The Top Ten Problems in Coagulation Testing

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Your Interactive Hemostasis Resource
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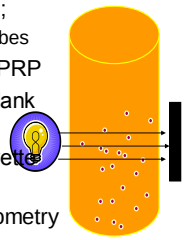
10

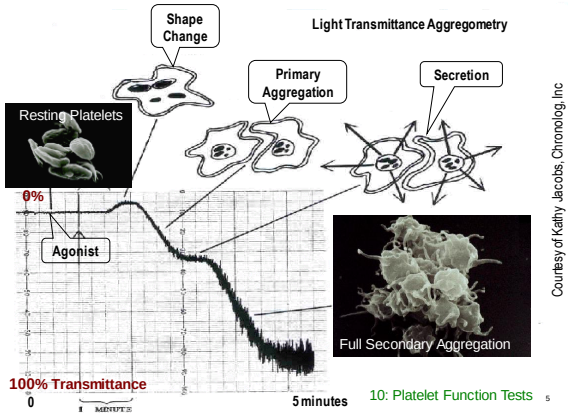
How to Monitor Platelet Function

Platelet Aggregometry
Platelet Function Testing

Light Transmittance Aggregometry

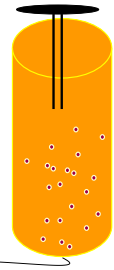
- Collect 12–15 mL blood;
  4–5 2.7 mL 3.2% citrate tubes
- Cfg 50×g 30”, remove PRP
- Cfg 1500×g 15”, PPP blank
- Wait 30” for “platelet shock”, dispense to cuvette
- Pipette agonist, record absorbance using photometry





Whole Blood Aggregometry (WBA)

- Collect 9 mL blood
  – 3 tubes 2.7 mL + 0.3 citrate
- Dilute 1:1 with saline
- Pipette agonist, timer starts
- Lower electrodes into suspension



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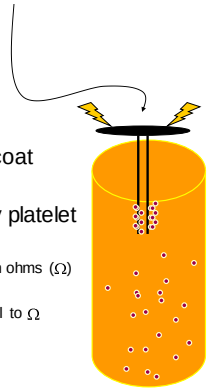
WBA



- Aggregating PLTs coat electrodes
- Current impeded by platelet layer
  - Impedance measured in ohms (Ω)
  - 0 Ω = no aggregation
  - Aggregation proportional to Ω

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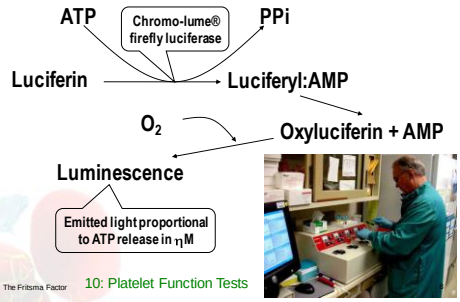
Secretion Measured Using the "Firefly" Reaction



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In Vitro Agonists and Receptors

Platelet Aggregometry

- Thrombin (IIa) or thrombin receptor-activating peptide (TRAP)
  - Binds protease activatable receptor (PAR-1) and GP V
  - Thrombin generates clot, measure secretion (release) only
  - Activates all pathways
- Arachidonic acid (AA)
  - Enters eicosanoid pathway as substrate to produce TXA_2
  - TXA_2 activates platelet by binding receptors TP_α or TP_β
- Collagen
  - Binds receptors GP Ia/IIa (integrin $\alpha_2\beta_1$), GP IV, GP VI
  - Requires intact eicosanoid synthesis pathway
- ADP binds receptors $P2Y_1$ & $P2Y_{12}$
  - Epinephrine binds α-adrenergic receptor
  - Require intact eicosanoid synthesis pathway
- Ristocetin
  - Ristocetin-induced platelet agglutination (aggregation, RIPA) tests for VWD

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Whole Blood Lumi-Aggregometry (WBLA) Reference Intervals; Mean ± 2 SD

Agonist	Conc	Aggregation Impedance	Secretion: ATP Luminescence
Thrombin	1 unit	N/A	1.0-2.0 ηM
Collagen	5 μg/mL	15-31 Ω	0.9-1.7 ηM
ADP	5 μM	1-17 Ω	0.0-0.7 ηM
	10 μM	6-24 Ω	0.4-1.7 ηM
AA	0.5 mM	5-17 Ω	0.6-1.4 ηM
Ristocetin	1 mg/mL	> 10 Ω	[< 70 sec lag]

Courtesy of Kathy Jacobs, Chronolog, Inc

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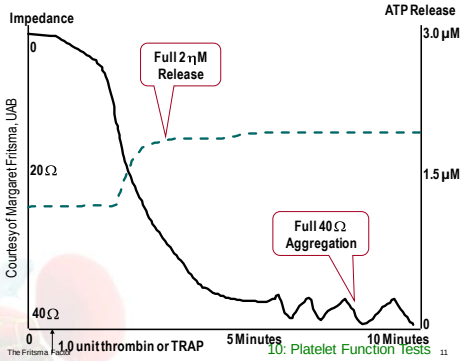
Normal WBLA



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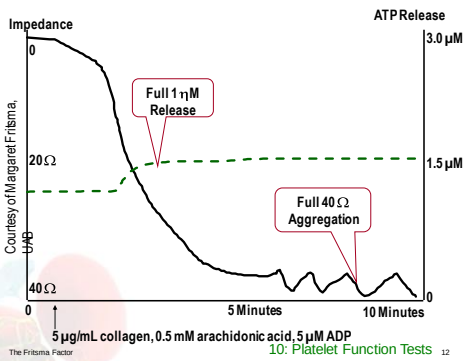
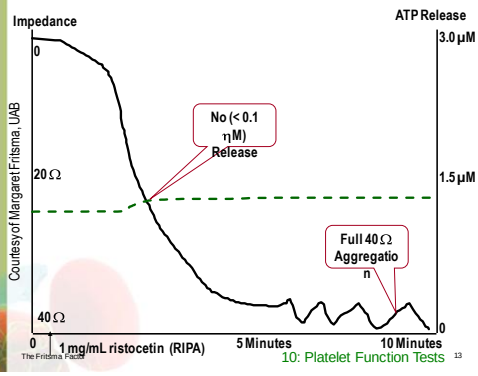
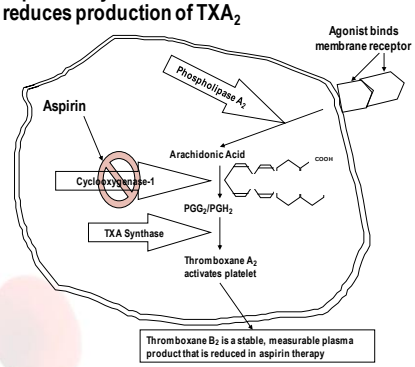
Normal WBLA



Normal WBLA



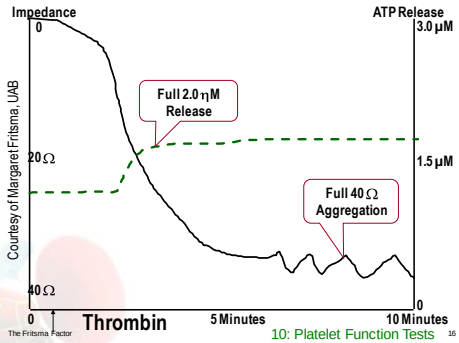
Aspirin acetylates COX1 and reduces production of TXA_2



