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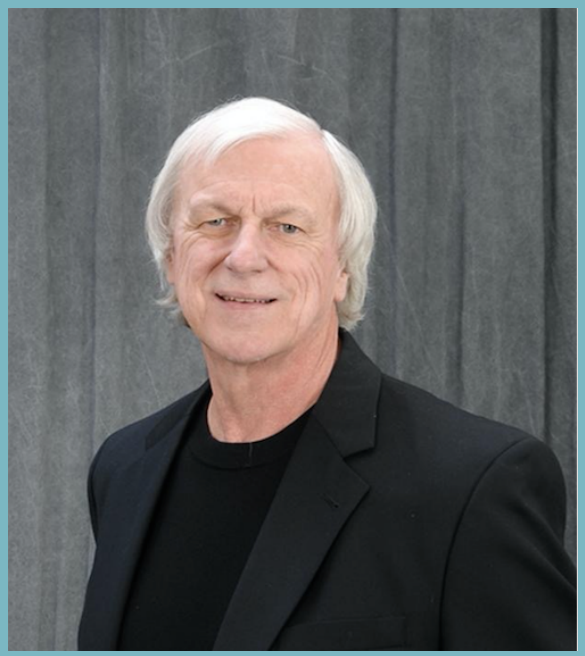
Coagulation Factor Inhibitors and the Nijmegen Bethesda Assay



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Coagulation Factor Inhibitors

Bottom Line at the Start (BLATS); The Participant…

- Explains the origin of anti-factor VIII (FVIII inhibitor)
- Detects FVIII inhibitors using factor assays and mixing studies
- Measures FVIII inhibitors using the Bethesda titer, Nijmegen Bethesda assay, chromogenic Bethesda assay, enzyme immunoassay and fluorescence immunoassay
- Describes coagulation factor bypass therapy to resolve bleeding in inhibitor patients
- Describes immune tolerance induction therapy
- Lists new factor concentrates designed to prevent inhibitor formation

2-yo Hemophilic Boy

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

Mixing Study: 2-yo Hemophilic Boy

NP: commercial pooled normal plasma from 20 normal donors with ~100 U/dL factor levels

Assay	Result	RI	Comment
Patient PTT	63 s	25–35 s	
Immediate PTT of patient/NP 1:1 mix	34.5 s	NP PTT 30 s	Limit: NP + 10%: Incomplete correction
PTT of Pt/NP 1:1 mix incubated 1 h at 37°C	47.9 s	NP PTT 35 s	Incubate mix and NP: Uncorrected

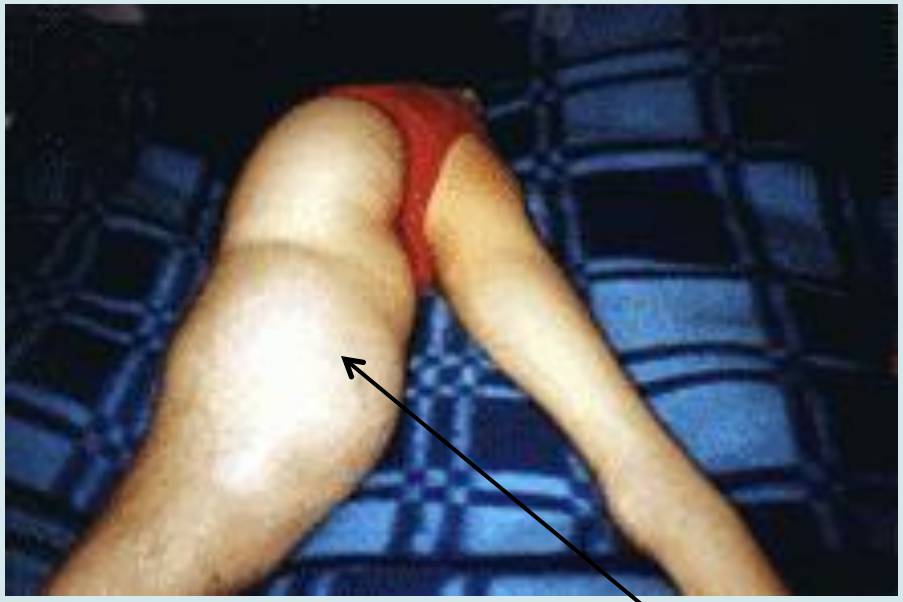
- R/O lupus anticoagulant (LAC): pediatric, bleeding
- Specific coagulation factor VIII (FVIII) inhibitor; Bethesda titer

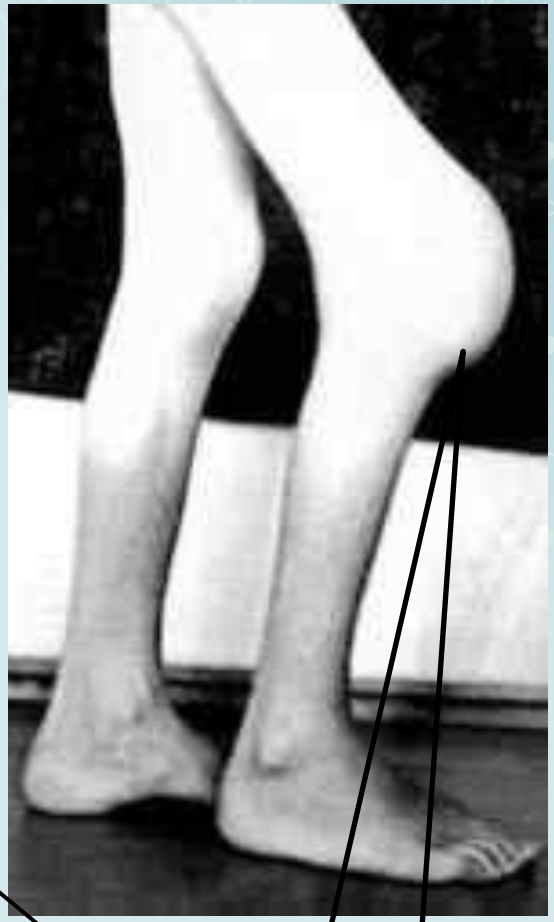
Hemophilia A Symptoms

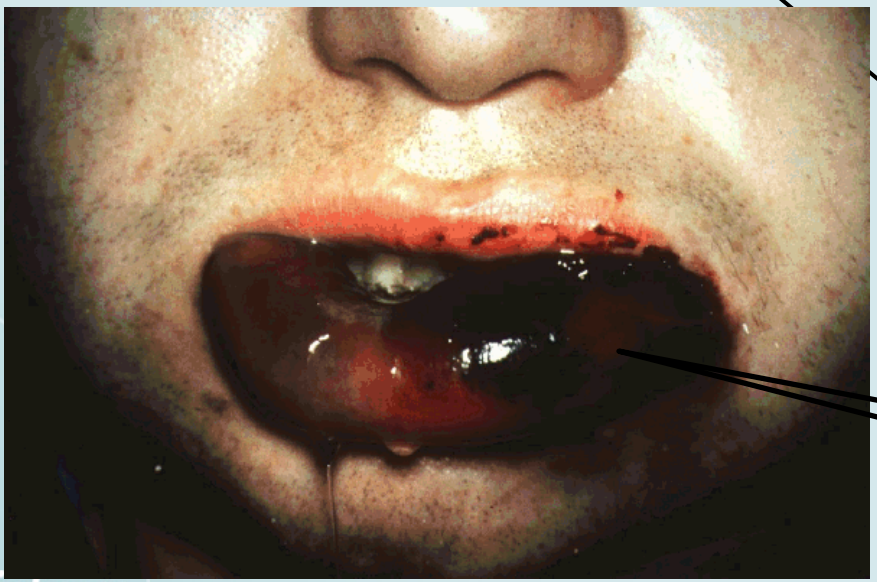
Spontaneous anatomic (soft-tissue) bleeds

- Bleeding at umbilical stump and circumcision
- Delayed bleeding triggered by injury
  - Joints, large muscles, body cavities, GI, soft tissue, tongue, kidney, testicles, brain, CNS
- Spontaneous bleeds, especially into joints
- Inflammation, hematomas, hemarthroses

	Severe	Moderate	Mild
Prevalence	70%	15%	15%
FVIII U/dL	< 1	1–5	6–30
Bleeding	Spontaneous	Minor trauma	Major trauma







Hemarthroses

Airway obstruction

40 YO Hemophilic

- Bleeding into ankle at midnight
  - Attempted DDAVP (Stimate®) inhaler
- Ran out of therapeutic FVIII concentrate
  - In US, Medicare subsidy via ~100 national hemophilia centers
  - Canadian Hemophilia Society Treatment Cntrs
- Night tech and on-call path resident
  1. Determine residual patient factor VIII activity
  2. Compute FVIII dosage from package insert to prevent potential thrombotic overdose and to avoid waste
  3. Order FVIII concentrate from transfusion service
  4. Reconstitute with sterile water, administer as IV push
  5. Subsequently determine therapeutic factor VIII level

If Factor Assay not Available

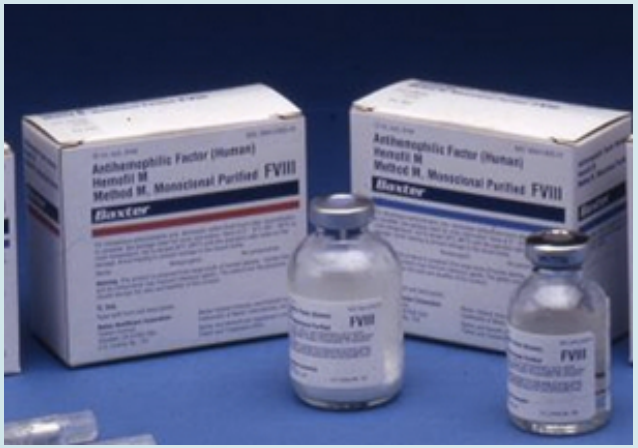
- When timing is critical, assume <1 U/dL FVIII activity or…
- Estimate residual FVIII from PTT:

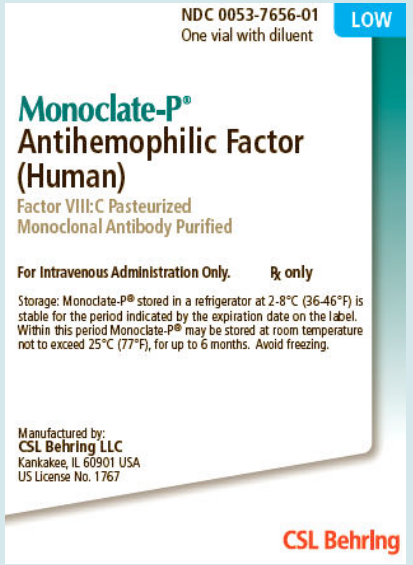
FVIII	PTT
40 U/dL	35 s
30 U/dL	50 s
20 U/dL	65 s
10 U/dL	90 s
1 U/dL	120 s
Example only, do not use *Varies by reagent sensitivity*	

