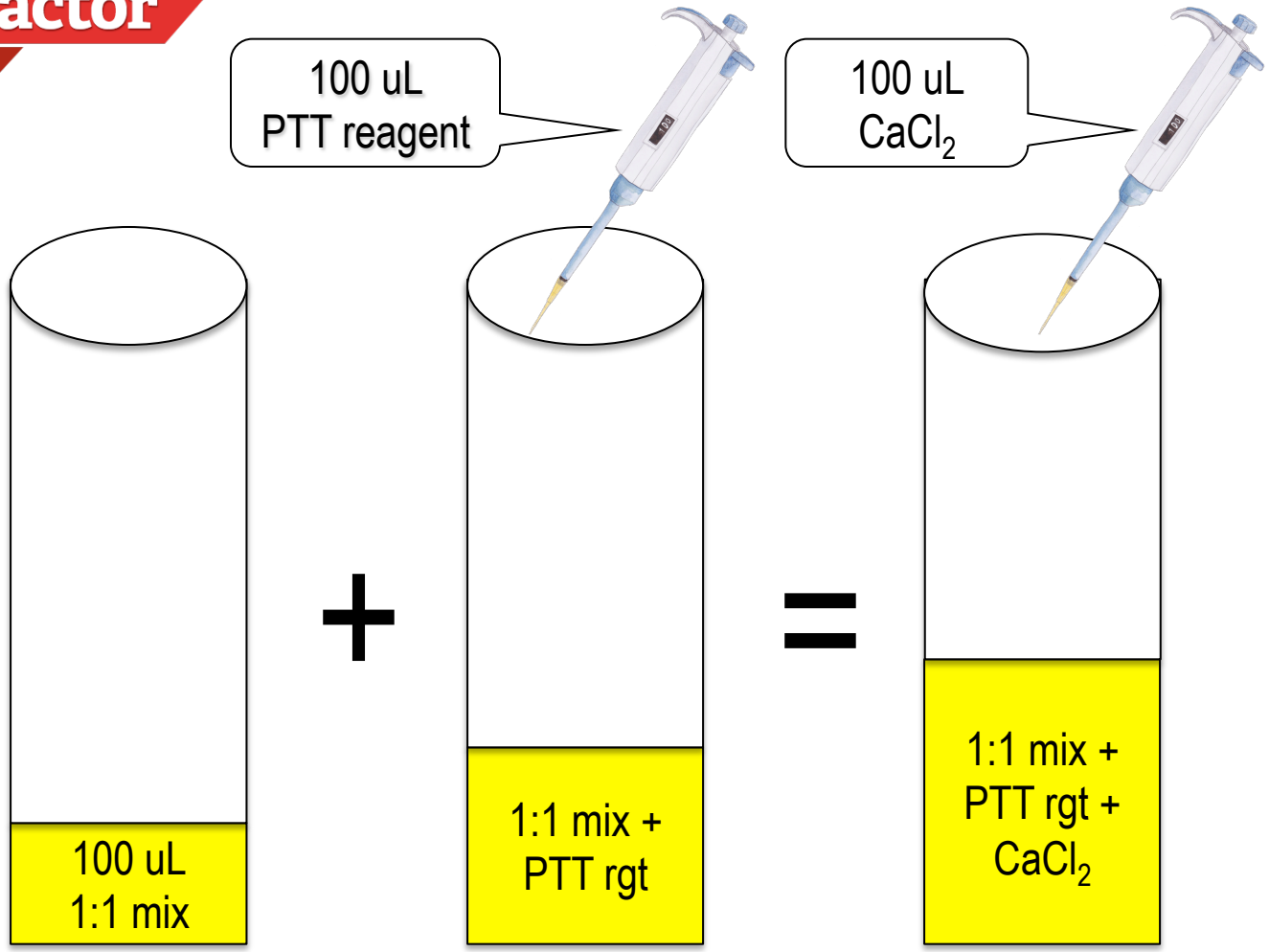
- Intravenous DTI
  - Argatroban; no brand name in US, generic available, PTT
  - Bivalirudin; Angiomax, PTT

PTT Mixing Study: Cheap and Simple

- Mix patient plasma 1:1 with pooled normal plasma (NP) and perform immediate PTT on mixture
- No correction: If PTT of 1:1 mix is >10% longer than NP PTT
  - Lupus anticoagulant (LAC)
- Correction: If PTT of 1:1 mix is ≤10% longer than NP PTT
  - Factor deficiency?
  - Specific inhibitor (anti-FVIII)?, requires 37°C incubation

PTT Mixing Study





PTT Mixing Study Using 10% Limit

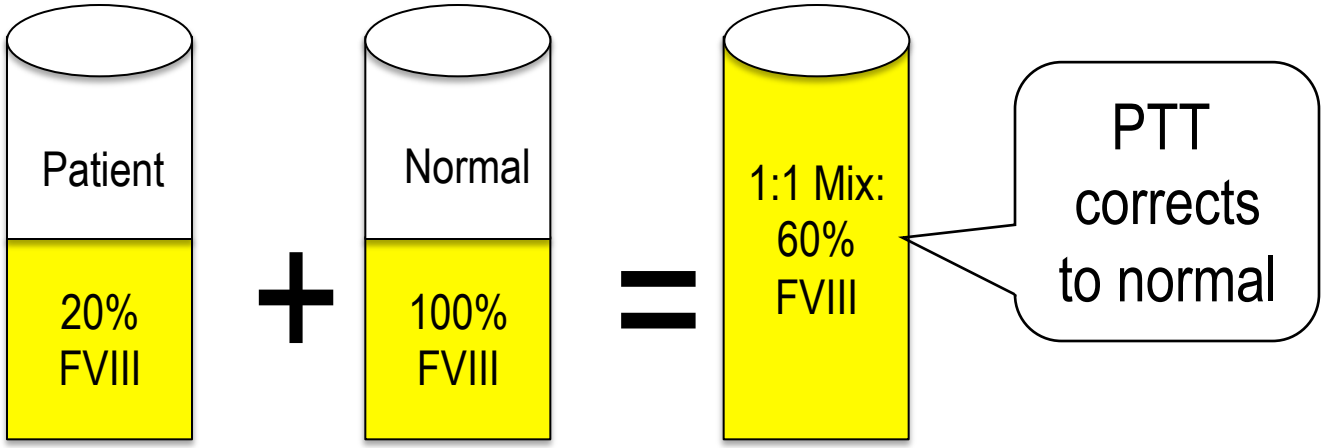
PTT

≤33 s: Correction

>33 s: No correction

PTT Mix: Why Does This Work?