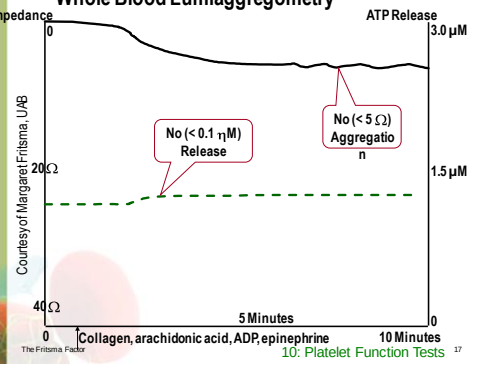
WBLA Anticipated Aspirin or Aspirin-like Disorder (Secretion Disorder) Ranges

Agonist	Conc	Aggregation Impedance	Secretion: ATP Luminescence
Thrombin	1 unit	N/A	> 0.5 ηM
Collagen	5 µg/mL	21-25 Ω	0.3-0.7 ηM
ADP	5 µM	1-13 Ω	< 0.1 ηM
AA	0.5 mM	< 0.5 Ω	< 0.1 ηM
Ristocetin	1 mg/mL	> 10 Ω	< 70 sec lag

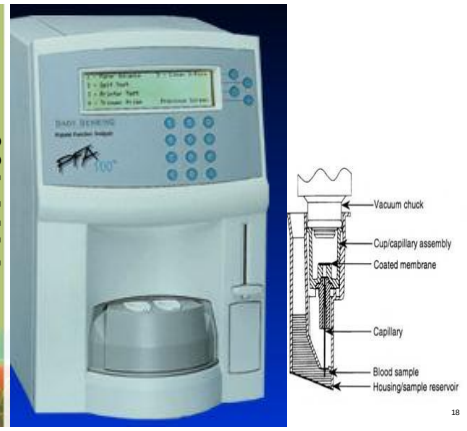
Whole Blood Lumiaggregometry Aspirin, Aspirin-like Disorder (Secretion Defect)



Aspirin, Aspirin-like Disorder (Secretion Defect) Whole Blood Lumiaggregometry



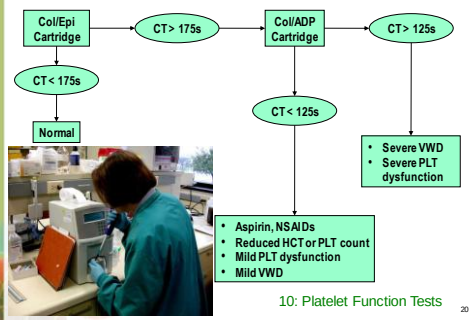
PFA-100



Siemens PFA-100

- Transfer 800 μL citrated whole blood to each of two cartridges, load and record interval to closure time (CT)
  - Coated membrane: agonists
- Collagen and epinephrine (Col/Epi, CEPI)
  - Initial screen cartridge
  - "Weak:" normal CT 98–175s
- Collagen and ADP (Col/ADP, CADP)
  - 50 μM ADP
  - Normal CT 77–125s
  - Confirmatory

PLT Function Screen for Variable Aspirin Response

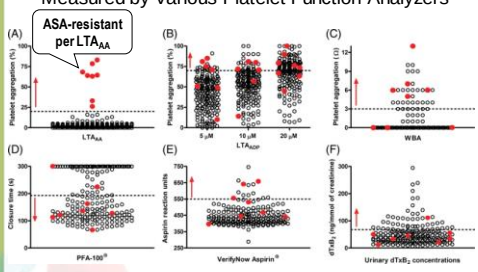


Aspirin Resistance Prevalence

Overall		27.1%
By method	PFA-100	29.0%
	Ultegra VerifyNow	26.2%
	LTA	21.3%
By population	CAD	22.9%
	Stroke	32.1%
By ASA dose	< 100 mg/d	35.6%
	101–299 mg/d	28.2%
	> 300 mg/d	18.6%

Hovens MMC, Snoep JD, Eikenboom CJ. Prevalence of persistent platelet reactivity despite use of aspirin: a systematic review. Am Heart J 2007;153:175–81.

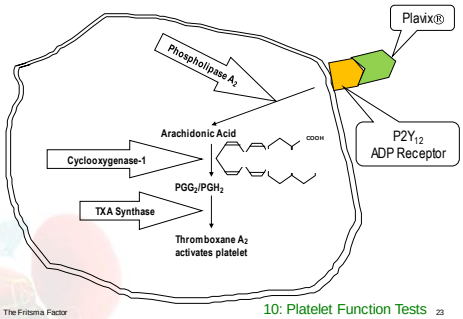
Distribution of Aspirin Responses Measured by Various Platelet Function Analyzers



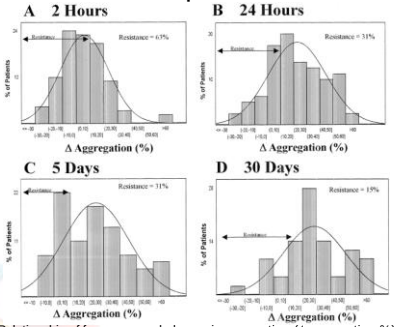
Lordkipanidze M. Eur Heart J 2007 28:1702-8; doi:10.1093/eurheartj/ehm226

European Heart Journal

Clopidogrel Occupies $P2Y_{12}$ ADP Receptor



Variable Response to Plavix



Gurbel, P. A. et al. Circulation 2003;107:2908-2913

Relationship of frequency and change in aggregation (Δ aggregation %) in response to 5 umol/L ADP at 2 h, 24 h, 5 days, and 30 days after stenting

#9

Unreliable D-dimer Assay Results

Variations in reporting units and computation

Unreliable D-dimer Results

- D-dimer purposes
  - Exclude (R/O) venous thromboembolism (VTE)
    - Pulmonary embolus, deep venous thrombosis
    - Only when there is low pre-test probability
  - Monitor DIC
  - Predict need to continue anticoagulation
- CAP survey results (unpublished): 2008
  - Per Dr. John Olson
  - 28% CV among reporting laboratories

D-dimer Units Are Confusing

- Fibrinogen equivalent units Vs. D-dimer units based on relative MW
  - FEU: VTE limit is ~500 ng/mL
  - DDU: VTE limit is ~250 ng/mL
  - Some laboratories conflate the two reporting systems
- Unit confusion: ng/mL, ug/mL, mg/dL, mg/L
  - Many labs compute report by hand with high error rate
- 39% of labs report a VTE limit higher than the MFR-established limit
  - Many don't know how they generated the limit
  - Often the limit is within the lab-generated reference range

Cunningham MT, Olson JD. Proficiency testing finds too-high cutoffs. CAP Today. 2005;19:54, 58

Fibrin Degradation Products and D-dimer

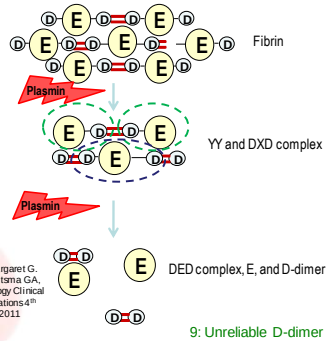


Figure courtesy of Margaret G. Fritsma, Rodak, BF, Fritsma GA, Keohane EM. Hematology Clinical Principles and Applications 4th Edition, Elsevier, 2011

D-dimer Technology Variability

- Monoclonal antibodies (MABs) vary in affinity and specificity
  - The range of MABs bind FDPs of varying MW
  - Identifying all as D-dimer
- No international standard or calibrator
- No limit established for DIC
  - Does 2000 ng/mL FEUs (1000 DDUs) indicate DIC?
  - D-dimer is an acute phase reactant that rises in inflammation
- Many labs attempt to exclude VTE using semiquantitative card test

Asakura H, Wada H, Okamoto K, et al. Evaluation of haemostatic molecular markers for diagnosis of disseminated intravascular coagulation in patients with infections. Thromb Haemost 2006;95:282-7.