- Collect baseline specimen
  - Freeze, confirm next day shift with FVIII assay
- Maintain patient database

Plasma-derived FVIII Concentrates

- <25% of FVIII concentrate in industrialized countries
- Theoretical risk of HBV, HCV, HIV transmission 1/60,000
  - Human and animal plasma matrix
  - Purification: immunoaffinity column, solvent-detergent, Pasteurization, viral filtration, combinations
  - Hemofil-M, Monarc-M, Monoclate-P
- Seroconversions per 2003 CDC surveillance: 0





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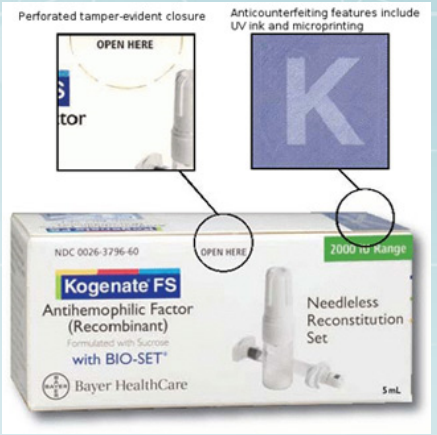
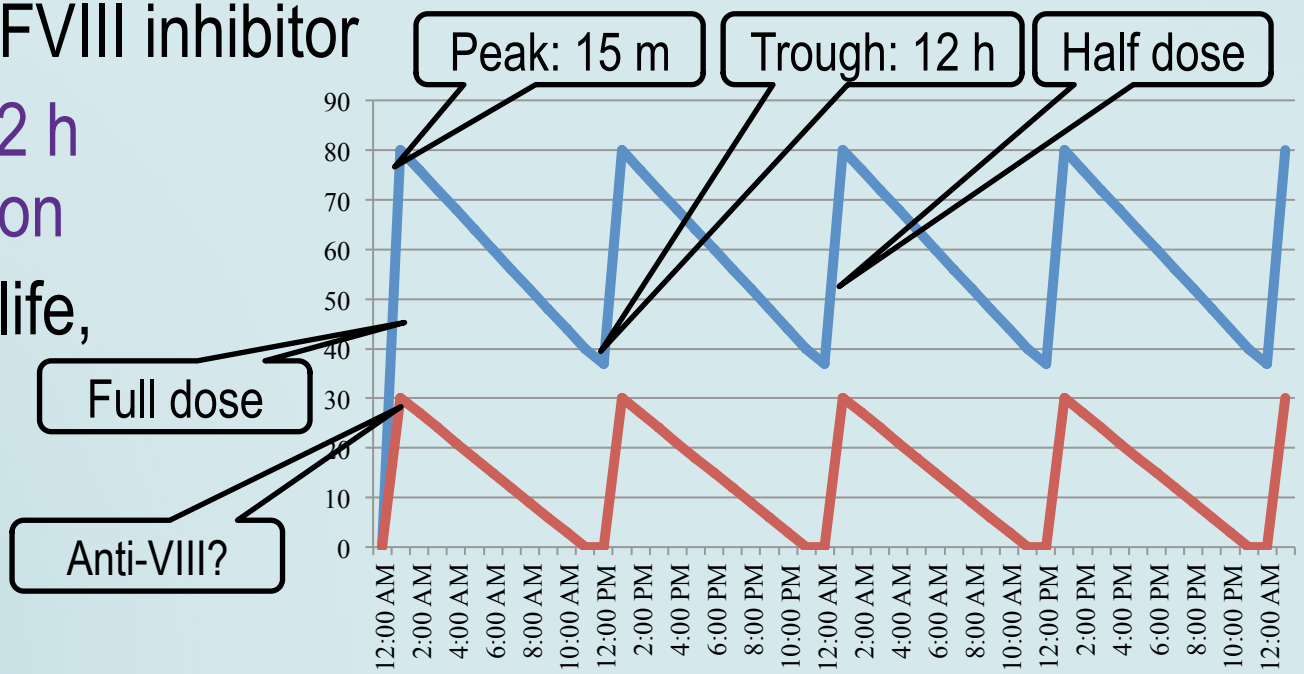
Recombinant FVIII Concentrates

- Serum or albumin in culture medium
  - Helixate, Kogenate, Recombinate
  - Abundant, used for prophylaxis
  - No HBV, HCV, HIV seroconversions
- No protein in culture or preparation
  - No theoretical viral risk: Advate
- B-domain-deleted FVIII concentrate
  - Human albumin in culture: ReFacto
  - No protein: Xyntha
  - FVIII clot assay unreliable



40 YO Receives Advate

- Peak: collected 15 m after administration
  - If peak reaches expected value, plan for next administration at 12 hours
  - Factor assay result: 30 U/dL
  - Should have reached 80 U/dL, what happened?
  - Suspect anti-FVIII inhibitor
- Trough: collect 12 h after administration
  - Reflects half-life, 50% of peak
  - Continue w/ half doses



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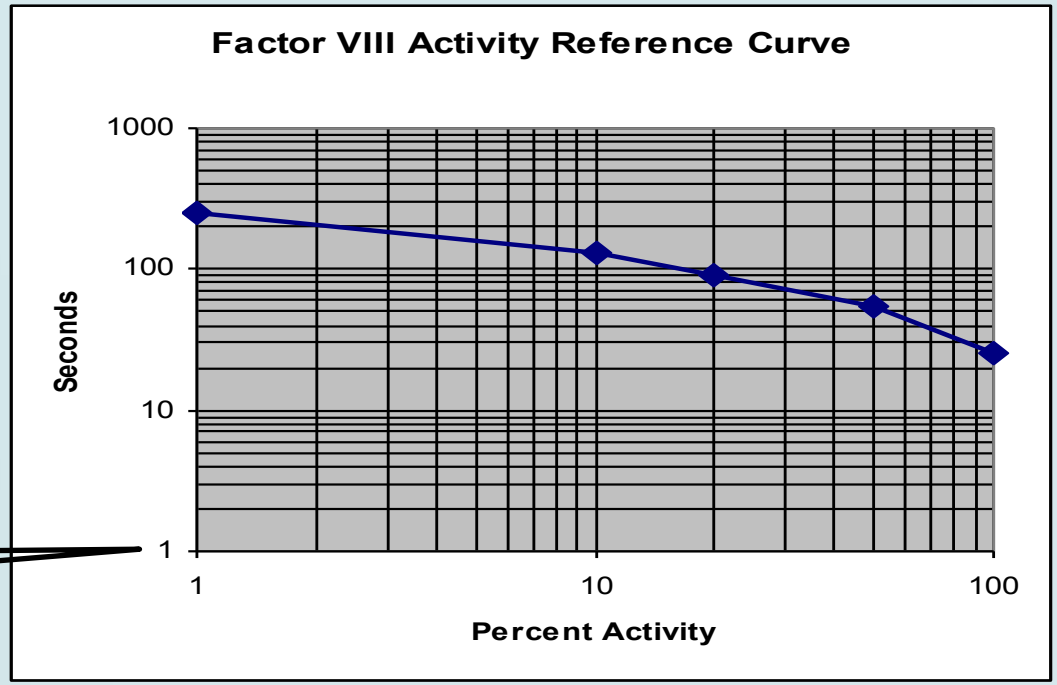
One-stage Factor VIII Assay

1. Dilute pt plasma 1:10 in imidazole buffered saline (IBS)
2. Add FVIII-depleted reagent plasma 1:1 (FVIIIDP)
   - Provides all factors except FVIII
   - Typical: 100 uL patient plasma dilution + 100 uL FVIIIDP
3. Add PTT reagent, incubate 3 m
4. Add $CaCl_2$, record interval to clot formation
5. Compare result in seconds to calibration curve to derive plasma activity:



1% activity = 1 U/dL

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Factor VIII Assay Plasma Dilutions Parallelism Indicates No Inhibitor

Automated Plasma Dilution	Seconds	Raw Factor VIII Activity	Computed Factor VIII Activity (× dilution)
1:10 “undiluted”	90 s	20 U/dL	20 U/dL
1:20	105 s	10 U/dL	20 U/dL (parallel)*
1:40	107 s	5.5 U/dL	22 U/dL (parallel)
1:80	110 s	2.6 U/dL	20.8 U/dL (parallel)
* <10% difference from undiluted indicates parallelism, no inhibitor			

FVIII Assay Plasma Dilutions non-Parallelism Indicates Inhibitor

Plasma Dilution	Seconds	Raw Factor VIII Activity	Computed Factor VIII Activity (× dilution)*
1:10 (undiluted)	95 s	10 U/dL	10 U/dL
1:20	99 s	8 U/dL	16 U/dL
1:40	107 s	5 U/dL	20 U/dL
1:80	108 s	4 U/dL	32 U/dL
• >10% difference from undiluted = non-parallel & rising, implies inhibitor			

- Inhibitor: IgG alloantibody to FVIII concentrate
  - 30% prevalence, most arise in severe hemophilia
  - Reflex to inhibitor assay

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

Why Measure Inhibitors?