- Hypothetical 20% F VIII level prolongs PTT
  - PTT rgts are 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 its ability to prolong the mix



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PT and PTT Results can predict Inherited Single-factor 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 only when fibrinogen is <100 mg/dL, perform fibrinogen assay (fibrinogen assay often added to initial screen)

[2]Contact factor deficiencies XII (1–3% prevalence), prekallikrein (PK, Fletcher), or high molecular weight kininogen (HMWK, Fitzgerald) also prolong PTT results, but not associated with bleeding

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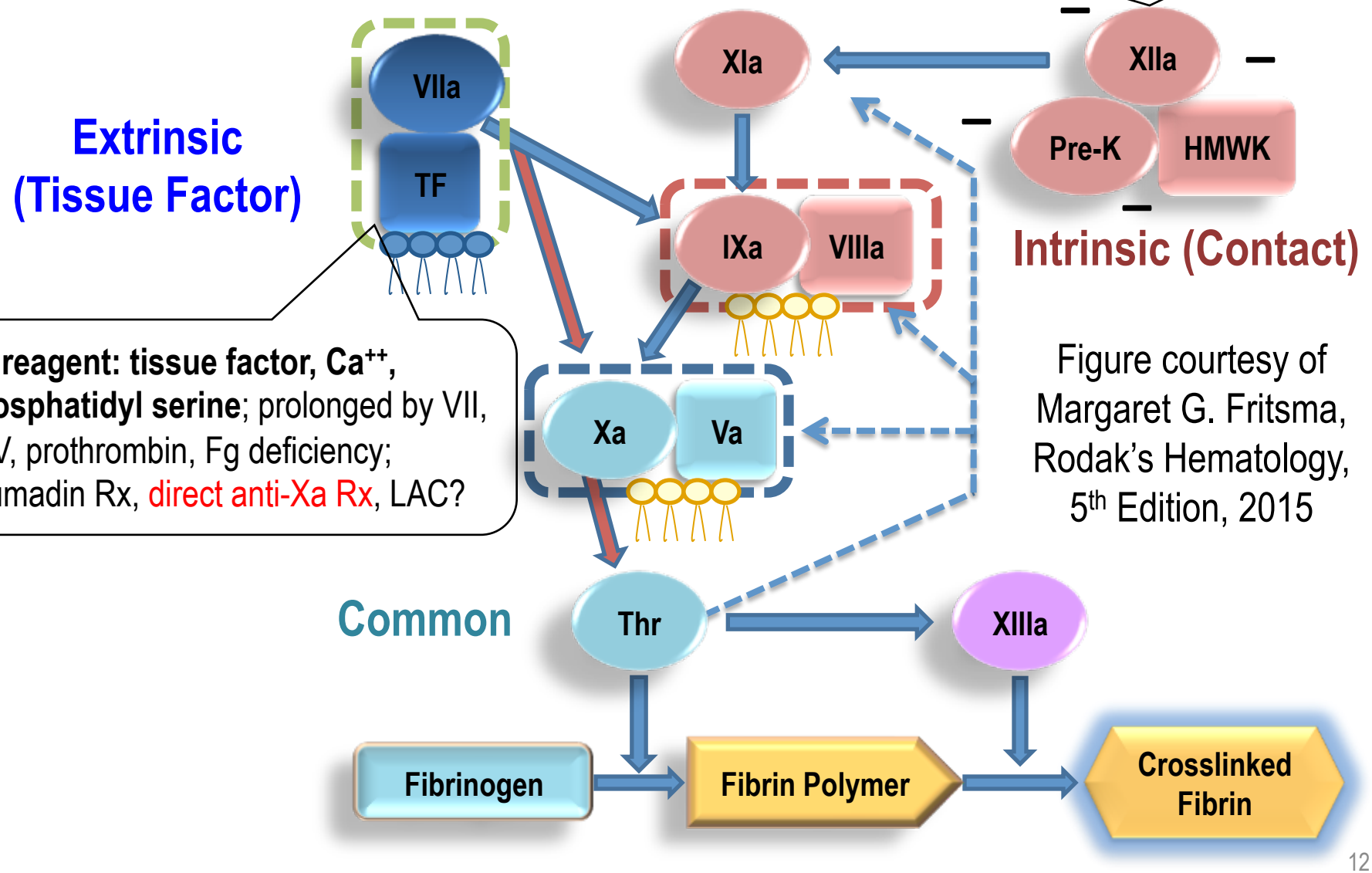
PTT reagent: Ca^{++}, particulate negative charge activator, phosphatidyl serine; test prolonged by XII, PK, HMWK, XI, IX, VIII, X, V, prothrombin, Fg deficiency, UFH, DTI, LAC



PT reagent: tissue factor, Ca^{++}, phosphatidyl serine; prolonged by VII, X, V, prothrombin, Fg deficiency; coumadin Rx, direct anti-Xa Rx, LAC?

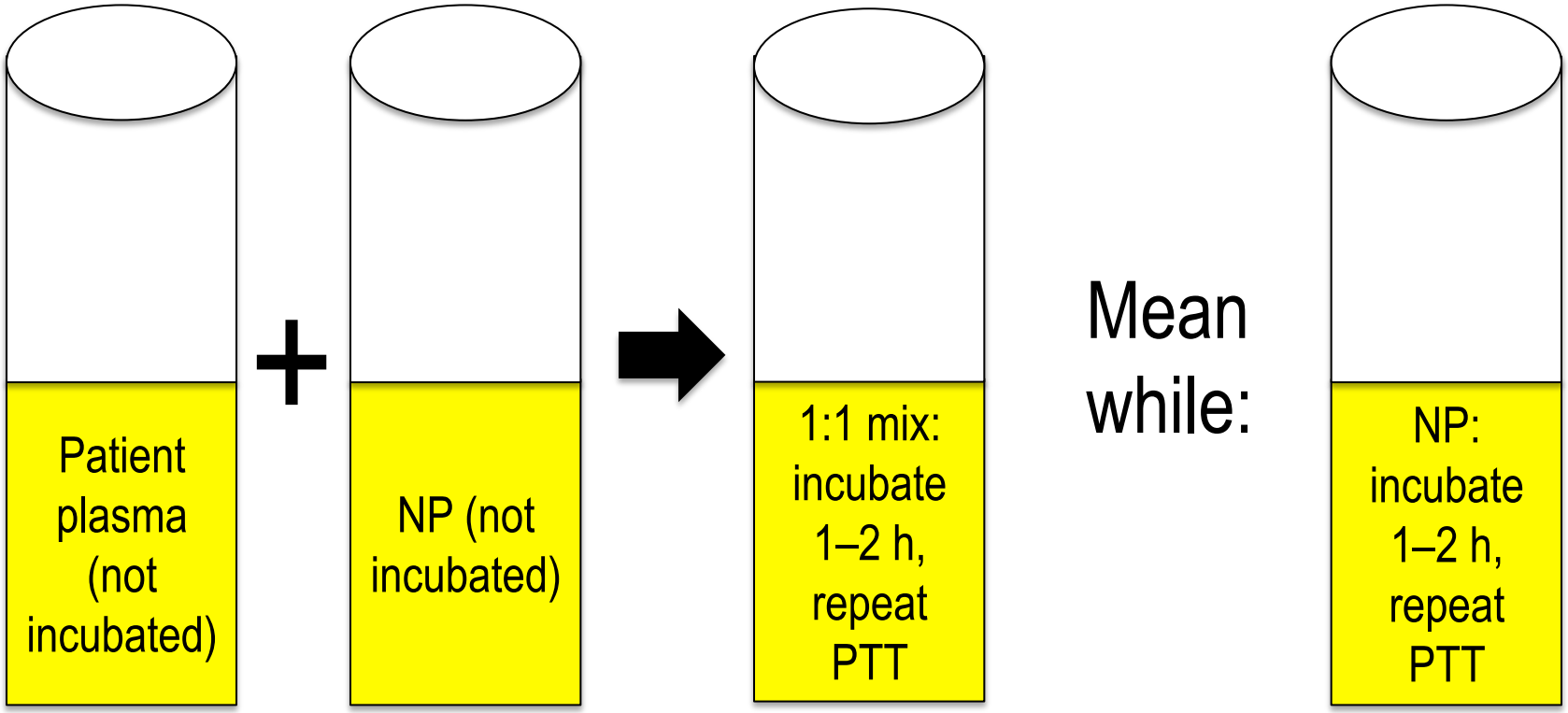
Figure courtesy of Margaret G. Fritsma, Rodak's Hematology, 5th Edition, 2015

1:1 PTT Mix with Incubation

- PTT of immediate mix ≤10% longer than NP
  - Correction: factor deficiency? But first…
  - Incubate 1:1 mix 1–2 hours and repeat
- Correction after incubated mix = factor deficiency
- No post-inc correction: PTT remains >10% above NP
  - Specific inhibitor such as anti-FVIII;
  - IgG_4: Temp dependent, requires incubation
- NP must be incubated. Why?