Solutions to D-dimer Variability A Missed-diagnosis Crisis

- Laboratories *must validate* reporting method, math formulas, VTE thresholds
- Need international D-dimer standard
- Need a single reporting convention
- Need electronic formulas that enable laboratories to report consistent results

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#8

PT/INR Variability

Instrument Variation
Reagent Variation
MNPT and ISI Variation

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INR Variation Among Reagents

Diagnostica Stago 1997 STA®				
PT, sec	Rgt A	Rgt B	Rgt C	Rgt D
Mean of 9	16.1	16.9	13.7	17.5
Patient 10	67.3	81.9	169	78.3
INR	Rgt A	Rgt B	Rgt C	Rgt D
Mean of 9	1.5	1.3	1.2	1.5
Patient 10	31.3	8.7	15.4	9.3

Sandy Harmon and Lynne Quarles, 1997, unpublished

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$INR = (PT_{Patient}/MNPT)^{ISI}$

- Where…
  - INR = international normalized ratio
  - $PT_{patient}$ = patient prothrombin time
  - MNPT = mean normal PT (computed at site)
  - ISI = international sensitivity index
- ISI computation
  - PT on ≥60 patient specimens in all ranges
  - PT on ≥ 20 normals representing both sexes
  - Test all ≥ 80 using international reference preparation (IRP) or surrogate and mfr's thromboplastin
  - Regress paired results

Ansell J, Hirsh J, et al. Pharmacology and management of antithrombotic therapy: ACCP evidence-based clinical practice guidelines (8th ed). Chest 2008;133:299S–339S

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What Affects the INR?

$$CV(INR) = CV(PT/MNPT)^{CV(ISI)}$$

- CV could be ≤15% How?
  - PT assay CV ≤5%, 205 when INR >4.5
  - MNPT local computation CV ≤5%
  - ISI manufacturers computation ≤5%. Why?
- IRP PTs are determined visually
  - Manufacturers use 2° thromboplastin to compute ISIs
  - Some are 2–3 calibration steps away from IRP
- The ISI is generalized from representative coagulometers to local instruments
- CLSI: ISI must be locally validated

Clinical and Laboratory Standards Institute. Procedures for Validation of INR and Local Calibration of PT/INR Systems; Approved Guideline. CLSI document H54-A. 2005.

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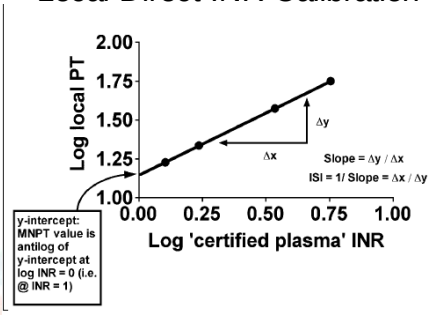
Local Direct INR Calibration



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Local Direct INR Calibration

- Manufacturer distributes certified plasma set
  - 1 normal, 3–5 coumadin plasmas INR 1.5 –4.5
  - INR assigned by visual analysis from at least two labs
- Perform duplicate PTs for 3 runs
  - Compute CV% for run to run variation, reject over 5%
  - Plot mean PTs on Y scale against INR on X on log-log
- Convert PT directly to INR from the graph
  - Eliminates ISI and MNPT
  - Performed worldwide except USA
  - FDA-cleared: Beckman-Coulter calibration set prepared only for BCI instruments and methods

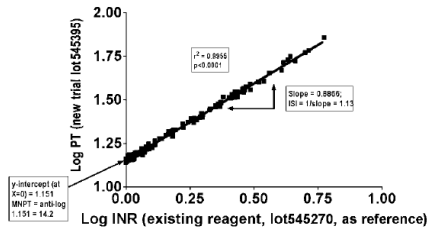
BECKMAN COULTER

Comparative Regression Analysis

- Compare old and new thromboplastin
  - Assay 100 regular run plasma samples of wide PT range
  - Compute regression
  - Plot log INR of existing reagent on x-axis
  - Plot log PT of new reagent on y-axis
- Purpose
  - Verify manufacturer ISI
  - Establish local INR direct from PT
- Verify by external quality assessment

Funk DM. Tips from the clinical experts. MLO 2009;April,38–39.

Comparative Regression Analysis



Favaloro EJ, McVicker W, Zhang Y, et al. Improving the inter-laboratory harmonization of the INR: Utilizing the concept of transference to estimate or validate ISI and MNPT values or to eliminate measurement bias. Clin Lab Sci 2012 in press.

#7

von Willebrand Disease Variability

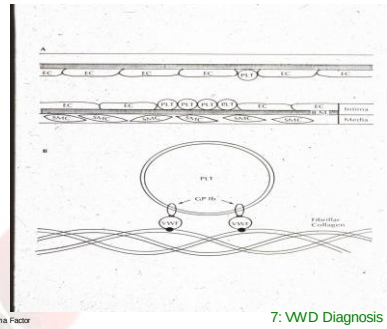
Biological and Technical Variation affection von Willebrand Factor Assays

Von Willebrand Disease (VWD)

- Autosomal mucocutaneous bleeding caused VWF deficiency or dysfunction
- VWF is a 5–20 million Dalton MW glycoprotein that binds platelets to injury sites and stabilizes coagulation factor VIII
- VWF deficiency impairs platelet adhesion and reduces FVIII activity
- Prevalence 0.6–1.3% in random population

Association of Hemophilia Clinic Directors of Canada. Hemophilia and von Willebrand disease: 1. Diagnosis, comprehensive care and assessment. Can Med Assoc J 1995;153:19–25.

Von Willebrand Factor Function



VWD Clinical Manifestations

- Mucocutaneous bleeds traceable to event
- Severity varies over time & within kindred
- Symptoms intensified by NSAIDs
- Symptoms reduced by oral contraceptives
- Symptoms disappear in 2nd–3rd trimester

Sadler JE, Mannucci PM, Berntorp E, et al. Impact, diagnosis and treatment of von Willebrand disease. Thromb Haemost 2000;84:160–74.

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Bleeding Reported by Healthy Subjects and All Types of VWD

Symptoms	Normals (%)	VWD (%)
Epistaxis	4.6–22.7	38.1–62.5
Menorrhagia	23–68.4	47–60
Bleeding after dental extraction	4.8–41.9	28.6–51.5
Ecchymoses	11.8–50	49.2–50.4
Bleeding from minor cuts or abrasions	0.2–33.3	36
Gingival bleeding	7.4–47.1	26.1–34.8
Postoperative bleeding	1.4–28.2	19.5–28

Federici AB. Clinical diagnosis of von Willebrand disease. Haemophilia 2004;10 (Suppl 4):169–76.