- Confirm refractory response to factor therapy
- Monitor efficacy of prophylactic FVIII or FIX therapy
- Monitor factor bypass preparation efficacy and safety
- Distinguish low response from high response patients
  - Low responders: treat with factor concentrate
  - High response when bleeding: treat with bypass preparations
  - High response when not bleeding: immune tolerance therapy
- Detect factor-induced anamnesis
- Monitor immune tolerance therapy efficacy
- Establish prevalence and population trends
  - Hemophilia severity, correlate to mutation, correlate to therapy

Courtesy of Connie Miller, PhD, CDC

Limitations of Inhibitor Measurement

- Median 40% of severe hemophilia patients in US hemophilia treatment centers are tested for inhibitors
- Require therapeutic “wash-out” before sampling
- Local laboratory expertise and experience
- Median 32% FP rate, 5% FN rate, interlab CV 50%
- The need for method standardization

- Soucie et al. National surveillance for hemophilia inhibitors in the United States: Summary report of an expert meeting. Am J Hematol 2014; 89:621.
- Favaloro et al. Haemophilia 2014; 20: Suppl 4

CVs of NASCOLA Inhibitor Measurement

Year	Expected	Average	Min	Max	CV
2010	0	0.1	0	5.0	178
2011	0	0.4	0	2.9	150
2010	0.5-1.0	0.5	0	2.0	83
2011	0.9	1.3	0	3.7	46
2012	0.6	4.0	0	25.5	40
2010	1-1.5	1.0	0	2.0	48
2011	2.0	2.8	0	15.0	47
2012	2.0	3.3	0	15.0	48
2012	1.1	1.3	0	2.7	46
2010	3.5	5.0	0.5	30.0	36
2011	3.9	4.0	0	25.5	40
2012	5.0	7.5	0	30.0	39

53 labs

78 labs

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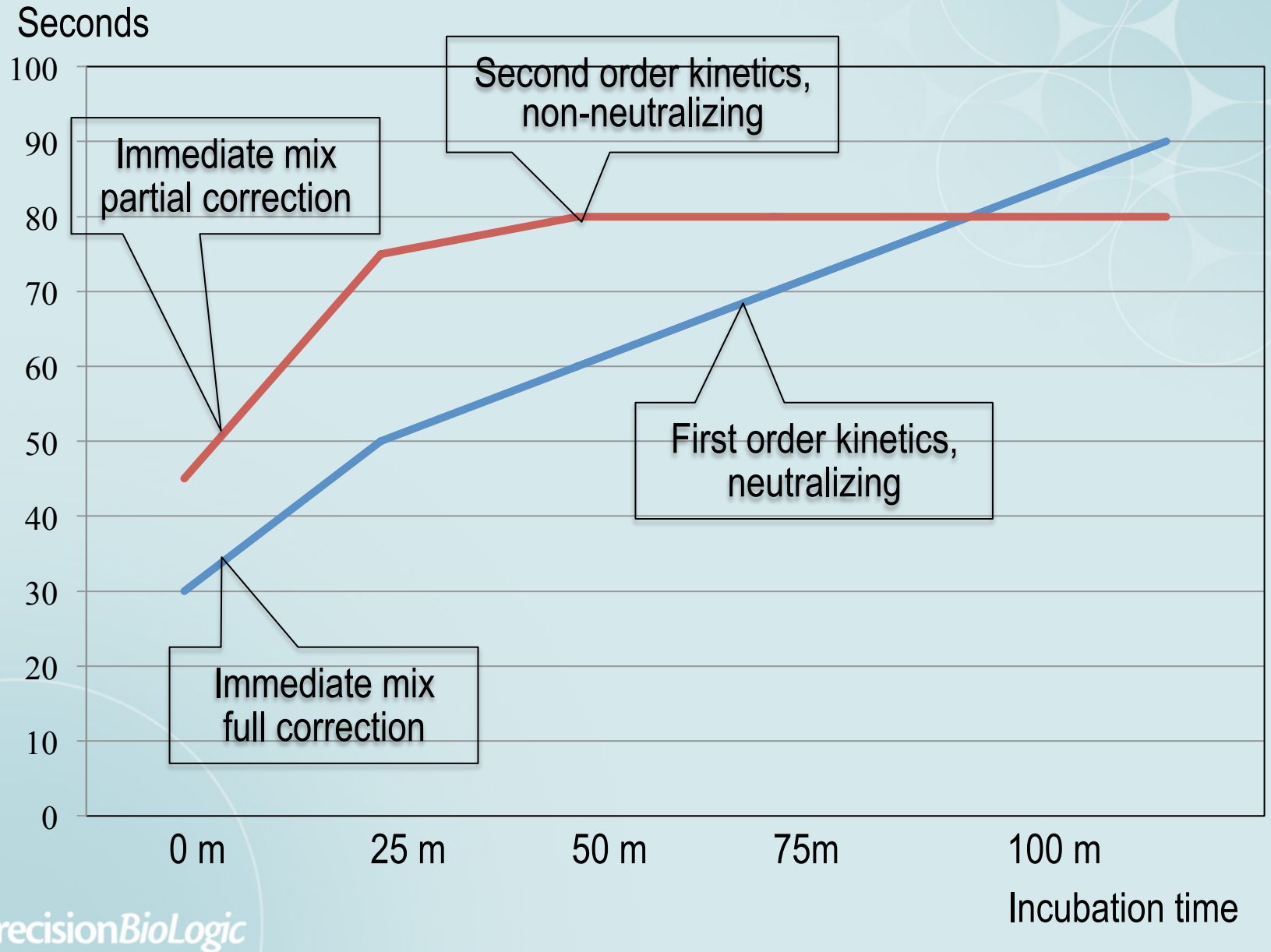
Pruthi et al. Quality of factor VIII inhibitor testing in North American specialized coagulation laboratories. *Am J Hematol* 2014; 89: E27.

Variations That Affect Inhibitor Measurement

- Residual patient plasma coagulation factor
- Presence of anticoagulants: dabigatran, heparin
- Presence of other inhibitors: lupus anticoagulant
- Differences in inhibitor epitope specificity
- Elevated coagulation factors shorten PTT results
- Inhibitor kinetics: neutralizing and non-neutralizing

- Soucie et al. National surveillance for hemophilia inhibitors in the United States: Summary report of an expert meeting. Am J Hematol 2014; 89:621.
- Favaloro et al. Haemophilia 2014; 20: Suppl 4

PTT Mix Inhibitor Kinetics



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Inhibitors Effects

- Severe hemophilia A: 30%, moderate, 10%
- Hemophilia B: 1–3%
- Render replacement therapy ineffective
- Most occur in early age factor administration
- Raise rates of hemarthrosis
- More catastrophic bleeds, mortality
- Delay physical maturation
- Raise healthcare costs five-fold

Arruda VR, Samelson-Jones BJ. Gene therapy for immune tolerance induction in hemophilia with inhibitors. J Thromb Haemost 2016; 14: 1121–34. Slide added 6-16-16

Inhibitor Measurement Methods

- Functional, clot-based factor activity inhibition
  - Bethesda titer, 1975
  - Nijmegen Bethesda assay (NBA), 1995
- Functional: chromogenic Bethesda assay (CBA)
- Qualitative immunoassay
  - Solid state enzyme immunoassay (EIA, ELISA)
  - Flow-based fluorescence immunoassay (FLI)
- CDC modification of the NBA, 2012

Courtesy of Connie Miller, PhD, CDC

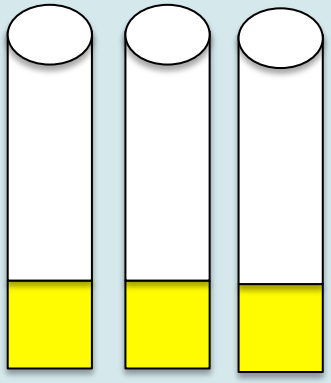
1975 Bethesda Titer

1. Prepare serial twofold patient plasma dilutions in imidazole buffered saline (IBS)
2. Mix patient plasma dilutions 1:1 with commercial NP
3. Prepare 1:1 NP/IBS for normalization
4. Incubate patient dilutions and 1:1 NP/IBS 37°C 2h
5. Add FVIII-depleted plasma to all dilutions after incubation
6. Perform FVIII assay on all dilutions using PTT methodology
7. Report Bethesda units (BU)/mL as reciprocal of dilution that neutralizes 50% FVIII compared to the incubated 1:1 NP/IBS

- NP is the source for FVIII, should be ~100 FVIII U/dL
- Incubated NP/IBS compensates for FV & FVIII deterioration

Kasper CK, Aledort LM, Counts RB, et al. A more uniform measurement of factor VIII inhibitors. Thromb Diath Haemorrh 1975;34:869–72.

1975 Bethesda Titer



Serial dilutions of patient plasma in IBS

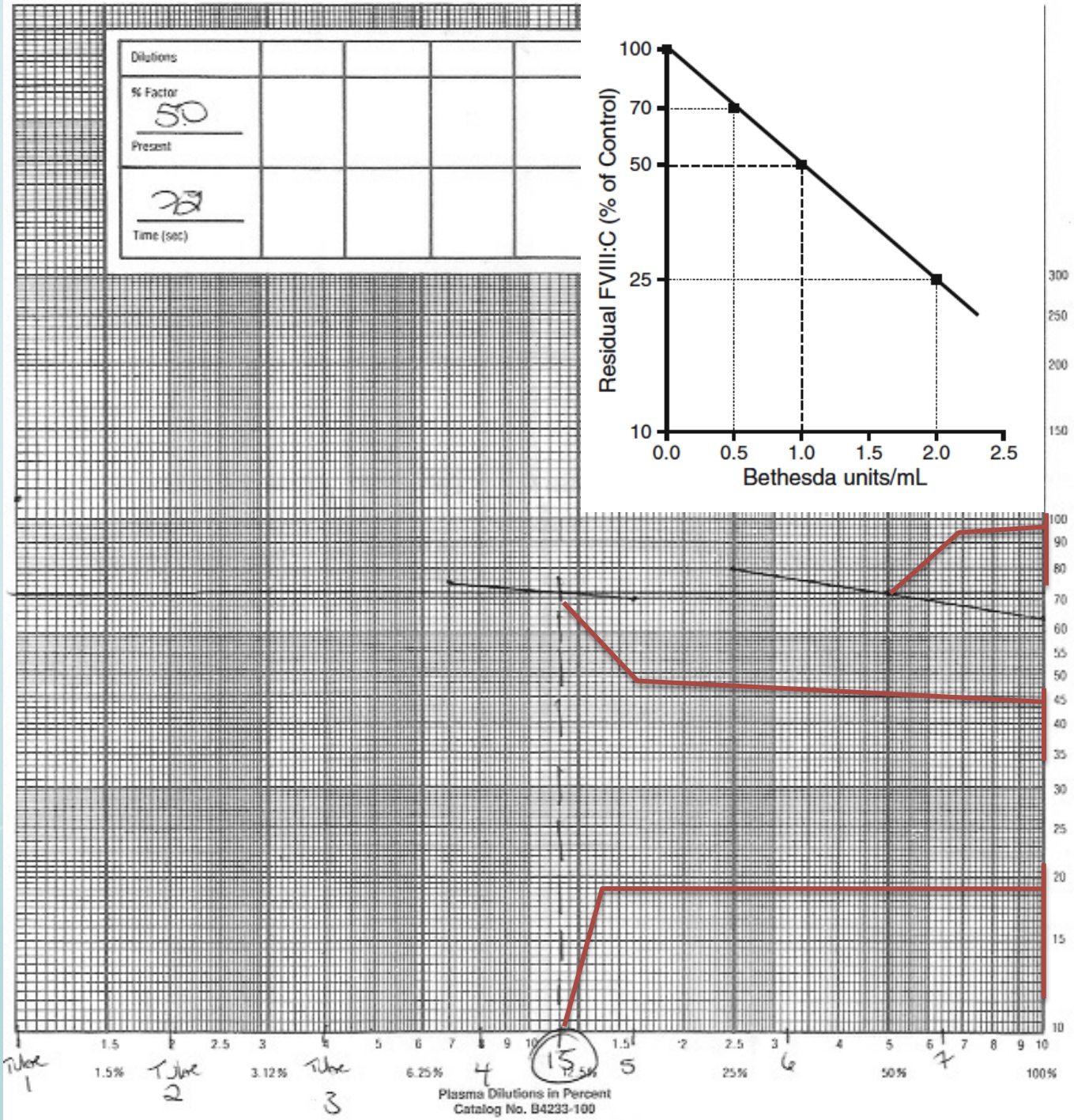
Patient mix
Mix 1 part patient dilution with 1 part NP

NP/IBS 1:1 mix

Incubate 37C/2h, perform FVIII assay

Express reciprocal of patient dilution that yields 50% FVIII as BU/mL

Miller CJ, Platt SJ, Rice AS, et al. Validation of Nijmegan-Bethesda assay modifications to allow inhibitor measurement during replacement therapy and facilitate inhibitor surveillance. J Thrombos Haemostas 2012;10:1055–61.