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

37°C Incubated 1:1 PTT Mix



PTT
42.5 s

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

Compare to incubated PTT: 35 s

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Incubated Mixing Study Result

32-yo Female, 2 Weeks Post-partum

Assay	Result		Comment
PTT	42.5 s	RI: 25–35 s	Confirms previous PTT
PTT/NP1:1 mix immediate	32.1 s	Limit: 33 s	NP: 30s
PTT/NP1:1 mix 1–2 h at 37°C	37.3 s	Incubated NP: 35s, limit 38.5 s (10%)	
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-yo Female, 2 Weeks Post-partum

<table>
<tr><th>Factor</th><th>Result</th><th>RI</th><th>Comment</th></tr>
<tr><td>VIII</td><td>32%</td><td rowspan="4">50–150%</td><td rowspan="2">  </td></tr>
<tr><td>IX</td><td>92%</td></tr>
<tr><td>XI</td><td>131%</td></tr>
<tr><td>XII</td><td>113%</td><td rowspan="3">XII, HMWK & PK deficiency not associated with bleeding</td></tr>
<tr><td>HMWK</td><td rowspan="2">ND</td><td rowspan="2">65–135%</td></tr>
<tr><td>PK</td></tr>
</table>

Expanded PT and PTT Results

Deficiency/Disorder	PT	PTT	PT Mix	PTT Mix
VWF[1], VIII, IX, XI	N	Long	ND	Correc
VII	Long	N	Correc	ND
FG, II, V, X	Long	Long	Correc	Correc
Liver Dis, VK Def, DIC, Amyloid[2]				
Nephrotic Syndrome	N	Long	ND	Correc
LAC, VIII, IX Inh	N	Long	ND	No Cor
DOACs[3]	Long	Long	No Cor	No Cor
UFH	N	Long	ND	No Cor

1: if VIII < 35 U/dL; 2: if X deficient; 3: Anti-Xa prolongs PT, DTI prolongs PTT

52-yo Athletic Female

Screen Prior to Hip Replacement Surgery

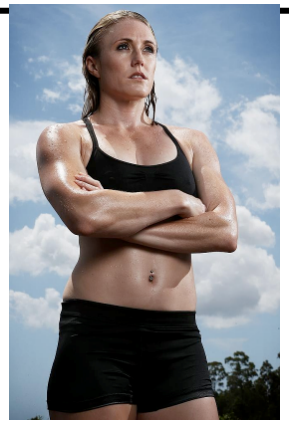
Test	Result	RI
HGB	14.1 g/dL	12–15 g/dL
PT	11.2 s	9.8–12.6 s
PTT	58 s	25–35 s
PLT	170,000/μL	150–400,000/μL
Fibrinogen	410 mg/dL	220–498 mg/dL
She reports no bleeding or bruising, no thrombosis		

Again: Isolated Prolonged PTT: Differential

- Could be nothing: 5% of normals exceed limit (± 2SD)
- Outpatient: DOAC, esp Pradaxa (dabigatran) a DTI
- 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 or IX inhibitor with severe bleeding
  - Auto-anti-factor VIII, “Acquired hemophilia”
- Lupus anticoagulant (LA, LAC)

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% longer than NP = no correction
What is the next step?		



PTT Reagent LAC Response

Fritsma Factor 2011–12 Survey: LAC Sensitivity			
LAC Response	**FF**	**CAP 2011**	**Examples**
High	10%	46%	Siemens Actin FSL HemosIL aPTT-SP
Intermediate	43%	47%	Stago STA-PTT-LA HemosIL SynthASil
Low	18%	3%	Siemens Actin FS Stago CK–Prest
Don't know	29%	Other: 4%	

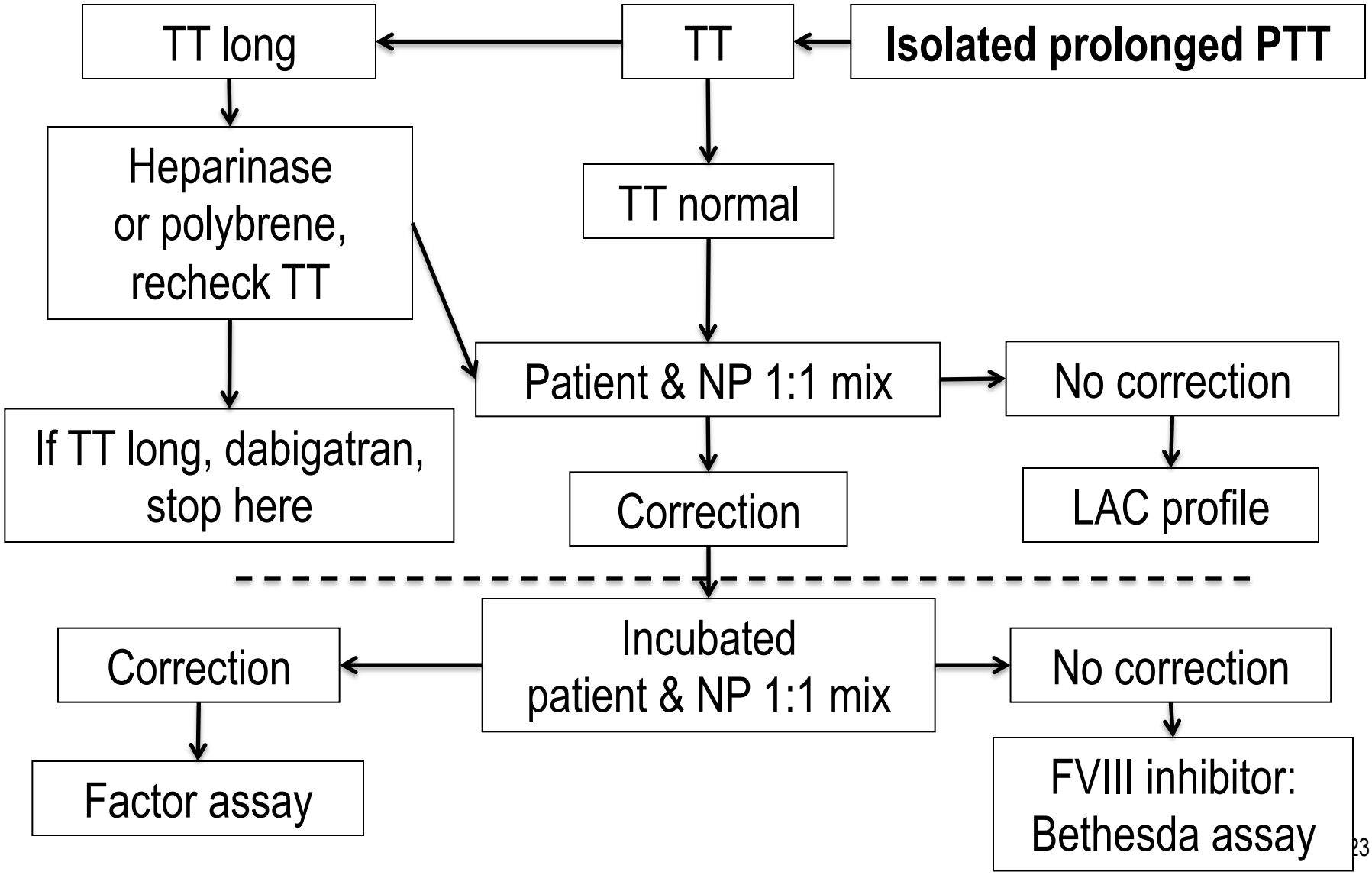
Fritsma GA, Dembitzer FR, Randhawa A, et al. Recommendations for appropriate activated partial thromboplastin time reagent selection and utilization. Am J Clin Pathol 2012; 137: 904–8.