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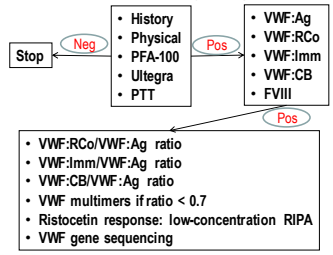
VWD Laboratory Algorithm



- VWF:Ag: VWF antigen (concentration, protein immunoassay)
- VWF:RCo: VWF ristocetin cofactor (function, activity)
- VWF:CB: VWF collagen binding assay (function, activity)
- VWF:Imm: VWF functional immunoassay (function, activity)
- FVIII: Coagulation factor VIII

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Type 1 VWD Profile (70%)

Assay	Patient	RI
VWF:Ag	34%	65–140 Units/dL
VWF:RCo	37%	
VWF:CB	39%	
VWF:Imm	32%	
VWF:RCo/VWF:Ag	1.08	Type 2 <0.7
VWF:Imm/VWF:Ag	0.94	Type 2 <0.7
VWF:CB/VWF:Ag	1.14	Type 2 <0.7
Factor VIII	38%	55–150%
RIPA	< 60% Agg	> 60% Agg
VWF multimers	Not done; use only when VWF:RCo/VWF:Ag is < 0.7	

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2004–2009 NASCOLA VWD Proficiency Test CVs

Assay	Normals (7 Surveys)	Type 1s (7 Surveys)	Type 2s (3 Surveys)
VWF:Ag	11%	15%	19%
VWF:RCo	20%	30%	42%
VWF:Imm	15%	14%	49%
VWF:CB	21%	14%	30%
VWF:RCo/VWF:Ag	22%	32%	49%
VWF:Imm/VWF:Ag	19%	20%	53%
VWF:CB/VWF:Ag	23%	12%	43%

Chandler WL, Peerschke EI, Castellone DD, Meijer P; NASCOLA Proficiency Testing Committee. Von Willebrand factor assay proficiency testing. The North American Specialized Coagulation Laboratory Association experience. Am J Clin Pathol 2011;135:862-9.

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2004–2009 NASCOLA VWD Proficiency Test Interpretations

Assay	Interpretation	Normal (7 Surveys)	Type 1 (7 Surveys)	Type 2 (3 Surveys)
VWF:Ag	Normal	325	38 (14%)	11 (9%)
	Abnormal	0	272	130
VWF:RCo	Normal	246	10 (4%)	1 (1%)
	Abnormal	5 (2%)	226	106
VWF:Imm	Normal	47	1 (2%)	0
	Abnormal	0	42	22
VWF:CB	Normal	35	2 (6%)	0
	Abnormal	0	32	19

Marques MB, Fritsma GA. Von Willebrand disease laboratory diagnosis: the saga continues. Am J Clin Pathol 2011;135:818–20.

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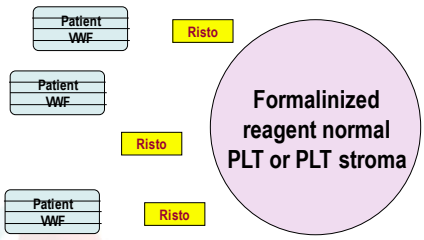
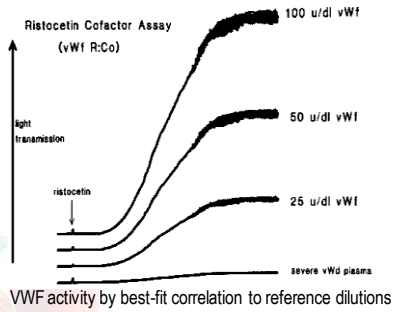
Ristocetin Cofactor (VWF:RCo) Aggregometry



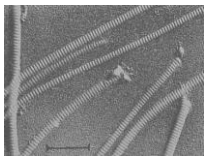
Quantitates VWF activity (function), reduced in all types of VWD

Ristocetin Cofactor Standard Curve

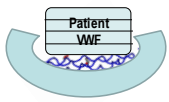


VWF activity by best-fit correlation to reference dilutions

Collagen Binding Assay; VWF:CB



corgenix Online



Favaloro EJ. Detection of VWD and identification of qualitative VWF defects: Direct comparison of commercial ELISA-based VWF activity options. Am J Clin Pathol 2000;114:608-18.

VWF Activity Immunoassay HemosIL® VWF Activity

- Automated 12-minute immunoassay
- Anti-VWF monoclonal Ab on latex particles
  - Directed against patient VWF GP Ib receptor ligand
  - Agglutination proportional to VWF activity
  - Transmitted light scattered by agglutinates
- Compares favorably with the VWF:RCo

De Vleeschauwer A, Devreese K. Comparison of a new automated von Willebrand factor activity assay with an aggregation von Willebrand ristocetin cofactor activity assay for the diagnosis of von Willebrand disease. Blood Coagul Fibrinolysis 2006; 17:353-8.

Laboratory and Clinical Solutions

- Improve VWF:Imm, VWF:CBA
- Use WHO standard for VWF:RCo
- Repeat testing to confirm, test kindred
- Physical stress, surgery, exercise, anxiety, inflammation, pregnancy, OCs raise VWF
- Lowest on days 1–4 of menstrual cycle
- Clinical consideration: 30%–50% is named "Low VWF"

US Department of Health and Human Services National Institutes of Health National Heart, Lung, and Blood Institute. The Diagnosis, Evaluation, and Management of von Willebrand Disease. 2007. NIH Publication 08-5832

#6
Lupus Anticoagulant Testing

Variation and Interpretation
Lupus Anticoagulant Profiles

Lupus Anticoagulant Testing

- Mix plasma with platelet-poor normal plasma (NP) and perform PTT on mix
  - 1:1 sensitive to deficiencies, may miss inhibitor
  - 1:4 sensitive to weak inhibitors, may miss mild deficiency
- Correction of PTT of the mix to within 10% of NP (Rosner index)
  - Factor deficiency
  - 1 or 2 hour incubated mixing study follow-up
- No correction: PTT remains >13% of NP
  - Inhibitor is present

Rosner E, Pauzner R, Lusky A, et al. Detection and quantitative evaluation of lupus circulating anticoagulant activity. Thromb Haemost 1987; 57: 144–7.

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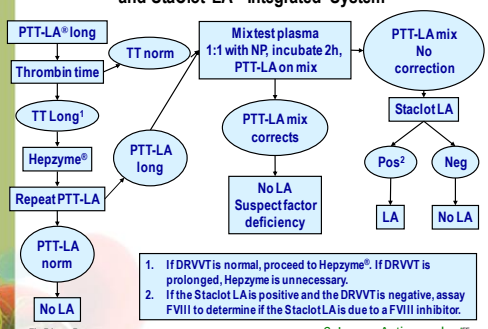
LA Flow Chart
LA-sensitive Partial Thromboplastin Time (PTT-LA®) and StaClot LA® Integrated System



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

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Lupus Anticoagulant Testing
PTT Screening Reagent Survey

LAC Responsiveness	Example Reagents	CAP[a] 2008	CAP[a] 2009	CAP[a] 2010	CAP[a] 2011	Survey[b]
High	Siemens Actin FSL® Beckman-Coulter HemosIL® APTT-SP	2019 (53%)	2015 (51%)	2023 (48%)	1902 (46%)	9 (10%)
Intermediate	Stago STA®-PTT-LA Beckman-Coulter HemosILSynthASil®	1491 (39%)	1647 (41%)	1854 (44%)	1954 (47%)	39 (43%)
Low	Siemens Actin FS® Stago C. K.Prest®	150 (4%)	127 (3%)	132 (3%)	125 (3%)	17 (18%)
Don't Know						26 (29%)

Fritsma GA, Dembitzer FR, Randhawa A, et al. Appropriate APTT reagent selection and utilization. Submitted to Clin Chem 8/31/11

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Lupus Anticoagulant Testing
PTT Screening Reagent Survey

Table 3: APTT reagent usage among laboratories that use both low and high LAC responsive reagents: Fritsma Factor survey respondents

	Number of Laboratories*	
	Low LAC Responsiveness	High LAC Responsiveness
Screen for intrinsic coagulation factor deficiency to predict bleeding risk	20	1
Monitor standard unfractionated heparin therapy	17	2
Screen for LAC to predict thrombosis risk	2	18
All three	7	

*Laboratories represent a subgroup (32%) of all survey respondents

Fritsma GA, Dembitzer FR, Randhawa A, et al. Appropriate APTT reagent selection and utilization. Submitted to Clin Chem 8/31/11