Duncan E, Collect M, Street A. Nijmegen-Bethesda assay to measure factor VIII Inhibitors. In Monagle P. Haemostasis: Methods and Protocols, Methods in Molecular Biology 992, Springer Science, 2013

NP/IBS 50% FVIII

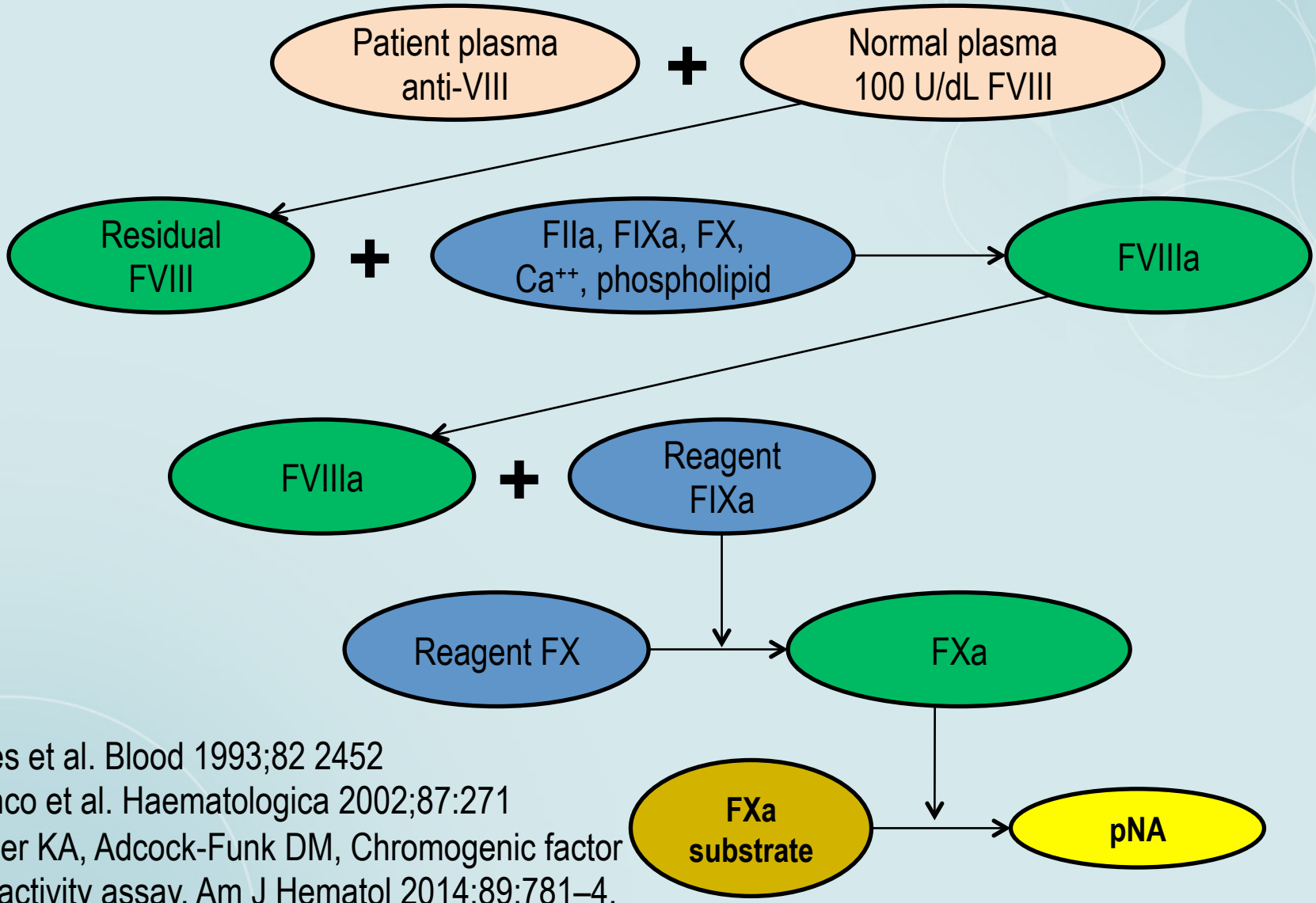
Patient or control dilution 50% FVIII

BU = reciprocal of dilution

Chromogenic Bethesda Assay

1. Prepare pt plasma *and control* dilutions as for Bethesda titer
2. Mix pt plasma and control dilutions 1:1 with NP, incubate 2 h.
3. Dilute incubated pt/control plasma/NP 1:31 in IBS.
4. Add reagent: bovine FX, FIXa, thrombin, $CaCl_2$, & PL.
5. Incubate 90s at 37°C, generates FXa
6. Add FXa chromogenic substrate with thrombin inhibitor and stopping buffer to measure FXa surrogate to FVIII activity
7. Measure FXa: read ΔA/m at 405 nm.
8. 1 chromogenic Bethesda unit (CBU) = level of inhibitor/mL of patient plasma that inactivates 50% of FVIII in 1 mL NP.
9. Limit: ≥0.5 CBU = positive

Chromogenic Bethesda Assay



- Gilles et al. Blood 1993;82 2452
- Blanco et al. Haematologica 2002;87:271
- Moser KA, Adcock-Funk DM, Chromogenic factor VIII activity assay. Am J Hematol 2014;89:781–4.

pNA intensity at 405 nm is proportional to FXa activity and inversely proportional to patient anti-VIII

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Bethesda Titer Vs Chromogenic Bethesda Assay

Bethesda Titer

- Variable non-specific endpoint: clot detection
- Insensitive to inhibitors ≤ 0.5 BU/mL
- Interference from lupus anticoagulant
- therapeutic A/Cs interfere: heparin and dabigatran

Chromogenic Bethesda

- Quantitative endpoint detection system
- Sensitive to inhibitors at 0.1 BU/mL
- Specific for factor inhibitors
- No LAC interference, distinguish LAC from inhibitor

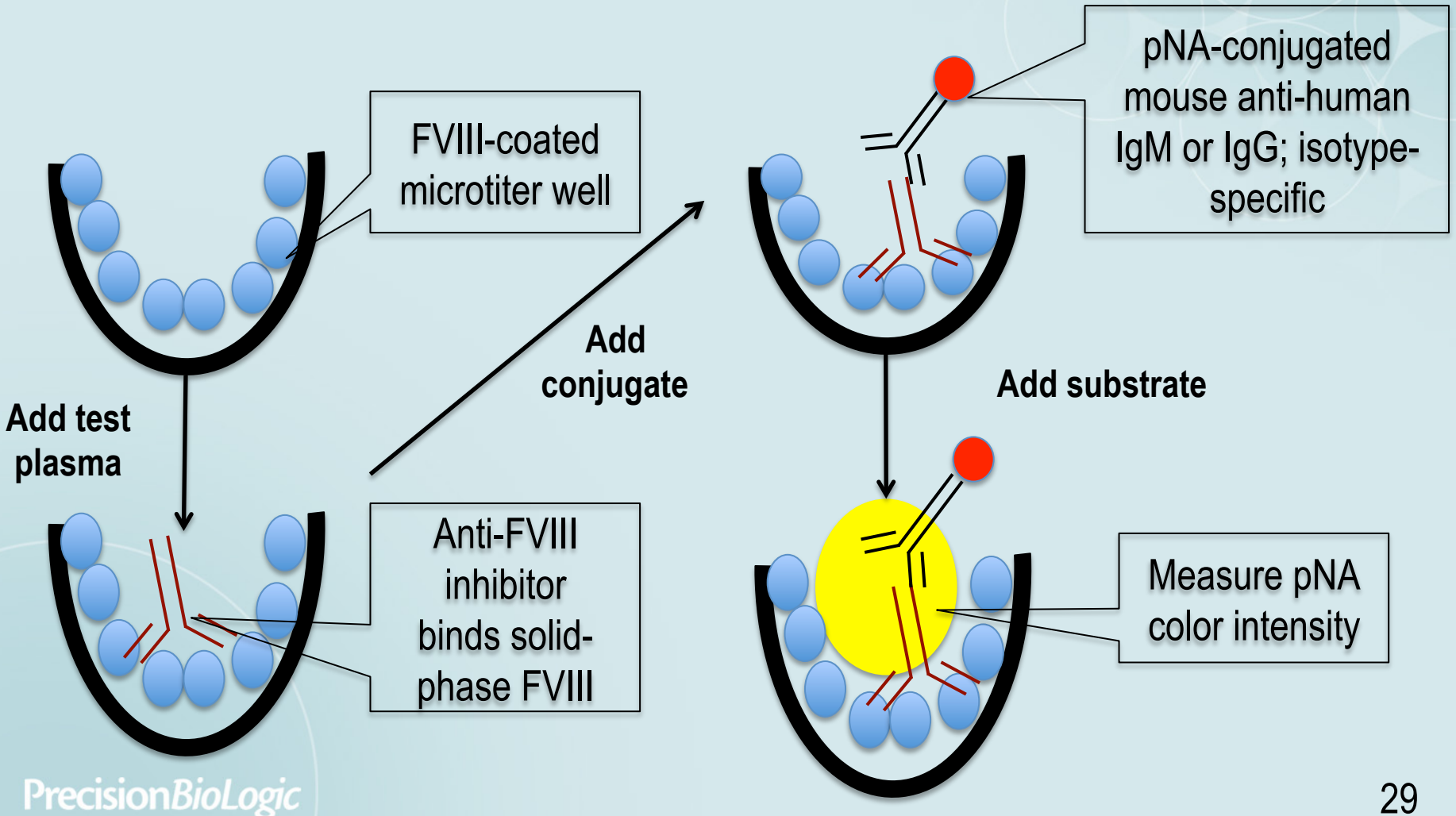
Miller CH, Rice AS, Boylan B, et al. Comparison of clot-based, chromogenic, and fluorescence assays for measurement of factor VIII inhibitors in the US Hemophilia Inhibitor Research Study. J Thromb Haemost 2013; 11: 1300–9.