Variation in PTT Reagent Response to LAC

LAC Pos Pat	Triniclot APTT S	Triniclot APTT HS	Actin FSL	Actin FS	PTT-LA
1	34	61	65	31	132
2	62	74	74	34	138
3	88	186	164	47	>300
4	234	64	69	30	253
5	60	171	136	66	258
Limit	**154**	**35**	**40**	**36**	**54**

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.

Acute Care PTT Mixing Study Algorithm



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Specimen Collection Factors

- Hemolysis: reject
- Prolonged tourniquet application raises VWF concentration
- Lipemia and icterus: use electro-mechanical coagulometer
- Wrong anticoagulant: EDTA (lavender), heparin
  - Run a potassium and a lithium
- Underfilled tube: AC proportionally increased
- Combining two tubes: AC proportionally increased
- Partially activated or clotted specimen: reject
- Specimen must be platelet-poor, <10,000/µL
  - Platelets release platelet factor 4, neutralizes heparin, also factor V

Ernst DJ, Ernst C. The Lab Draw Answer Book, 2nd Edition, Center for Phlebotomy Education, 2017

More Preanalytical Variables

- Start within 2 h to avoid in vitro prolongation
  - FV and FVIII deteriorate
- Coumadin, anti-Xa rivaroxaban, apixaban, edoxaban, betrixaban prolong PTT, PT
- Dabigatran, IV DTIs, and UFH prolong PT, PTT
  - Heparinase neutralizes ≤1 unit/mL UFH
- 15% of anti-FVIII inhibitors detected in immediate mix
- 15% of LAs require incubation
- Weak LAs may be missed in 1:1 mix
  - Select a more LA-responsive PTT reagent or prepare a 4:1 mix

Mostly

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Normal Plasma Source?

- Home brew: pool ~20 normal plasmas, male ≅ female
  - Ensure plasma is platelet-poor; < 10,000/uL; PTT ≅ mean of RI
  - Ensure NP has ~100% of all factors, especially VIII, IX, and XI
  - Elevated FVIII causes false negative results (patient specimens)
  - Screen each for LA, specific factor inhibitors. HBV, HCV, HIV
  - Aliquot and freeze
- Or purchase commercial plasma
  - GMP & frozen meets all criteria: Precision BioLogic Inc.
  - Lyophilized plasma 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.

What Limit Defines Correction?

No Consensus; Fritsma Factor 2015 Quick Question Answers

- Limits based on a fixed PTT value such as reference interval
  - 1:1 mix within RI upper limit (95% or 99% confidence interval, 39%)
  - 1:1 mix within RI upper limit + 5 seconds (8%)
- Limits based on the pooled normal plasma PTT value
  - 1:1 mix within NP PTT value + 5 seconds (14%)
  - 1:1 mix within NP PTT + 10% (32%)
- Rosner or Chang limit formula using patient, NP, and 1:1 mix results
  - Rosner formula produces a ratio
  - Chang's formula produces % deviation, requires incubation of patient plasma
- Other (7%): some combination of RI and Rosner, or…
  - A dedicated RI using ~20 healthy volunteer samples tested in 1:1 mixes. then computing 95th or 99th percentile.

Chang Index: Limit Based on % Correction

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

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

Incubate patient plasma, NP, and mix

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.

Rosner Index

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

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

Have to incubate patient specimen

Inhibitor ≥ 11

Correction < 11

Slightly more conservative than 10

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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Limit Levels and Correct Interpretation

Method	Result	Factor Def	Inhibitor
Mix secs – NP secs	<4 s	88%	100%
	<8 s	100%	77%
$\frac{\text{Mix secs}}{\text{NP secs}}$	<1.1	81%	100%
	<1.2	98%	82%
Rosner Index	<11	100%	82%
	<15	100%	61%
Chang Index	>72%	100%	59%

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 kaolin clotting time?
  - Use dilute PT?

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

59-yo Male
Former Hockey Player: TKR

Test	Result	Comment
TT	17 s	RI: < 21 s, rules out dabigatran
Chromo anti-Xa	0.0	0.4–0.7 U/mL, r/o direct anti-Xa
PTT	38 s	RI: 25–35 s
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 medical conditions, go on to TKR
- Prior thrombotic events (VTE)
  - Perform mix using 4:1 patient plasma to NP
  - Or choose PTT reagent that is LA-sensitive
- If anatomic bleeding, test for FVIII, FIX, FXI
  - Check sensitivity of the PTT reagent for deficiencies
  - Vitamin K deficiency: factor VII
  - Renal insufficiency
  - Liver disease (factor V), malignancy, VWD

2-yo Hemophiliac

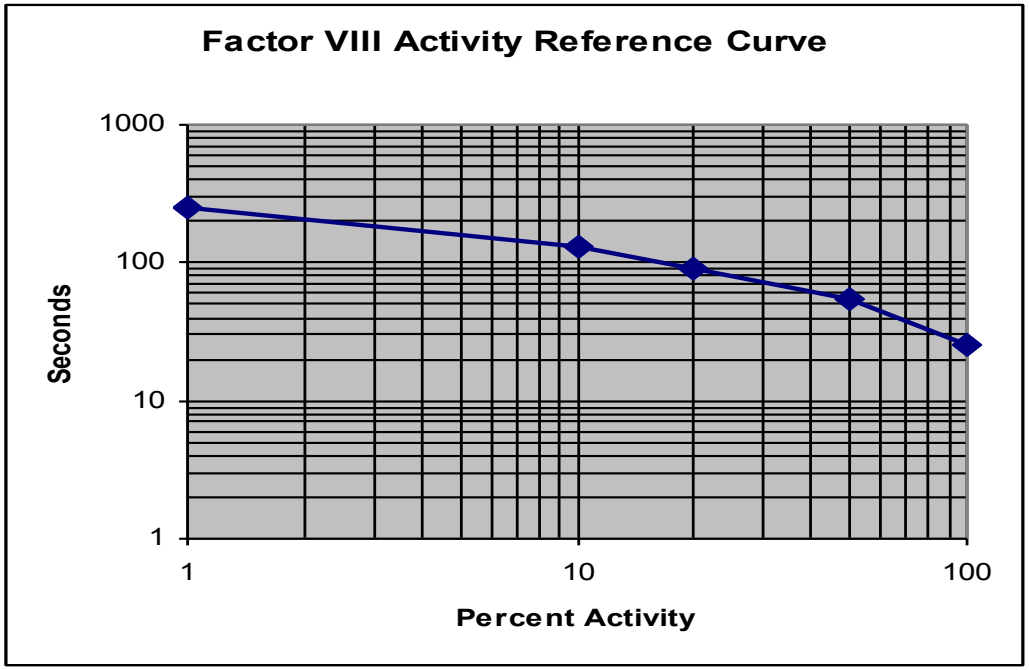
Test	Result	RI
HGB	11.8 g/dL	9.6–15.6 g/dL
PT	11.2 s	9.8–12.6 s
PTT	65 s	25–35 s
PLT	310,000/μL	150–400,000/μL
Fibrinogen	390 mg/dL	220–498 mg/dL
Inflamed, swollen knee and ankle		