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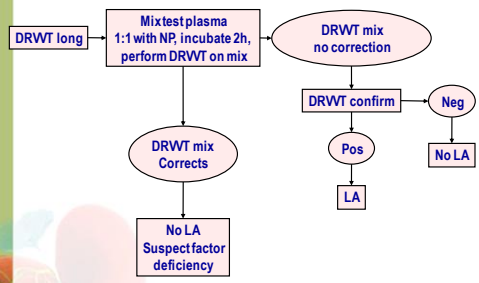
LA Flow Chart
Dilute Russell Viper Venom Time (DRVVT) and Confirmation



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Effective Interpretation of Antiphospholipid Syndrome Reports

- Lupus antibody, not detected
- Protime: 26.4
- PTT-LA: 52 H
- APTT immediate:corrected see also (@b) *[there is no @b]*
- Mixing interpretation: corrected: results consistant (sic) with common pathway factor deficiencies
- dRVVT Screen: 78 H
- Thrombin time: 18 H
- Hexagonal Phase: negative
- PHOS NEUT: Positve (sic) H
- Cardiolipin IgG Less than 10
- cardiolipin IgM Less than 10

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#5
Monitoring Heparin Therapy

The PTT and Anti-Xa

Monitoring Heparin Therapy
Physician/Nurse Standard Dosage

- Standard unfractionated heparin (UFH)
- Perform "baseline" PTT to ensure patient has no deficiencies or inhibitors
- Initiate therapy: 5–10,000 U bolus
- IV 1600 U/Kg/H
- 4–24 hours after bolus, collect 2nd PTT
- Adjust dose to PTT therapeutic range
- Traditional range: 1.5–2.5 × MNR
  - Never use the traditional range

Lab Tests Used to Monitor UFH

- PTT: responds to effect of heparin-antithrombin on thrombin and Xa
- Activated clotting time (ACT, surgical suite)
  - Normal mean 120 sec
  - Angioplasty: 200–300 sec
  - Coronary bypass, heparin at 5 Units/mL: 480 sec
- Chromogenic anti-Xa heparin assay
  - Therapeutic range 0.3–0.7 Units anti-Xa heparin/mL

Anti-Xa Heparin Assay
Performance Characteristics

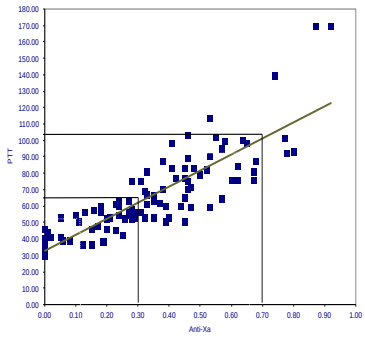
- May be calibrated for UFH, LMWH, or pentasaccharides
- Not affected by inhibitors, factor deficiencies, or elevated factor levels
- Reliance on patient antithrombin means test is sensitive to antithrombin deficiency

Brill-Edwards P, et al. Establishing a therapeutic range for heparin therapy. Ann Intern Med 1993;119:104-109

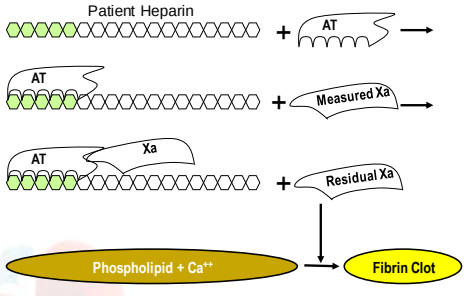
Establishing The PTT Heparin Therapeutic Range

- Collect ≥ 50 heparin patient specimens
  - All dosage ranges
  - No oral anticoagulant, PT normal
- Collect ≥ 20 normals, both sexes
- Perform PTT and anti-Xa heparin on all
- Prepare linear graph of paired results
- Correlate PTT range to the anti-Xa therapeutic range of 0.3–0.7 Units/mL
- Prophylactic range 0.1–0.4 Units/mL

HEPARIN THERAPEUTIC RANGE



American Diagnostica HEPTEST®



Clot time inversely proportional to heparin concentration

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#4
Confusing Test Names

How Hard Can We Make It?

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Factor V or Factor V Leiden Mutation?

- Factor V activity assay is rarely ordered
  - Liver disease diagnosis
  - Congenital single factor V deficiencies
  - Bovine fibrin glue inhibitor
- Factor V Leiden mutation is often ordered
  - The factor V Leiden mutation is present in 3–8% of Caucasians, Arabs and Hispanics
  - Confers a 2xto 8x risk of thrombosis
  - 19-fold when homozygous
  - Part of a thrombophilia profile
  - Screen using activated protein C resistance ratio (APCR)

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Factor II or Factor II 20210 Mutation?

- Factor II activity assay is rarely ordered
  - Congenital single factor II (prothrombin) deficiencies
- The factor II 20210 mutation test is ordered often
  - The factor II 20210 (prothrombin) mutation is present in 2–3 % of Caucasians, Arabs and Hispanics and confers a 2- to 4.8-fold risk of thrombosis
  - Ordered as part of a thrombophilia profile, same volume as the FVL
- Or: prothrombin Vs prothrombin mutation

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Protein C Activity, C-reactive Protein, or Activated Protein C Resistance?

- The protein C activity assay and the APCR are part of the thrombophilia profile
  - Protein C deficiency
  - Reduced APCR predicts factor V Leiden mutation
- C-reactive protein (CRP, hsCRP) is a marker of inflammation ordered as part of a cardiovascular risk profile

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Factor X or anti-Xa Heparin Assay?

- Factor X assay is rarely ordered
  - Congenital single factor X deficiencies
- The anti-Xa heparin assay often ordered
  - The anti-Xa test (anti-factor Xa,hep-test) is used to monitor UFH therapy when the PTT is ineffective
  - The anti-Xa test is the only test that works for monitoring LMWH or pentasaccharides
- Rename heparin assay?
- Also—transpositions and deletions
  - IX and XI; VII, VIII

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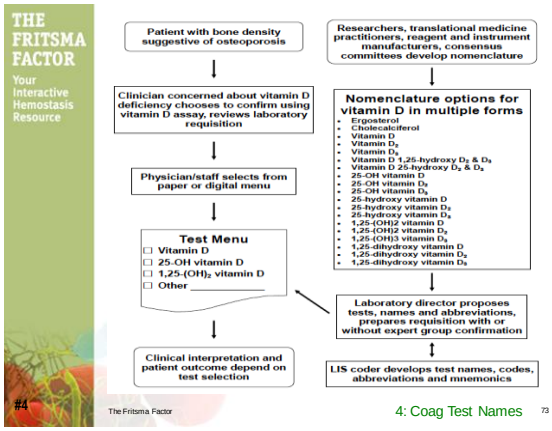
Coagulation Test Name Solution?