Immunoassay Measurement

- EIA: FVIII antigenic target immobilized in well
- FLI: FVIII target immobilized on fluorescent beads
- More sensitive than functional assays
- Detect neutralizing & non-neutralizing inhibitors
- Detect non factor-inhibiting immunoglobulins
- Non-specific, require functional assay follow-up
- Confirm FVIII reactivity in clot-based assays and distinguish isotypes IgG_1, IgG_2, IgG_4

- Lavigne-Lissalde et al. Thromb Haemost 2008;99: 1090
- Krudysz-Amblo et al. Blood 2009;113:2587

Solid-phase Enzyme Immunoassay



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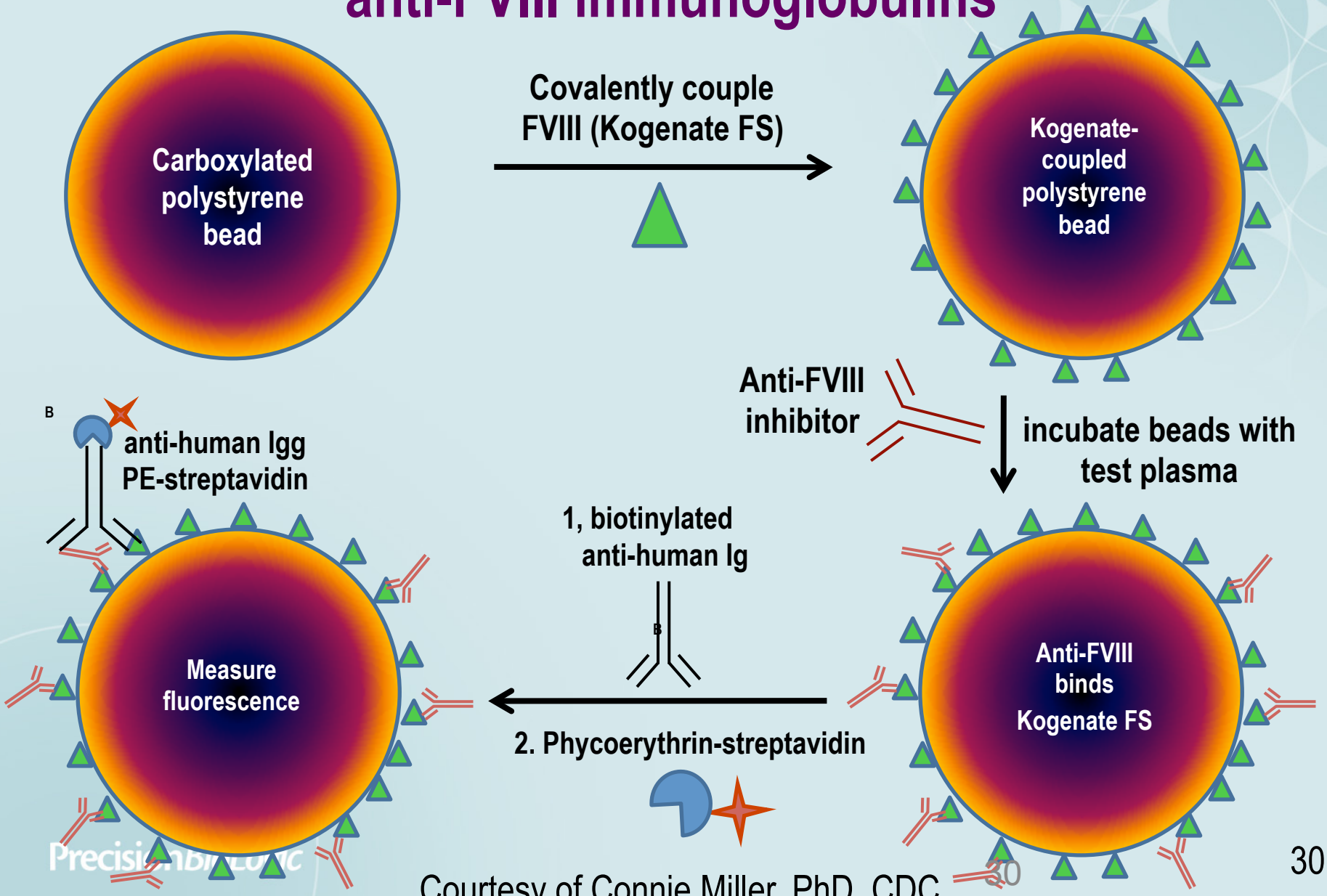
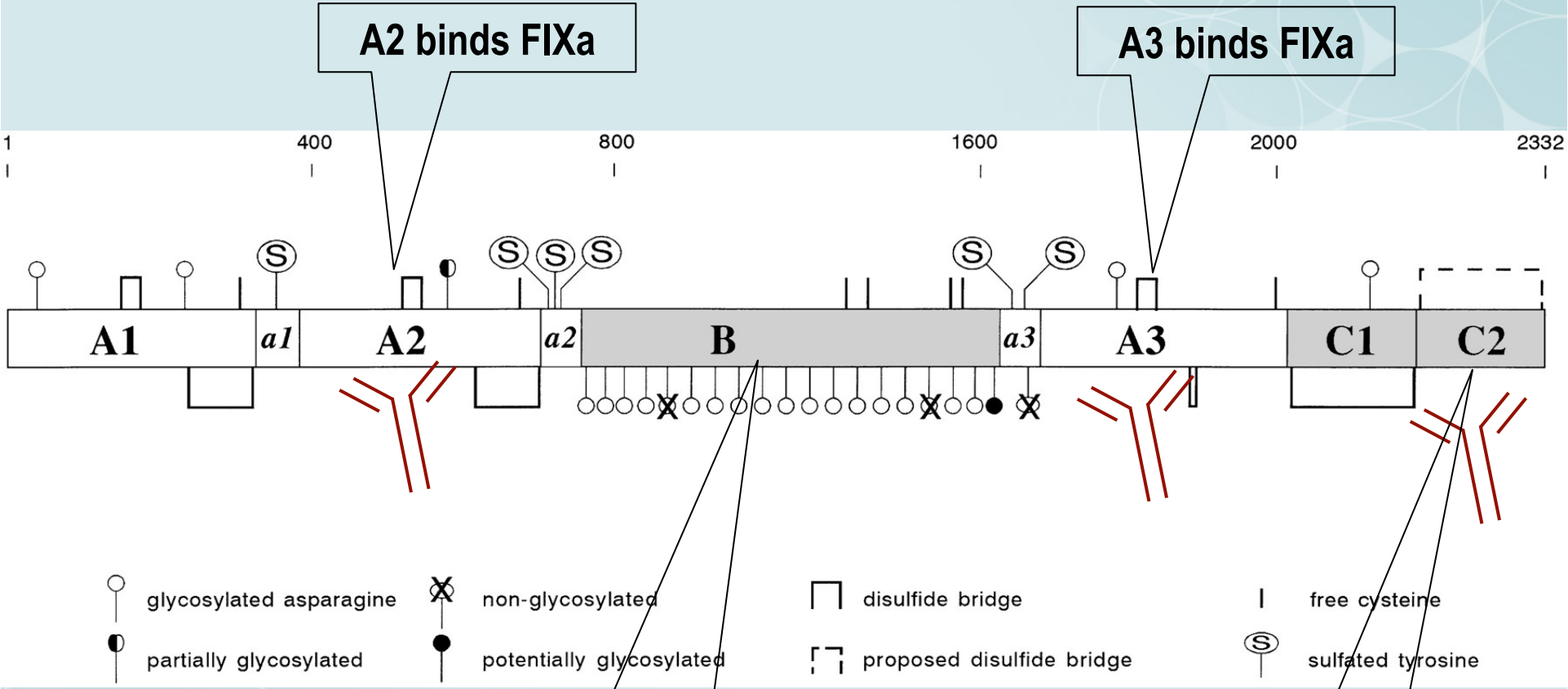
Fluorescence-based immunoassay detects anti-FVIII immunoglobulins



Courtesy of Connie Miller, PhD, CDC

FVIII IgG Binding Domains: A_2>C_2>A_3



Inhibitor Isotype by Fluorescence Immunoassay

- Non-hemophilia subject samples
  - 5% IgG_1 and IgG_2; 2% IgG_3 and IgG_4
- 491 samples from 371 hemophilia A patients
  - 41% IgG_1, 17% IgG_2, 6% IgG_3, 27% IgG_4
  - Many patients with multiple isotypes
- Inhibitor-positive hemophilia A patient isotypes
  - 14% single, 84% multiple, 2% no IgG by FLI
- 7 patients developed NBA-positive inhibitors
  - 5 had prior IgG_1 antibodies, *added* IgG_4

Boylan B, Rise AS, Dunn AL, et al. Characterization of the anti-factor VIII immunoglobulin profile in patients with hemophilia A by use of a fluorescence-based immunoassay. J Thromb Haemost 2015; 13: 47–53.