Mixing Study Result
2-yo Hemophilic Boy

Assay	Result	RI/NP	Comment
PTT	65 s	25–35 s	Confirms previous PTT
PTT/NP 1:1 mix immediate	33.5 s	NP 30 s	Correction (ambiguous)
PTT/NP 1:1 mix 2 h at 37°C	47.9 s	NP 35 s	NP is incubated alone and with mix
Conclusion: Anti-FVIII inhibitor			

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



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

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 Afib Mixing Study

Assay	Result	RI
PTT	159 s	25–35 s
TT	> 150 s	< 21 s
PTT/NP 1:1 mix immediate	78 s	NP 30 s
PT/NP 1:1 mix immediate	15.2 s	NP 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
- Vitamin K deficiency: des-carboxy II, VII, and X
- Liver disease: PT prolongs before PTT due to des-carboxy II, VII, and X, reduced factor V

Develop Mixing Study Reliability

- PTT reagent sensitivities to factors and to LA
  - Activator: ellagic acid, silica, kaolin, celite
  - 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

DIY Local Mixing Studies—Why?

- Unexpected isolated prolonged PTT or PT may require immediate therapy
- Local results may immediately direct therapy
- Delayed specimen may deteriorate
- Forward mixing study results to ref lab to direct follow-up, for instance, LAC profile or Bethesda titer

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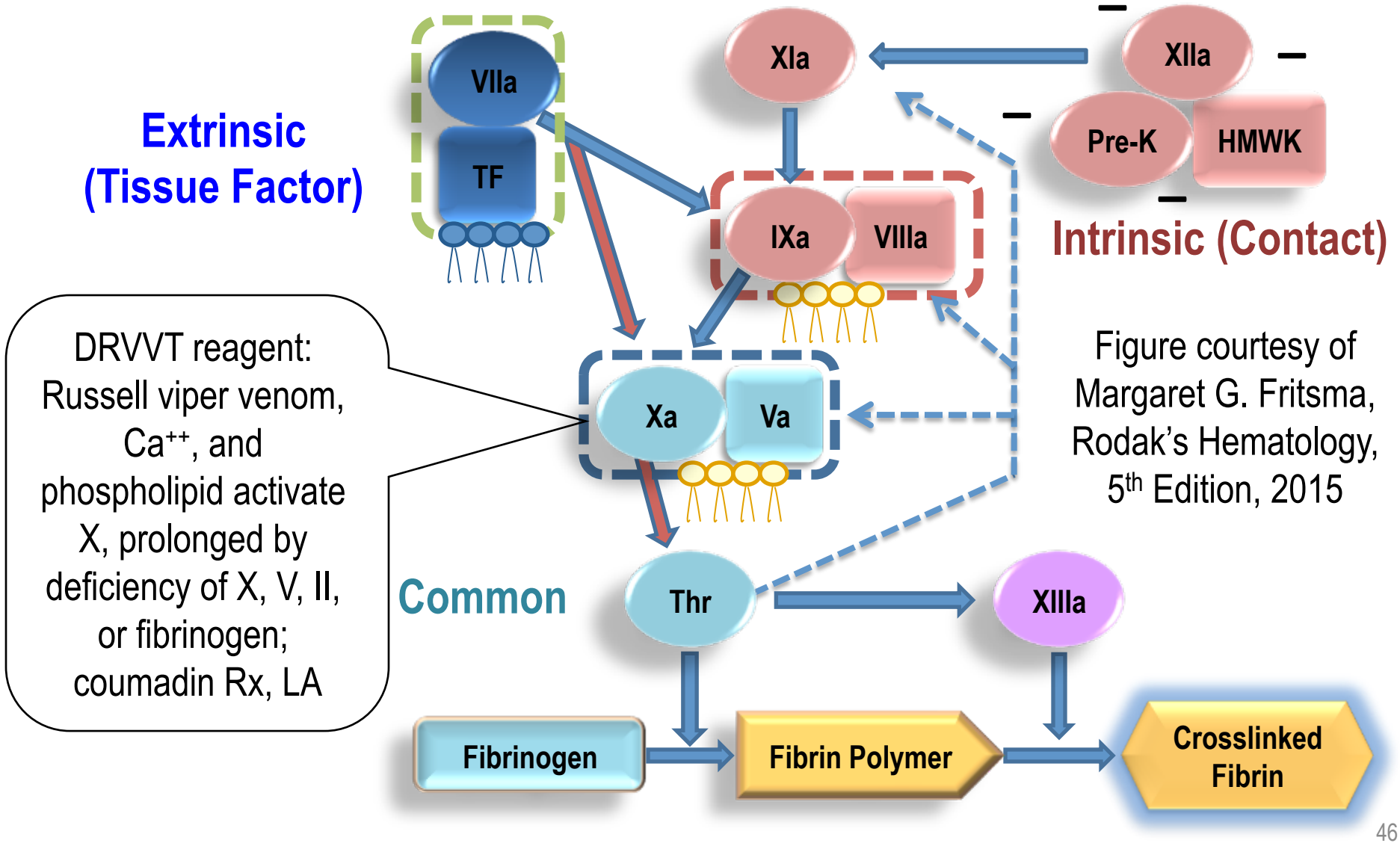
Lupus Anticoagulant (LA)