- Logical Observation Identifiers Names and Codes (LOINC®, www.LOINC.org)
  - Universal codes for identifying laboratory and clinical observations
  - Mapping terms to LOINC makes it possible to exchange and pool data from many systems for clinical care and research
- Laboratory Medicine Best Practices: CDC
  - "Appropriate Laboratory Test Selection: A Major Challenge": CDC "Thesaurus" project
  - www.futurelabmedicine.org/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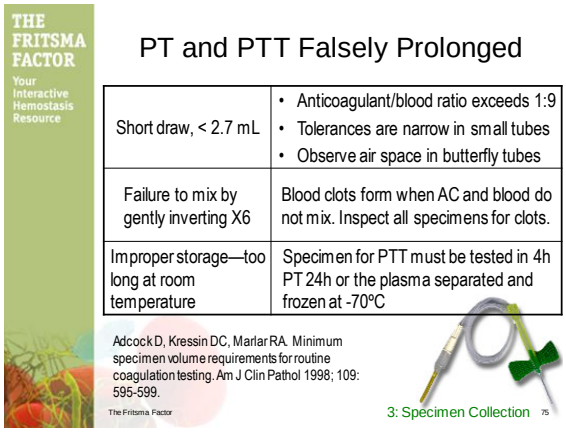
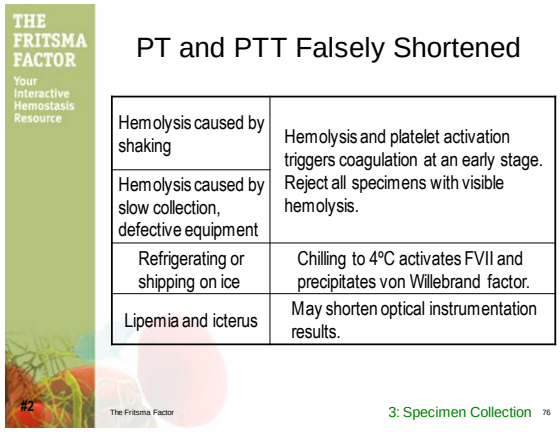
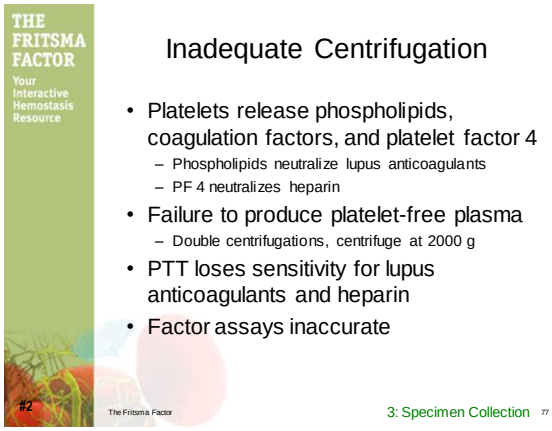
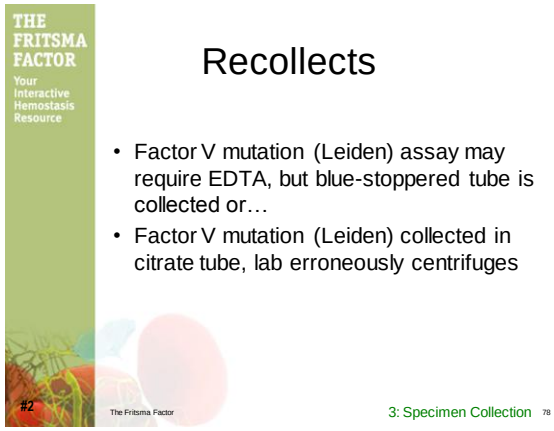
Your Interactive Hemostasis Resource
Patient with bone density suggestive of osteoporosis
Researchers, translational medicine practitioners, reagent and instrument manufacturers, consensus committees develop nomenclature
Clinician concerned about vitamin D deficiency chooses to confirm using vitamin D assay, reviews laboratory requisition
Nomenclature options for vitamin D in multiple forms
Ergosterol
Cholecalciferol
Vitamin D
Vitamin D2
Vitamin D3
Vitamin D 1,25-hydroxy D2 & D3
Vitamin D 25-hydroxy D2 & D3
25-OH vitamin D
25-OH vitamin D2
25-OH vitamin D3
25-hydroxy vitamin D
25-hydroxy vitamin D2
25-hydroxy vitamin D3
1,25-(OH)2 vitamin D
1,25-(OH)2 vitamin D2
1,25-(OH)3 vitamin D3
1,25-dihydroxy vitamin D
1,25-dihydroxy vitamin D2
1,25-dihydroxy vitamin D3
Physician/staff selects from paper or digital menu
Test Menu
Vitamin D
25-OH vitamin D
1,25-(OH)2 vitamin D
Other
Laboratory director proposes tests, names and abbreviations, prepares requisition with or without expert group confirmation
Clinical interpretation and patient outcome depend on test selection
LIS coder develops test names, codes, abbreviations and mnemonics
#4
The Fritsma Factor
4: Coag Test Names
73


THE FRITSMA FACTOR
Your Interactive Hemostasis Resource
Vampire blood
#3
Specimen Management
The Fritsma Factor
74


THE FRITSMA FACTOR
Your Interactive Hemostasis Resource
PT and PTT Falsely Prolonged
Short draw, < 2.7 mL
Anticoagulant/blood ratio exceeds 1:9
Tolerances are narrow in small tubes
Observe air space in butterfly tubes
Failure to mix by gently inverting X6
Blood clots form when AC and blood do not mix. Inspect all specimens for clots.
Improper storage—too long at room temperature
Specimen for PTT must be tested in 4h PT 24h or the plasma separated and frozen at -70ºC
Adcock D, Kressin DC, Marlar RA. Minimum specimen volume requirements for routine coagulation testing. Am J Clin Pathol 1998; 109: 595-599.
The Fritsma Factor
3: Specimen Collection
75

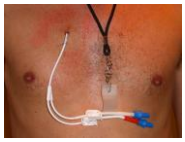

THE FRITSMA FACTOR
Your Interactive Hemostasis Resource
PT and PTT Falsely Shortened
Hemolysis caused by shaking
Hemolysis caused by slow collection, defective equipment
Hemolysis and platelet activation triggers coagulation at an early stage. Reject all specimens with visible hemolysis.
Refrigerating or shipping on ice
Chilling to 4ºC activates FVII and precipitates von Willebrand factor.
Lipemia and icterus
May shorten optical instrumentation results.
#2
The Fritsma Factor
3: Specimen Collection
76


THE FRITSMA FACTOR
Your Interactive Hemostasis Resource
Inadequate Centrifugation
Platelets release phospholipids, coagulation factors, and platelet factor 4
Phospholipids neutralize lupus anticoagulants
PF 4 neutralizes heparin
Failure to produce platelet-free plasma
Double centrifugations, centrifuge at 2000 g
PTT loses sensitivity for lupus anticoagulants and heparin
Factor assays inaccurate
#2
The Fritsma Factor
3: Specimen Collection
77


THE FRITSMA FACTOR
Your Interactive Hemostasis Resource
Recollects
Factor V mutation (Leiden) assay may require EDTA, but blue-stoppered tube is collected or…
Factor V mutation (Leiden) collected in citrate tube, lab erroneously centrifuges
#2
The Fritsma Factor
3: Specimen Collection
78

Order of Draw

- Collect discard tube?
  - NCCLS H21-A5 directs that the first tube may be used
- Do not follow additive tube
  - Plastic red-closure tubes have particulate activator
  - Serum separator tubes have particulate activator
- Vascular access device
  - Correct-fitting syringe
  - Flush with saline
  - Discard 5 mL blood



#2

Thrombophilia Awareness

#2
Thrombophilia Profiles

Ordering and Interpreting

Effective Ordering and Interpretation of Thrombophilia Profiles

- FVIII and VWF rise during infection and inflammation
- Protein C and antithrombin are consumed in sepsis and DIC
- Antithrombin drops in L-asparaginase Rx, hepatic sinusoidal veno-occlusion, nephrotic syndrome
- Acquired APC resistance in pregnancy and OCRs, raised estrogens
- Protein S drops in pregnancy, primary varicella infection
- OCRs odds ratio is 4x, in heterozygous factor V Leiden, 35x
- Obesity, smoking, and immobility have far greater impact than thrombosis risk factors