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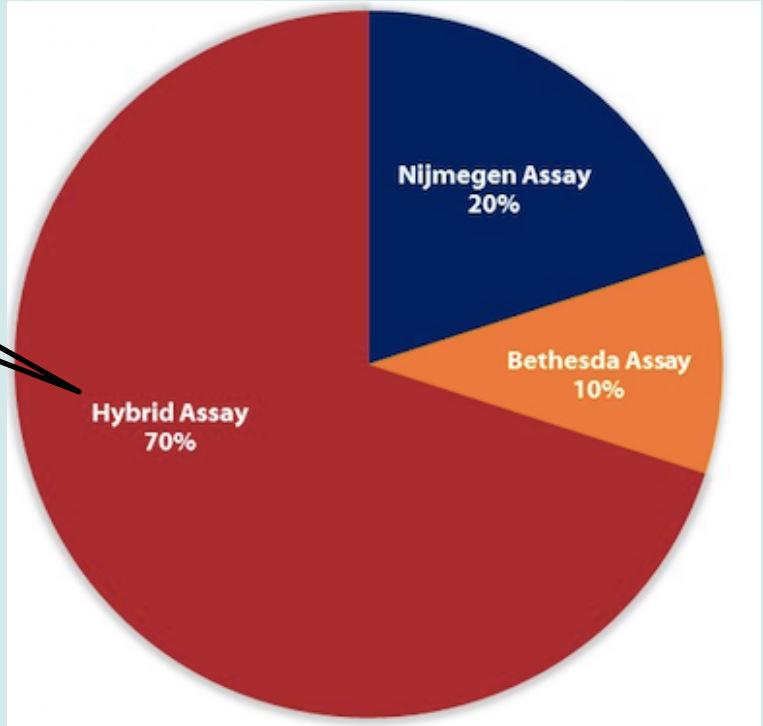
Nijmegen Bethesda Assay

1995 Nijmegen Bethesda Titer

1. Serially dilute patient plasma in FVIII-DP (New)
2. Mix dilutions 1:1 with IBS-pH 7.4 stabilized NP (New)
3. Incubate 37°C 2h, perform PTT-based FVIII assay
4. Report Nijmegen Bethesda units (NBU)/mL as reciprocal of dilution that neutralizes 50% FVIII

"Hybrid" assay uses IBS-pH 7.4 NP but substitutes IBS for FVIII-DP to reduce expense



Verbruggen B, Novakova I, Wessels H, et al. The Nijmegen modification of the Bethesda assay for factor VIII:C inhibitors: improved specificity and reliability. Thromb Haemost 1995;73:247–51.

CDC Standard NBA Protocol Updates

- Ship patient specimens on cold packs, not frozen
- Use IBS-pH 7.4 reagent normal plasma (NP) to stabilize FVIII
- Heat specimens 56°C 30” & centrifuge to remove FVIII
  - Residual FVIII from recent prophylaxis or on-demand Rx
  - Non-neutralizing antibody leaves behind residual VIII
  - Heat improves specificity for low-titer inhibitors
  - Factor IX titer, 58°C for 90”?
- Dilute heated pt plasmas and unheated 1 BU positive control in bovine serum albumin (BSA) or FVIII-DP, not IBS
- Confirm all <2 BU/mL samples with alternate assay

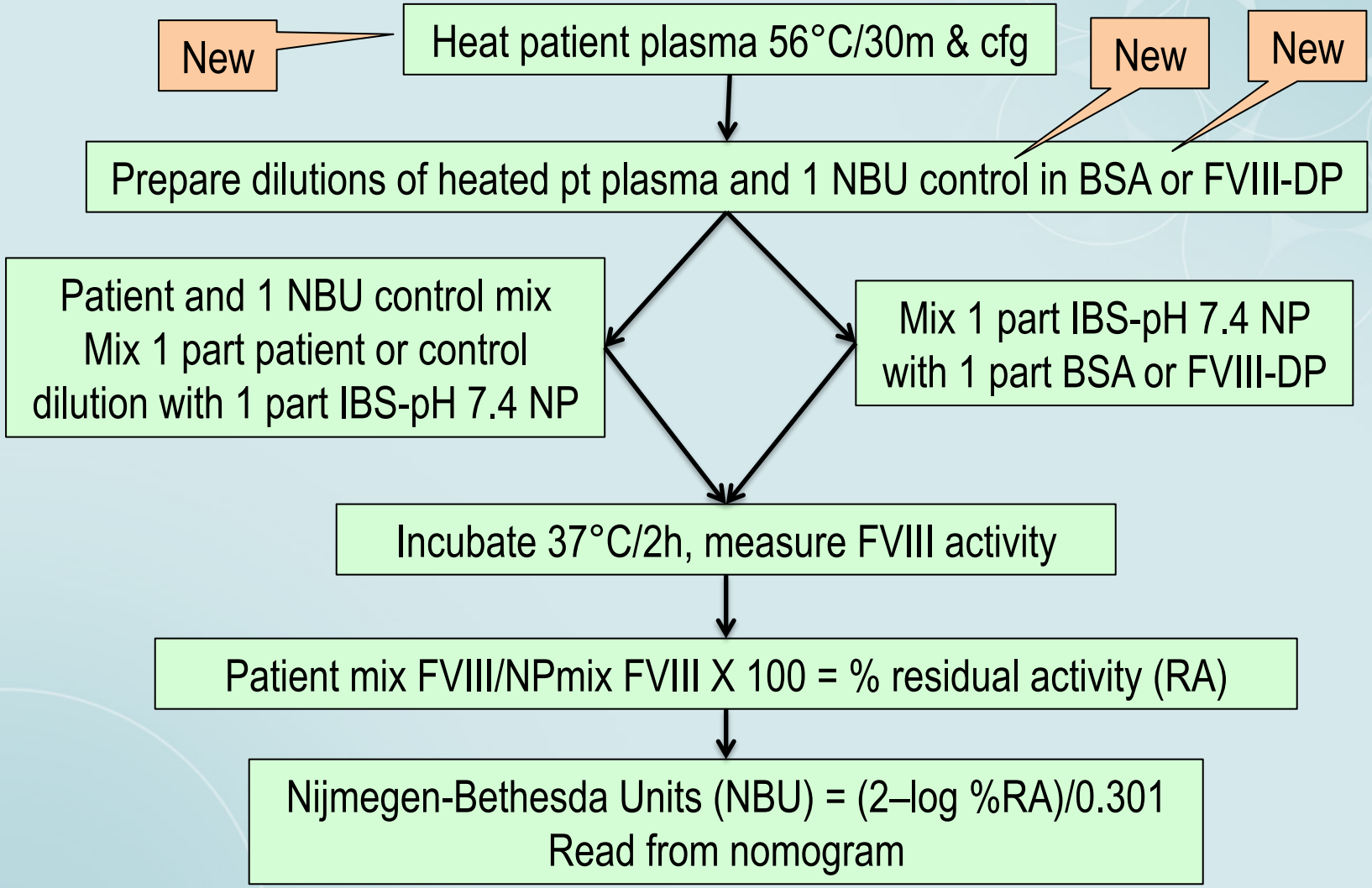
- Miller CH, Adcock DM. The need for standardization of factor inhibitor assays. 2016 THSNA Poster
- Verbruggen B, Novakova I, van Heerde W. Detecting and quantifying functional inhibitors in haemostasis. In: Kitchen S, Olson JD, Preston FE (eds) Quality in laboratory haemostasis and thrombosis, 2009. Blackwell, Oxford

CDC-modified Nijmegen Bethesda Assay

1. Heat patient plasma 56°C for 30” and centrifuge
2. Serially dilute heated patient plasma in BSA or FVIII-DP
3. Serially dilute unheated commercial 1.0 NBU-positive control in BSA or FVIII-DP
4. Mix dilutions 1:1 with IBS-pH 7.4 NP
5. Incubate 37°C 2h, perform PTT-based FVIII assay
6. Convert residual FVIII activity to NBU/mL using NBA nomogram and multiply the NBU/mL value by the dilution factor

Miller CJ, Platt SJ, Rice AS, et al. Validation of Nijmegan-Bethesda assay modifications to allow inhibitor measurement during replacement therapy and facilitate inhibitor surveillance. J Thrombos Haemostas 2012;10:1055–61.

CDC Nijmegen Bethesda Assay (NBA)