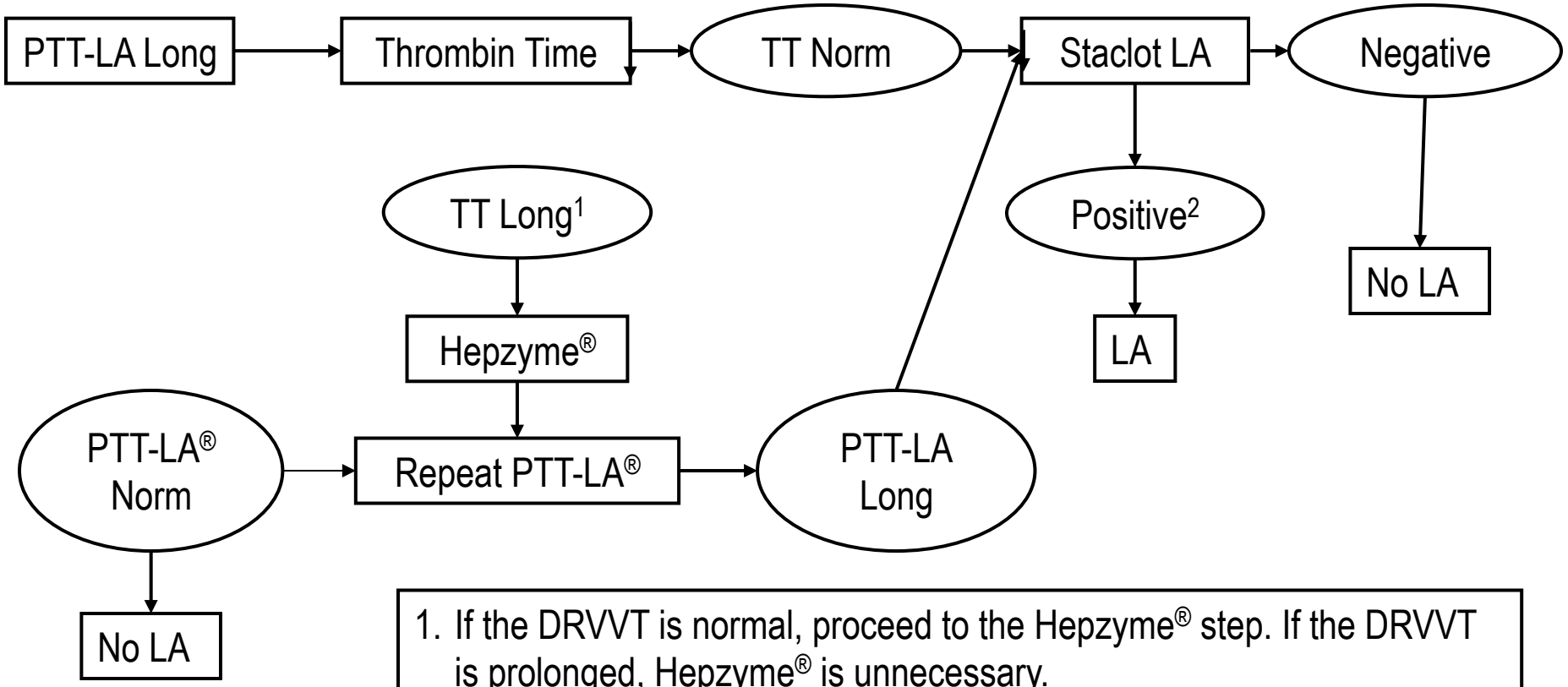
- Requires two initial assays
  - Reduced phospholipid concentration PTT (PTT-LA®)
  - Dilute Russell viper venom time (DRVVT, DVVtest®)
- LAC present if either PTT or DRVVT is…
  - Prolonged with no correction upon mixing with NP
  - Corrected by high phospholipid reagent
    - PTT neutralization by increased phospholipid (Sta-Clot LA®)
    - DRVVT confirmation by phospholipid reagent (DVVconfirm®)
- Other causes ruled out…
  - Acquired multiple factor deficiencies
  - Factor-specific inhibitors

Based on GOBSAT research: Pengo V, Tripodi A, Reber G, et al. Update of the guidelines of lupus anticoagulant detection. J Thromb Haemost 2009;7:1737–40

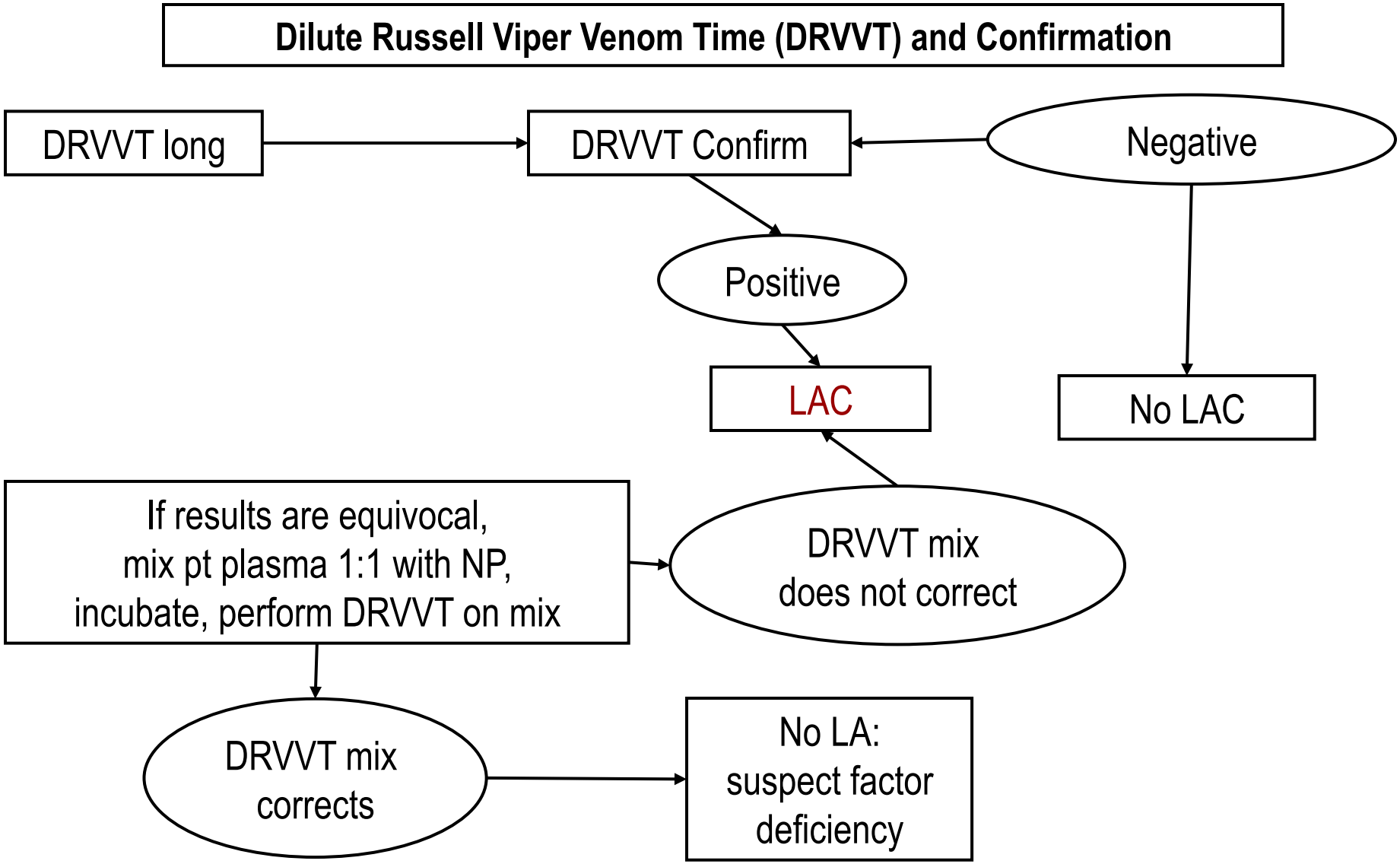
Coag Mechanism (Again)



Lupus Anticoagulant-Sensitive Partial Thromboplastin Time (PTT-LA®) and confirmatory StaClot LA®



1. If the DRVVT is normal, proceed to the Hepzyme® step. If the DRVVT is prolonged, Hepzyme® is unnecessary.
2. If the Staclot LA® is positive and the DRVVT is negative, assay FVIII to determine if the positive Staclot LA® is due to a FVIII inhibitor.