Thrombophilia Assays, Prevalence, Risk

Assay	Prevalence	Venous Thrombosis Prevalence	Odds for Thrombosis	Comment
APCR	3-8% of Caucasians, Hispanics, Arabs	20–25%	Het: 2–7× Hom: 18×	Confirm positives with FVL mutation test
F II20210		4–8%	Het: 2–6×	Molecular assay only
Antithrombin deficiency	1 in 2-5000	1%	10–20×	Do not test during A/C Rx or active clotting Perform immunoassay only when activity is consistently low
Protein C deficiency	1 in 300	1%	6×	
Protein S deficiency	Not known	2-10%	1.6–11.5×	
Homocysteinemia			2.5x	Reduction does not change outcome
LA	1-3%		30%	Acquired

#1

Common Errors in Thrombophilia Testing

- Failure to include all relevant profile members
- Irrelevant assays
  - MTHFR C677T: No clinical correlation
  - Homocysteinemia: Reducing does not change outcome
- Ordering PC, PS, AT soon after thrombotic event, during inflammation, or while patient is on anticoagulant therapy
- Failure to confirm abnormal screens

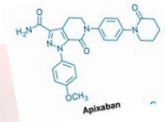
#1

Pradaxa dabigatran etexilate CAPSULES

Xarelto rivaroxaban

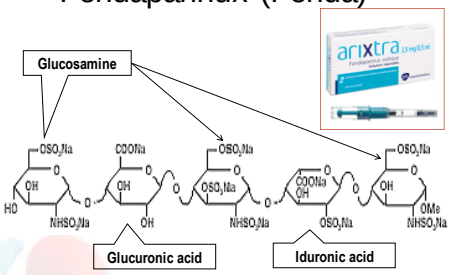
#1

How to Monitor Direct Thrombin Inhibitors and Oral Anti-Xa



ONCE DAILY Arixtra (fondaparinux sodium) for injection

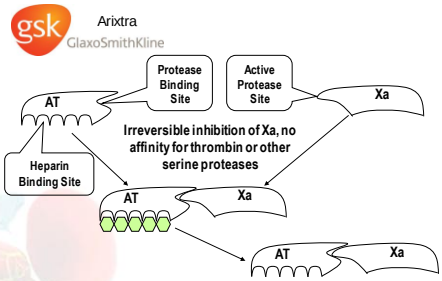
Synthetic Pentasaccharide, Fondaparinux (Fonda)



Turpie AGG. Pentasaccharides. Semin Hematol 2002;39:158-171

Fonda and Antithrombin

- Sulfate residues critical to high-affinity AT binding
- AT allostery raises Xa affinity 300X



Fonda and Antithrombin

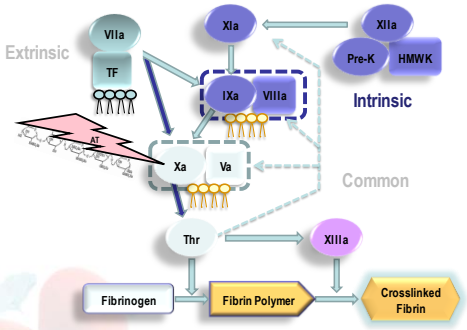


Figure courtesy of Margaret G. Fritsma

When to Monitor
Applies to all New Antithrombotics

- Compliance
- Bleeding patient in ED
  - Potential overdose, effects in co-medication
  - Detection; what anticoagulant is it?
- Bridging from one anticoagulant to another
- Renal disease: inadequate excretion
- Marginal fluid compartment
  - > 150 kg: proportionally reduced fluid compartment
  - < 40 kg or ped: proportionally increased fluid compartment
- Unstable coagulation system
  - Pregnancy, liver disease, malignancy, DIC

Monitoring Fonda

- SQ (never IM) injection; 2.5 mg
  - Peak plasma level at 3 h: 0.4–0.5 mg/L
  - Minimum steady state 0.14–0.19
- Chromogenic anti-Xa calibrated with pentasaccharide in mg/L
  - Standards available from Beckman-Coulter and Aniara for their kits
- Hep-test, PiCT
- Not PT or PTT, neither respond to fonda

Chromogenic Anti-Xa Heparin Curve

- Separate curves for UFH and LMWH?
  - Hybrid curve: one curve fits all
- Different LMWH formulations: Tinzaparin
  - Aventis lost Lovenox patent 5/1/09
- Separate curve for fonda?
  - mg/dL, not international units
  - Marilyn Johnston, McMaster: uses same curve as LMWH

McGlasson DL, Kaczor DA, Krasuski RA, et al. Effects of pre-analytical variables on the anti activated factor X chromogenic assay when monitoring unfractionated heparin and low molecular weight heparin. Blood Coagul Fibrinolysis 2005;16:173–6.

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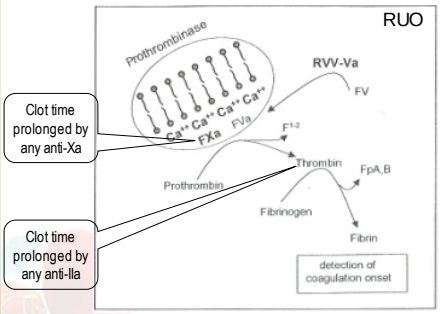
Pentapharm® Pefakit® Prothrombinase-induced Clot Time (PiCT)

RUO



The Fritsma Factor

1: New Oral A/C 91

THE FRITSMA FACTOR Your Interactive Hemostasis Resource

PiCT

pentapharm benefiting society through science

RUO

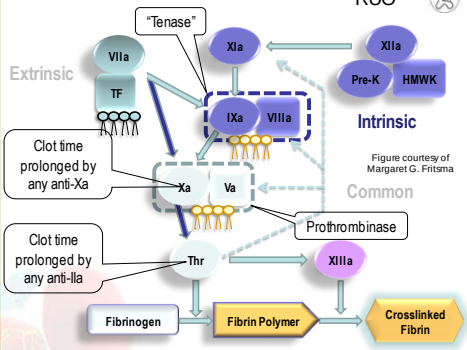


Figure courtesy of Margaret G. Fritsma

The Fritsma Factor

1: New Oral A/C 92

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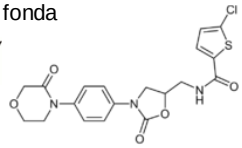
Rivaroxaban

- An oxazolininone derivative direct anti-Xa
- Safety and efficacy exceed Lovenox, fonda

Bayer

Johnson & Johnson PHARMACEUTICAL RESEARCH & DEVELOPMENT, L.L.C.

Xarelto rivaroxaban



Bauer KA, Homering M, Berkowitz SD. Effects of age, weight, gender and renal function in a pooled analysis of four phase III studies of rivaroxaban for prevention of venous thromboembolism after major orthopedic surgery. Blood 2008; 112: Abstract 436

The Fritsma Factor

1: New Oral A/C 93

THE FRITSMA FACTOR Your Interactive Hemostasis Resource

Rivaroxaban inhibits Xa independent of AT

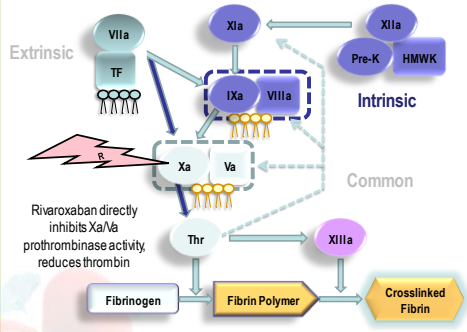


Figure courtesy of Margaret G. Fritsma

The Fritsma Factor

1: New Oral A/C 94

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Rivaroxaban

- Oral dose: 10 mg/day: steady state at 4 hours
- Neutralizes free, clot-bound, and IXa-bound Xa
  - Interacts with no other serine proteases
- Excretion: 66% renal, 28% fecal (liver)
- Monitoring: all the same reasons as LMWH, fonda
  - No PT or PTT: insensitive vary by reagent formulation
  - Hep-test, PiCT may monitor with incubation time modifications
  - Chromogenic anti-Xa: variability among formulations
  - Xa neutralization assay: see next slide
- Cleared for VTE prophylaxis; Canada & EU 5/2009

Laux V, Perzborn E, Kubitza D, Misselwitz F. Preclinical and clinical characteristics of Rivaroxaban: Anovel, oral, direct factor Xa inhibitor. Semin Thromb Hemost 2007;33:5115–5123.