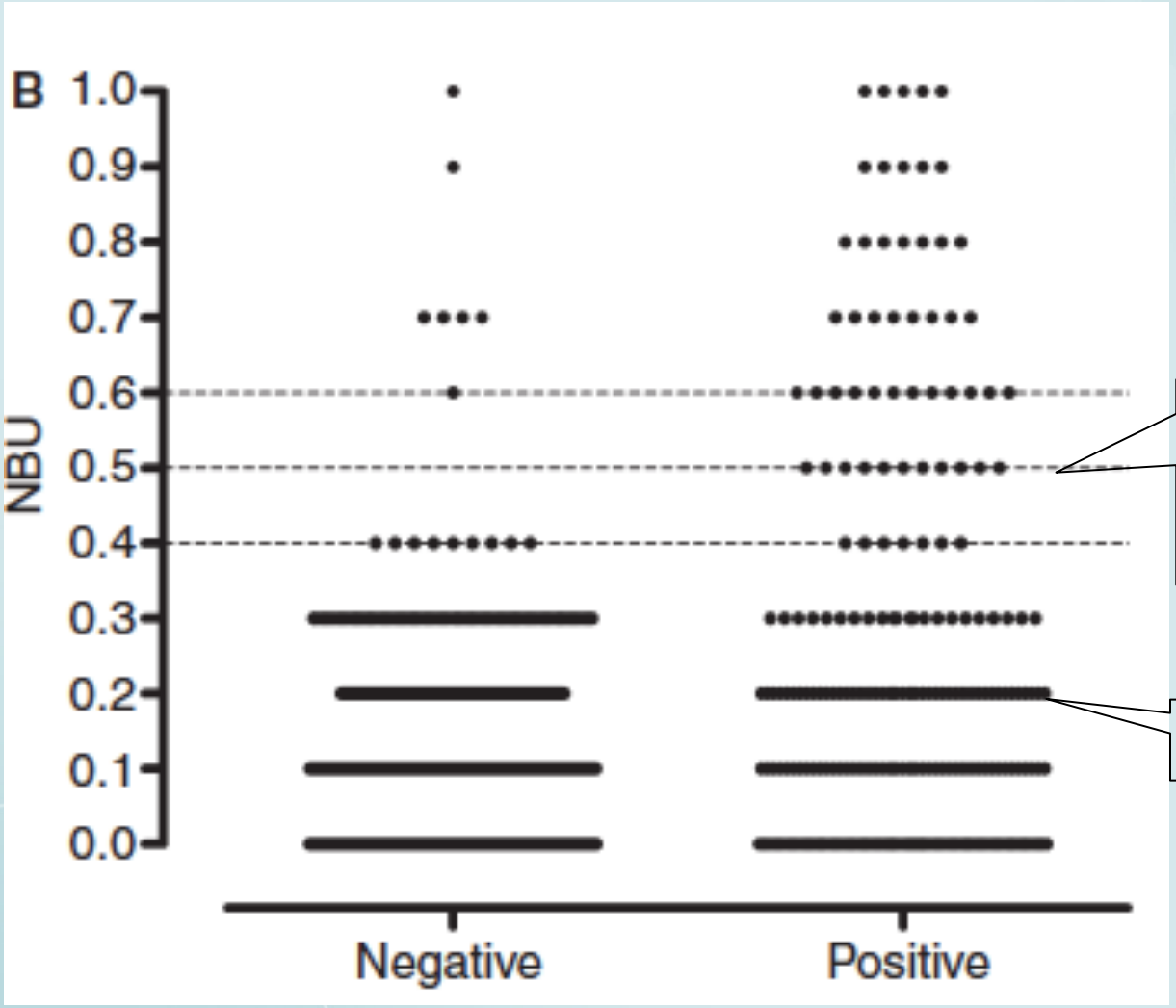
Courtesy of Connie Miller, PhD, CDC

The Value of Heating

- 126 (55%) of 228 severe HA samples had measurable FVIII.
  - All from patients treated with FVIII within 72 h of specimen collection
  - These had residual activity of >100 U/dL and a false inhibitor titer of 0
- Of 159 presumed inhibitor neg samples, 120 had unheated NBU of 0.
  - After heating, 45 (37.5%) remained 0
  - But 74 (61.7%) rose from 0 to 0.1–0.2 NBU, one rose from 0 to 0.7 NBU
- Of 30 documented inhibitor pos samples with results <0.5 NBU at enrollment, 5 (16.7%) rose to >0.5 NBU after heating.
- FVIII was in samples of patients infused within 24 h of collection.
  - in 15 severe patients, all had FVIII before heating and <1 U/dL after.
  - In 7 moderate patients, FVIII decreased to <1 U/dL after heating.

Miller CJ, Platt SJ, Rice AS, et al. Validation of Nijmegan-Bethesda assay modifications to allow inhibitor measurement during replacement therapy and facilitate inhibitor surveillance. J Thrombos Haemostas 2012;10:1055–61.

NBA FVIII Inhibitor Limits



Pos inhibitor limit ≥ 0.5 misclassified fewer specimens than ≥ 0.6.

Limit of detection 0.2

Miller CH, Boylan B, Hemophilia Inhibitor Research Study Investigators. Limit of detection of the NBA for factor VIII inhibitors. 2016 THSNA Poster

Purpose of NBA When Bleeding

- If ≤5 NBU, use high-dose FVIII concentrate
- If >5 NBU, prothrombin complex concentrate (PCC, 1980)
  - $BaSO_4$ extracted human plasma; II, VII, IX, X: Proplex
  - *Activated* PCC: FEIBA, Autoplex
- FEIBA or Autoplex
  - 50 U/kg/12 h prophylactic
  - 70 U/kg/8 h in hemorrhage
  - Limit 200 U/kg/24 h to avoid DIC
  - No direct monitor: repeat NBA, perform generalized coag evaluation using PTT

Ludlam DA, Morrison AE, Kessler C. Treatment of acquired hemophilia. Semin Hematol 1994;31 (Sup 4) 16−19

FEIBA Alternatives: NovoSeven, Obizur

- NovoSeven (1999): rFVIIa: 90 μg/kg, 6 h FVII half-life
  - Binds cell-bound tissue factor, no DIC risk
  - No direct monitor: repeat NBA, perform generalized coag evaluation using PTT
- Obizur (2014): recombinant porcine sequence FVIII
  - Indication: acquired hemophilia
  - Unlikely to cross-react with anti-FVIII IgGs
  - Initial dose 200 U/kg
  - Titrate dose and frequency based on FVIII recovery levels and clinical response
  - Monitor with chromogenic FVIII

NBA Purpose When Not Bleeding

- ≤5 NBU/mL in adults or ≤10 in peds, “low responders”
  - Use standard prophylactic FVIII concentrate Rx
- >10 NBU/mL, “high responder”
- If 5–10 NBU but consecutive Rx generates anamnestic pharmacokinetic response: “high responder”
- Immune tolerance FVIII therapy for high responders

Rivard GE, Rothschild C, Toll T, Achilles K. Immune tolerance induction in haemophilia A patients with inhibitors by treatment with recombinant factor VIII: a retrospective non-interventional study. Haemophilia. 2013;19:449–55

Immune Tolerance Induction (ITI) Rx

- Success rate 60%. Patient is good candidate when…
  - Historical peak < 200 NBU, < 10 BU at ITI initiation
  - < 2 years from inhibitor identification to ITI initiation
  - Age < 8 years; lower peak titer during ITI
- ITI: use 85–200 NBU FVIII/kg/day
  - Monitor using NBA; 0.6 NBU/mL = “negative”
  - 20% drop at 6 months Rx = satisfactory
  - Use maintenance dose throughout life
  - D/C if no response after 3 periods of 6 m

Arruda VR, Samelson-Jones BJ. Gene therapy for immune tolerance induction in hemophilia with inhibitors. J Thromb Haemost 2016; 14: 1121–34. Slide added 6-16-16

PrecisionBioLogic

New Factor Formulations

IDELVION®

Coagulation Factor IX (Recombinant), Albumin Fusion Protein

ELOCTATE™

[Antihemophilic Factor (Recombinant), Fc Fusion Protein]

Extended Half-life Factor VIII: Eloctate

- Recombinant B-domain deleted Fc fusion factor VIII
- Extended by Fc receptor and IgG recycling pathway
  - 96 HA adult males with >12 annual bleeds, 3–4 doses/week
  - rFVIIIFc half-life 19 h vs rFVIII 12h; 1.6–3.6 annual bleeds
- Prophylactic Rx interval up to 5 days versus 3–4 doses/week
- Monitor using clot-based FVIII assay with non-kaolin-based PTT
- Improved monitoring using chromogenic FVIII assay for all B-domain-deleted FVIII preparations

Biogen

- Shapiro AD, Ragni MV, Kulkarni R, et al. Recombinant factor VIII Fc fusion protein: extended-interval dosing maintains low bleeding rates and correlates with von Willebrand factor levels. J Thromb Haemost. 2014;12:1788–800.
- Mancuso ME, Mannucci PM. Fc-fusion technology and recombinant FVIII and FIX in the management of the hemophilias. Drug Des Devel Ther. 2014 28;365–71.

Additional rFVIII Preparations

Name	MFR		Progress
Bax 111	Baxter	rVWF (not rFVIII)	At FDA
Kovaltry	Bayer	Full-length rFVIII with no human or animal proteins, Reduced inhibitors, normal to slightly extended half-life	Approved
NovoEight	Novo Nordisk		
NuWiq	Octapharma		
Bay 94-9027	Bayer	Pegylated, plasma/albumin free, full-length rFVIII, up to 7.5 d frequency	At FDA
Bax 855	Baxter "Baxalta"	Pegylated plasma/albumin free, full-length rFVIII, 1.5 X Advate half-life	At FDA
rFVIII single-chain	CSL Behring	rFVIII covalently bonds VWF reduces clearance, extends half-life; no inhibitors	Phase II
ACE 910	Chugai & Genentech	Bispecific protein mimics FVIII cofactor, activates IX & X, bypasses inhibitors, SC 1/wk, generates no immune response	FDA breakthrough status

Extended Half-life Factor IX

- Recombinant Fc fusion factor IX Alprolix, FDA-approved 2014
  - rFIXFc half-life 70–80 h versus 24 h, 7–10 day intervals
  - Monitor using FIX assay with non-kaolin-based PTT
  - Chromogenic FIX valid but not available in the US
- Recombinant albumin fusion FIX Idelveon, FDA 3/4/2016
  - Patients <12 years old: 40–55 U/kg 7 day interval
  - Patients ≥12 years old: 25–40 U/kg 7 day interval
  - For ≥12 YO if controlled, go to 14-d interval at 50–75 U/kg

Biogen

CSL Behring
Biotherapies for Life®

PrecisionBioLogic

BioMarin BMN270 FVIII Transfer Trial

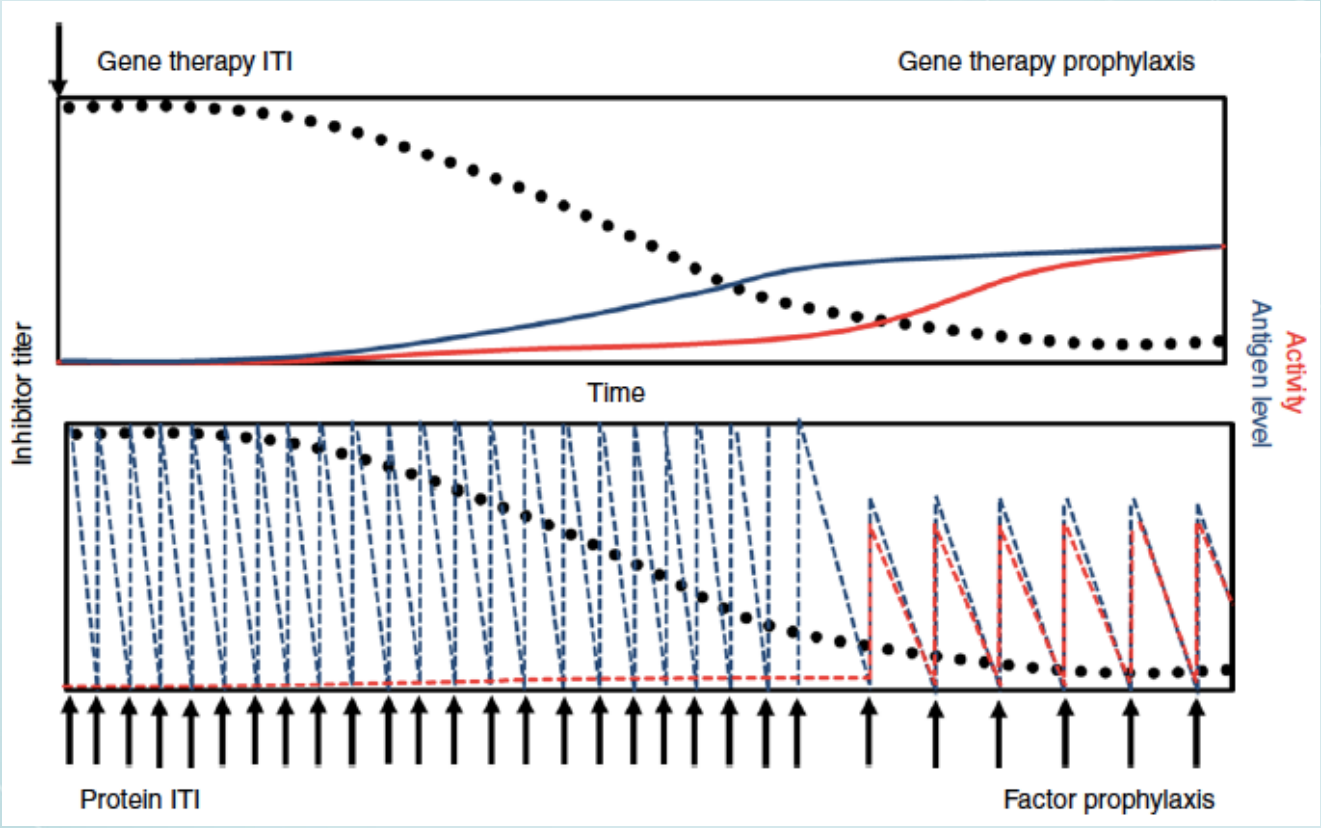
- B-domain-reduced FVIII gene with minimal glycosylation
- Vector optimization: adenovirus-associated vector “8” with adequate capacity for the FVIII gene
  - University College London
  - St. Jude’s Research Hospital
- Phase 1 & 2 trial completed
- Preparing phase III trial

- McIntosh J, Lenting PJ, Rosales C, et al. Therapeutic levels of FVIII following a single peripheral vein administration of rAAV vector encoding a novel human factor VIII variant. Blood. 2013 25;121:3335-44
- Ward NJ, Buckley SM, Waddington SN, et al. Codon optimization of human factor VIII cDNAs leads to high-level expression. Blood 2011;117:798–807.