Dilute Russell Viper Venom Time (DRVVT) and Confirmation
DRVVT long
DRVVT Confirm
Negative
Positive
LAC
No LAC
If results are equivocal,
mix pt plasma 1:1 with NP,
incubate, perform DRVVT on mix
DRVVT mix
does not correct
DRVVT mix
corrects
No LA:
suspect factor
deficiency

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LAC By The Numbers

PTT-LA RI: 34–50 s
When PTT-LA is >50 s R/O heparin using thrombin time
Thrombin time RI: 17–21 s
When Staclot-LA Δ is ≥ 8 seconds, LAC is confirmed
DRVVT: LA Check RI : 30.9–41.5 s
LA Sure ratio reference interval: 0.87–1.19
When LA Sure ratio is ≥ 1.2, LAC is confirmed
If Staclot LA confirms LAC but DRVVT does not, assay factor VIII If FVIII < 30%, use Nijmegen Bethesda assay for FVIII inhibitor

The "LAC Cofactor" Effect

- Initial PTT = 48 s, RI 25–35; 1:1 mix *prolongs* to 54 s
- LAC binds IIa, slows clot formation, NP in mix adds IIa?
  - Or placental annexin V?
  - Do we really know?

- 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

LAC Consequences

- LAC does not increase risk of surgical bleeding.
- LAC increases risk of post-surgical thrombosis.
- The PTT and ACT cannot be used to monitor UFH during surgery.
- Use chromogenic anti factor Xa heparin assay to monitor heparin.
  - Prophylactic range 0.1–0.4 U/mL
  - Therapeutic range 0.3–0.7 U/mL

The Fritsma Factor
YOUR INTERACTIVE HEMOSTASIS RESOURCE

When Do You Test for LAC?

- Low clinical suspicion
  - Venous or arterial thromboembolism ≥ 50 YO
- Moderate clinical suspicion
  - Unexpected prolonged PTT without symptoms
  - Recurrent spontaneous pregnancy loss
  - Provoked venous thromboembolism (VTE) < 50 YO
- High clinical suspicion
  - Unprovoked VTE or arterial thrombosis < 50 YO
  - Thrombosis in unusual sites
  - Late pregnancy loss, pregnancy-related thrombosis
  - Accompanied by SLE, rheumatoid arthritis, autoimmune thrombocytopenia, autoimmune hemolytic anemia

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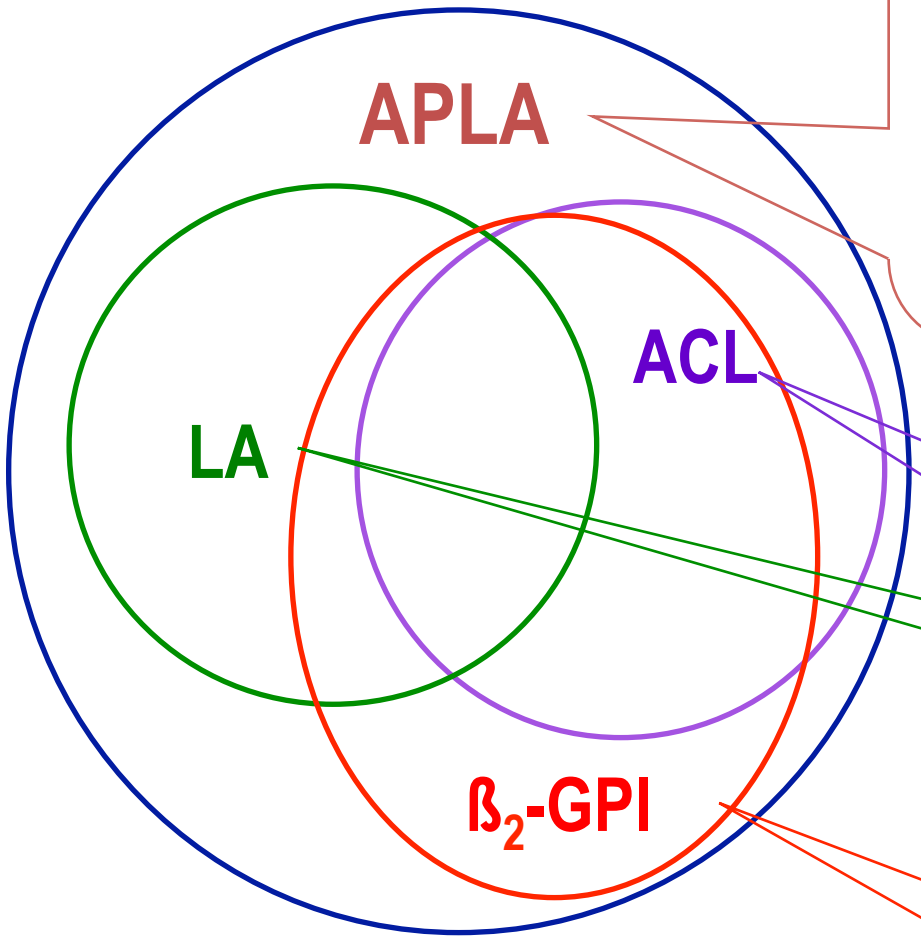
Anti-phospholipid Antibodies (APLs)



Immunoassays for low-incidence autoantibodies

- Anti-phosphatidylserine
- Anti-phosphatidylcholine
- Anti-phosphatidylethanolamine
- Anti-prothrombin
- Anti-annexin

Immunoassays for anti-cardiolipin antibody

Clot-based assays for lupus anticoagulant

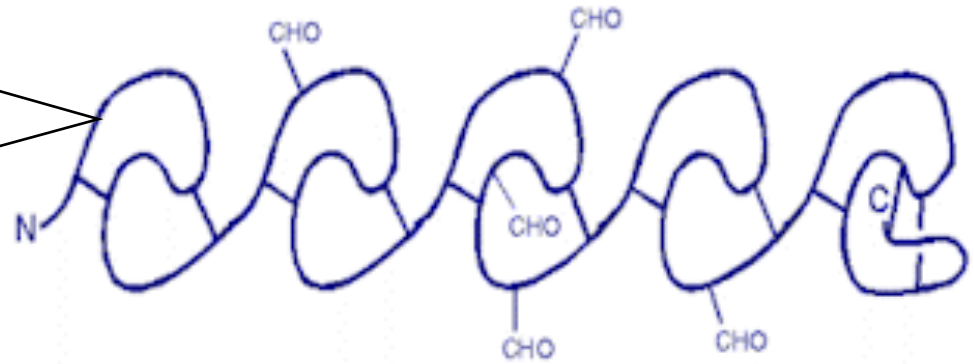
Immunoassays for anti-β_2-glycoprotein I antibody

APLs: Antibodies to Protein-Anionic Phospholipid Complexes

- Lupus anticoagulant (LA)
  - Defined by clot-based assays previously described
- Anti-cardiolipin antibody (ACL)
  - Defined by cardiolipin-based immunoassay
- Anionic phospholipid-binding proteins
  - $\beta_2$glycoprotein I (target for standard immunoassay)
  - Annexin V (placental)
  - Prothrombin

} Minor targets

β_2GPI 54KD single-chain glycosylated protein with five *sushi* domains binds anionic phospholipids



Anti-β_2GPI Vs. ACL

- ACL inter-laboratory standardization weak
- Pure β_2 glycoprotein I target antigen
  - No phospholipids in assay system
  - Antibodies associated with thrombotic events
- Ten β_2GPI kits
  - Pos agreement among kits IgG: 12/22, IgM: 5/22
  - No standard units nor cut-off values

Reber G, Tincani A, Sanmarco M, et al. Proposals for the measurement of anti-β2-glycoportein I antibodies. Standardization group of the European Forum on Antiphospholipid Antibodies. J Thromb Haemost 2004;2:1860–2.