The Fritsma Factor

1: New Oral A/C 95

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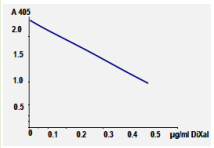
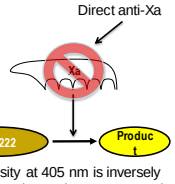
BIOPHEN DiXal® Chromogenic

RUO



mcg/mL Rivaroxaban or Apixiban



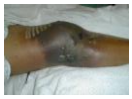
Intensity at 405 nm is inversely proportional to anti-Xa concentration

The Fritsma Factor

1: New Oral A/C 96

Direct Thrombin Inhibitors (DTIs)

- Indication: heparin-induced thrombocytopenia (HIT)
  - Do not generate or bind anti-heparin-PF4
  - Platelet counts recover within three days
- Rapidly reduce thrombin production in HIT
  - Warfarin too slow
  - LMWH may cross-react
  - Fondaparinux OK



- Kaplan KL, Francis CW. Direct thrombin inhibitors. Semin Hematol 2002;39:187–196.
- Prechel M, Walenga JM. The laboratory diagnosis and clinical management of patients with heparin-induced thrombocytopenia: an update. Semin Thrombos Hemostas 2008;34:86–96.

Direct Thrombin Inhibitors (DTIs)

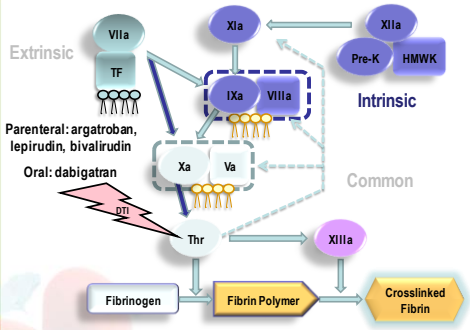
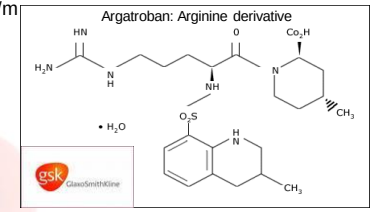


Figure courtesy of Margaret G. Fritsma

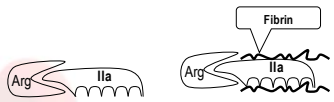
Argatroban (Novastan®)

- Raises nitric oxide, causing vasodilatation
- Metabolized and excreted by liver CYP450
- IV: 2 mcg/kg/m 5–7 d: immediate steady state
- PCI: bolus 350 mcg/kg; continuous infusion 15–40 mcg/kg/m



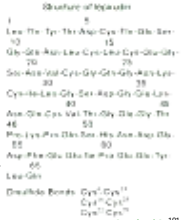
Argatroban Comments

- Safe to use in renal disease
- Liver disease
  - Reduce to 0.5 mcg/kg/h and monitor
  - Major bleeds 5.3%, minor 14.4%
- No antidote, but half-life is 40 minutes
- Inhibits free and fibrin-bound thrombin



Hirudin: Lepirudin

- Inhibits free, not bound thrombin
- Metabolized and excreted by kidney
  - Monitor when GFR <30 mL/min
- Immune response: anaphylaxis



Lepirudin Administration

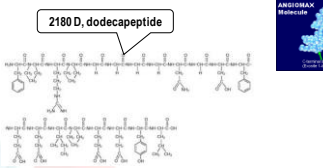
- IV bolus: 0.4 mg/kg/h
- Infusion 0.1–0.15 mg/kg/h 11–14 d
- Steady state within 2.5 hours
- Clearance half-life 20 minutes
- Coronary bypass: 0.25 mg/kg/h

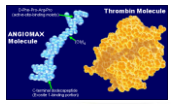
PHARMION®

Refludan® 50mg
Powder for solution for injection or infusion
lepirudin

Bivalirudin

- Thrombin active site-directed peptide, D-Phe-Pro-Arg-Pro, linked to an analogue of the carboxy-terminal of hirudin





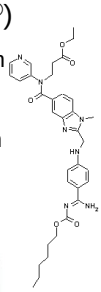
THE MEDICINES COMPANY®

Bivalirudin

- Neutralizes free and bound thrombin
- FDA-cleared spring, 2008
  - Reduced major hemorrhage by 41% to 61%
  - Cleared for use with aspirin
- Bolus 0.75 mg/kg + 1.75 mg/kg/h infusion
- Renal excretion, 25 m half-life
- If GFR is <30 mL/minute, reduce to 1 mg/kg/h, no reduction in bolus
- If a patient is on hemodialysis, reduce infusion to 0.25 mg/kg/h

Dabigatran (Pradaxa®)

- Oral DTI cleared for prophylaxis in Canada and Europe 2009
  - Indication: post-surgical VTE prevention
- Cleared for prevention of stroke in atrial fibrillation in US 2010
- 110 mg/d with wide safety range
  - Immediate steady state
  - Monitoring: same reasons as LMWH



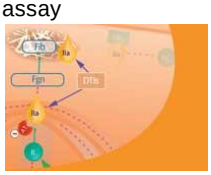
Boehringer Ingelheim

Dabigatran (Pradaxa®)

- Binds clot-bound and free thrombin
- Renal excretion 80%
  - Reduce dosage and monitor when GFR < 30 mL/min
- Half-life 12–17 hours
- No interaction with food
- Not metabolized by CYP450 pathway
- Levels raised by quinidine and verapamil
- Predictable efficacy
- No liver toxicity
- Dyspepsia

Monitoring DTIs

- PTT: use 1.5–3x mean of reference interval
  - Sensitivity varies by formulation
- ACT during coronary bypass
- Ecarin clotting time
- Ecarin chromogenic assay
- Thrombin inhibition assay



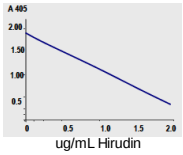
Ecarin Clotting Time/Chromogenic

Saw-scale Viper: Echis carinatus

Stago Ecarin Chromogenic Assay®
Intensity at 405 nm is inversely proportional to DTI concentration

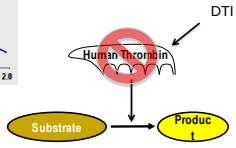
THE FRITSMA FACTOR
Your Interactive Hemostasis Resource

BIOPHEN DTI® Chromogenic



ug/mL Hirudin

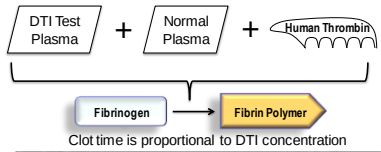
RUO



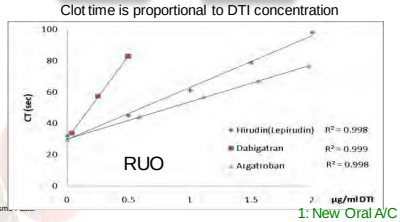
Intensity at 405 nm is inversely proportional to DTI concentration

THE FRITSMA FACTOR
Your Interactive Hemostasis Resource

BIOPHEN Hemoclot® Thrombin Inhibitors



Clot time is proportional to DTI concentration



RUO