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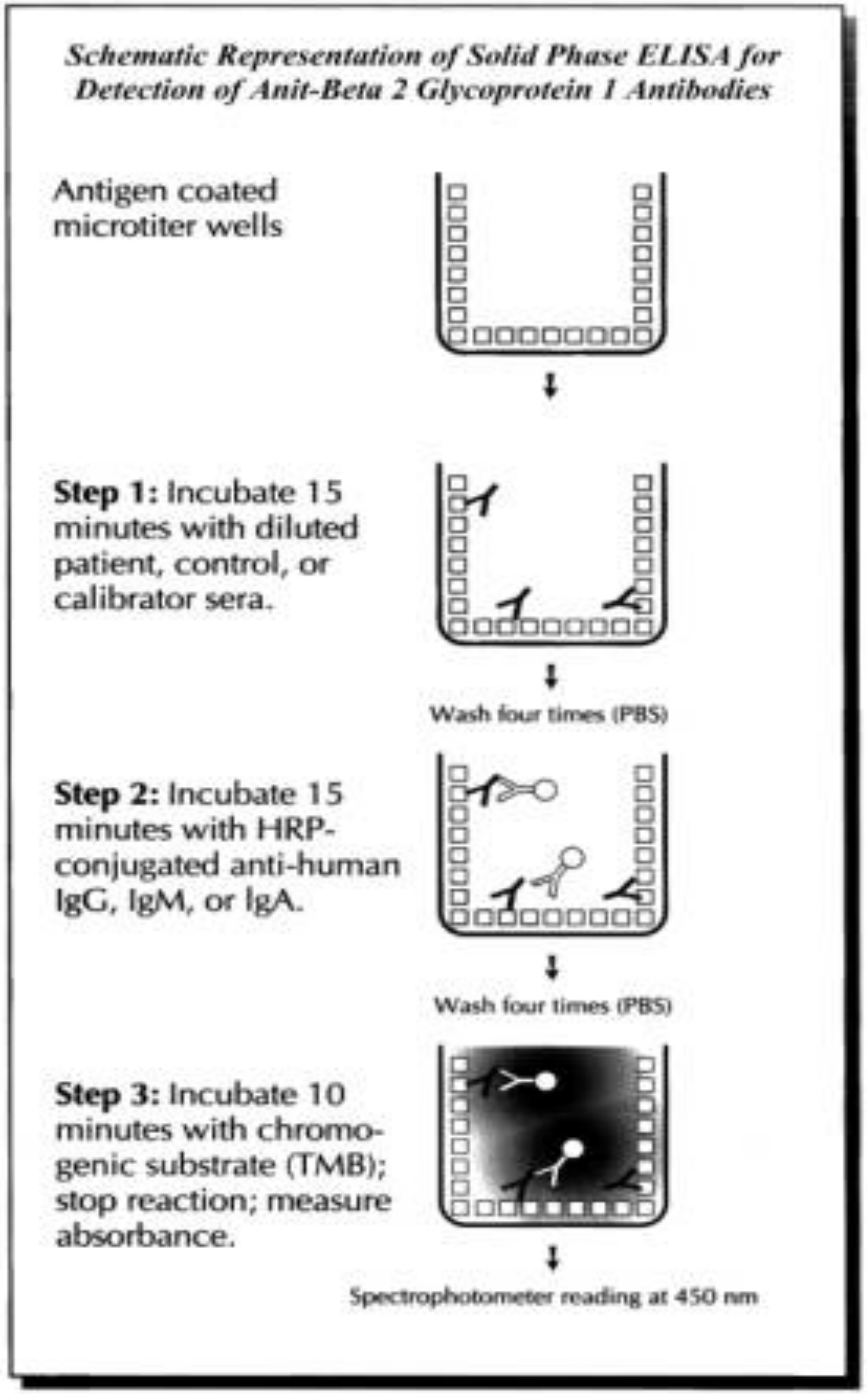
YOUR INTERACTIVE HEMOSTASIS RESOURCE

Enzyme Immunoassay Technology

- Anti-β_2GPI or ACL
- Report ACL as anticardiolipin antibody IgM or IgM units (APU)
- Report β_2GPI as β_2GPI IgM or IgM units



Alloimmune APLs

- Transient, no thrombosis
- 2–4% of unselected individuals, rises with age
- Associated with infections, malignancy, syphilis, HIV, drugs
  - Bacterial, viral, fungal, and parasitic infections
- LAC negative by DRVVT or Sta-Clot LA (?)
- Immunoassay: ACL or β_2GPI positive
- Require confirmation after 12 weeks

Dlott JS, Belter M, Ercolano E, et al. Evaluating the role of a new dilute prothrombin time assay ACTICLOT dPT in lupus anticoagulant testing. Blood 2007;110:1061A

Autoimmune APLs

- SLE, RA, AITP, AIHA, other autoimmune disorders
- Primary anti-phospholipid syndrome
- Persistent LAC, APL positive
- Repeated spontaneous abortion related to ACL
- 30% associated with thrombosis
- Reported as chronic APL if repeat positive after 12 weeks

Anti-phospholipid Syndrome (APS)

- Arterial thrombosis
  - Transient ischemic attacks (TIAs), strokes
  - Acute myocardial infarctions (AMIs)
- Venous thromboembolic disease (VTE)
  - Deep venous thrombosis (DVT)
  - Pulmonary emboli (PE)
- Recurrent 2nd trimester fetal loss
  - Infertility, prematurity, intrauterine growth retardation, low birth weight
- Occasionally thrombocytopenia

The Fritsma Factor
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Why detect LAC or APL?

- Early detection of autoimmune disorder
- Explanation of venous and arterial thrombotic episodes in neurological and rheumatological conditions
- Explain recurrent spontaneous abortions, chronic low birth weights

Clinical Conditions Associated with APL

- Autoimmune diseases: SLE, RA, Sjogren syndrome, ITP, autoimmune hemolytic anemia
- Neoplasms: Hodgkin disease, myelofibrosis, epithelial malignancies
- Lymphoproliferative disorders: hairy cell leukemia, malignant lymphoma, Waldenstrom macroglobulinemia
- Drugs: chlorpromazine, procainamide, hydralazine, quinidine, antibiotics—penicillins, cephalosporins, phenytoin
- Infections: bacterial, protozoan (*P carinii),* viral

APS Therapy

- Coumadin to prevent venous thrombosis
- Eliquis off-label (in clinical trials)
- Aspirin, Plavix, and LMWH to prevent arterial thrombosis
- Prednisone to reduce autoantibody