BioMarin Phase 1 and 2 Results 4/20/16: 8 Severe Hemophilics

Dose	Week	%	Outcome
$6X10^{12}$ vg/kg	20	<1	Severe
$2X10^{13}$ vg/kg	16	2	Moderate
$6X10^{13}$ vector genomes/kg	16	57	Normal
	8	60	Normal
	7	8	Mild
	7	4	Moderate
	6	21	Mild
	5	10	Mild
Prednisolone controls liver toxicity as measured by ALT			

Gene Transfer Therapy May Reduce Inhibitor Formation: Animal Models



Nichols TC, Hough C, Agerso H, Ezban M, Lillicrap D. Canine models of inherited bleeding disorders in the development of coagulation assays, novel protein replacement and gene therapies. J Thromb Haemost 2016; 14: 894–905. Slide added 6-16-16

Target Antithrombin to Decrease Inhibition

- Silencing RNA (siRNA): synthetic RNA complementary to mRNA sequence, blocks mRNA translation
- siRNA-AT3 binds antithrombin mRNA and silences hepatic antithrombin production
- siRNA can be produced against any gene product: first accomplished in petunias

- Napoli C et al, Plant Cell , 1990; Novina CD, Sharp PA, Nature 2004
- Margaret Ragni, MD, MPH, University of Pittsburgh, THSNA, Chicago 4/14/16

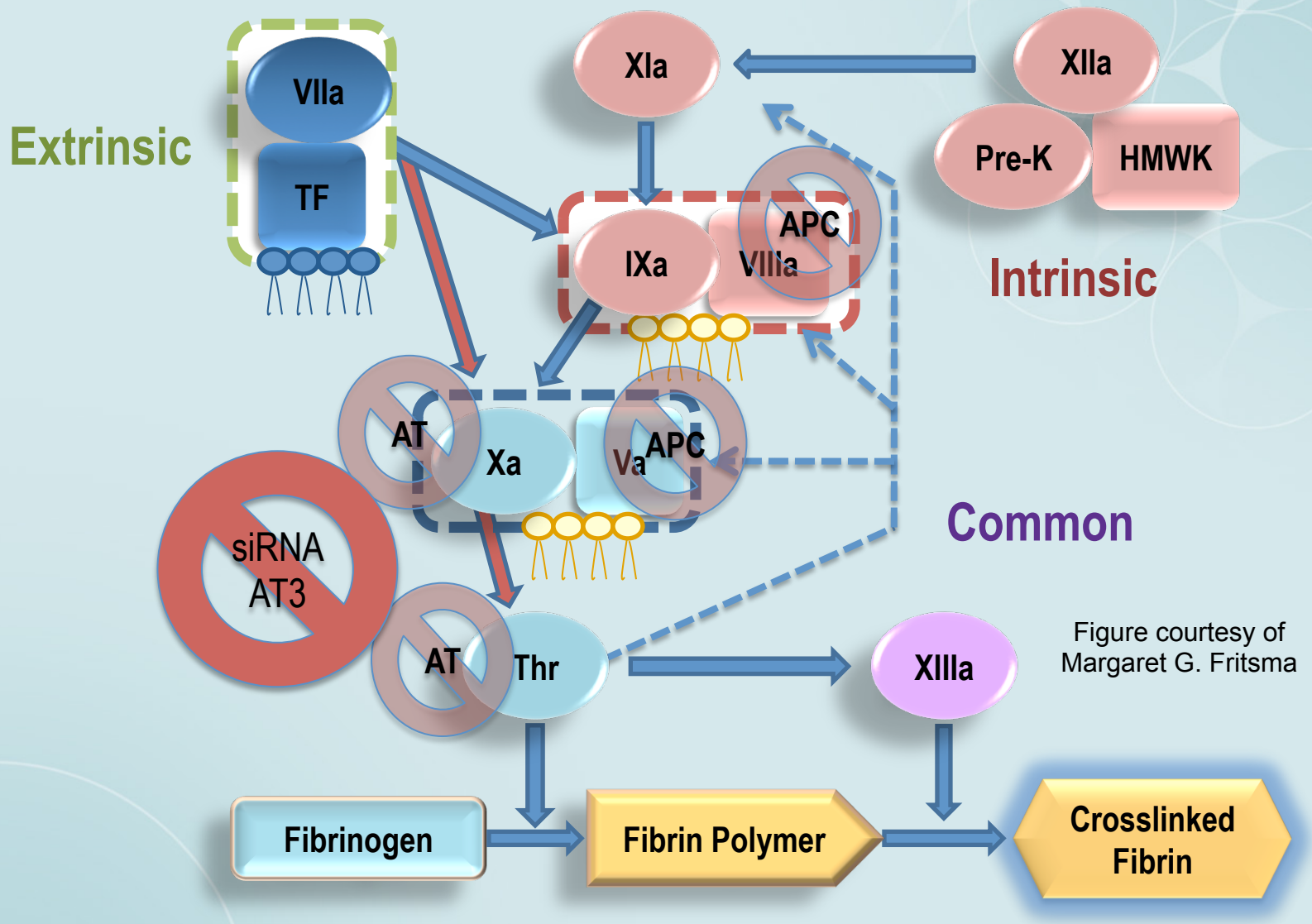
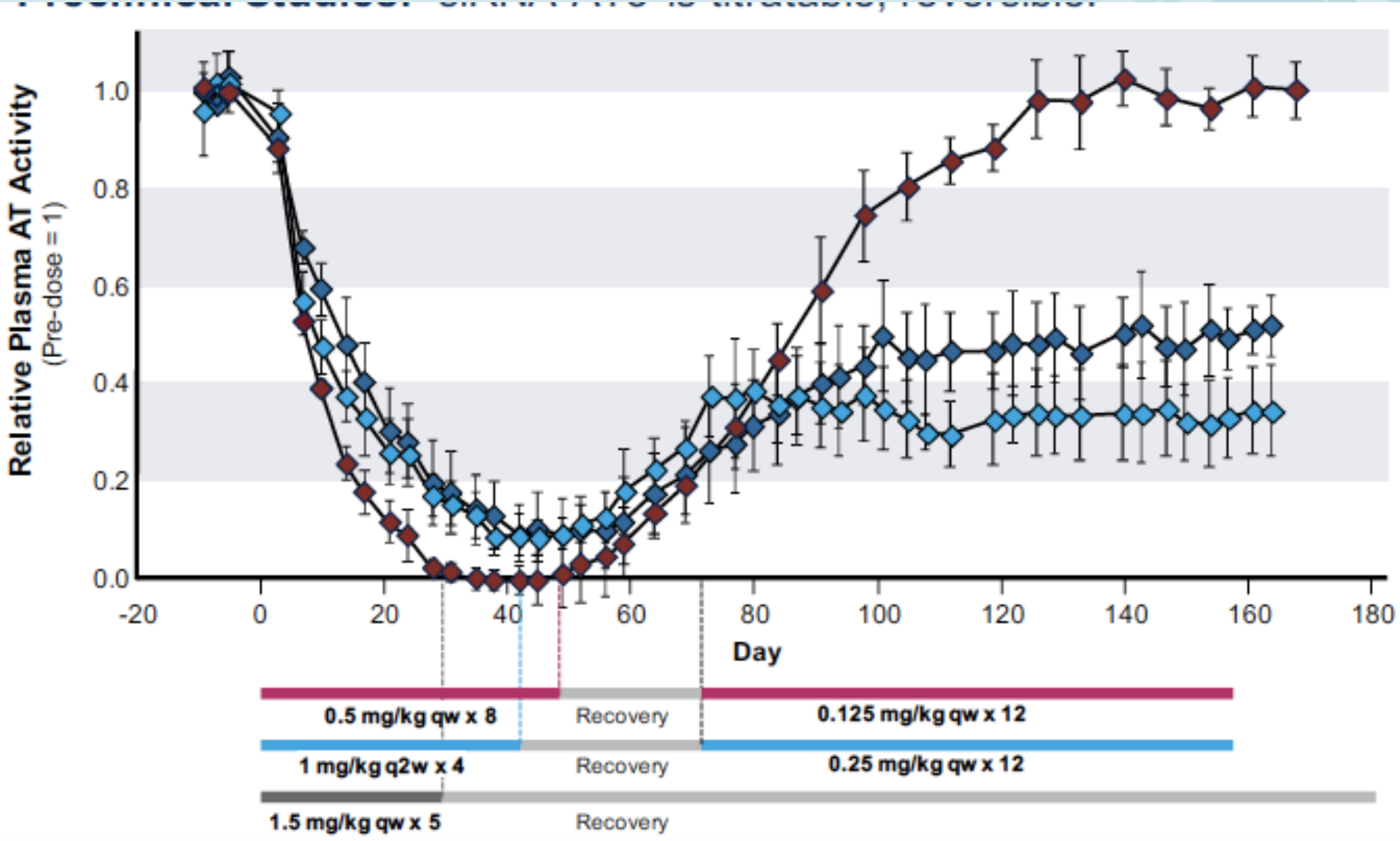
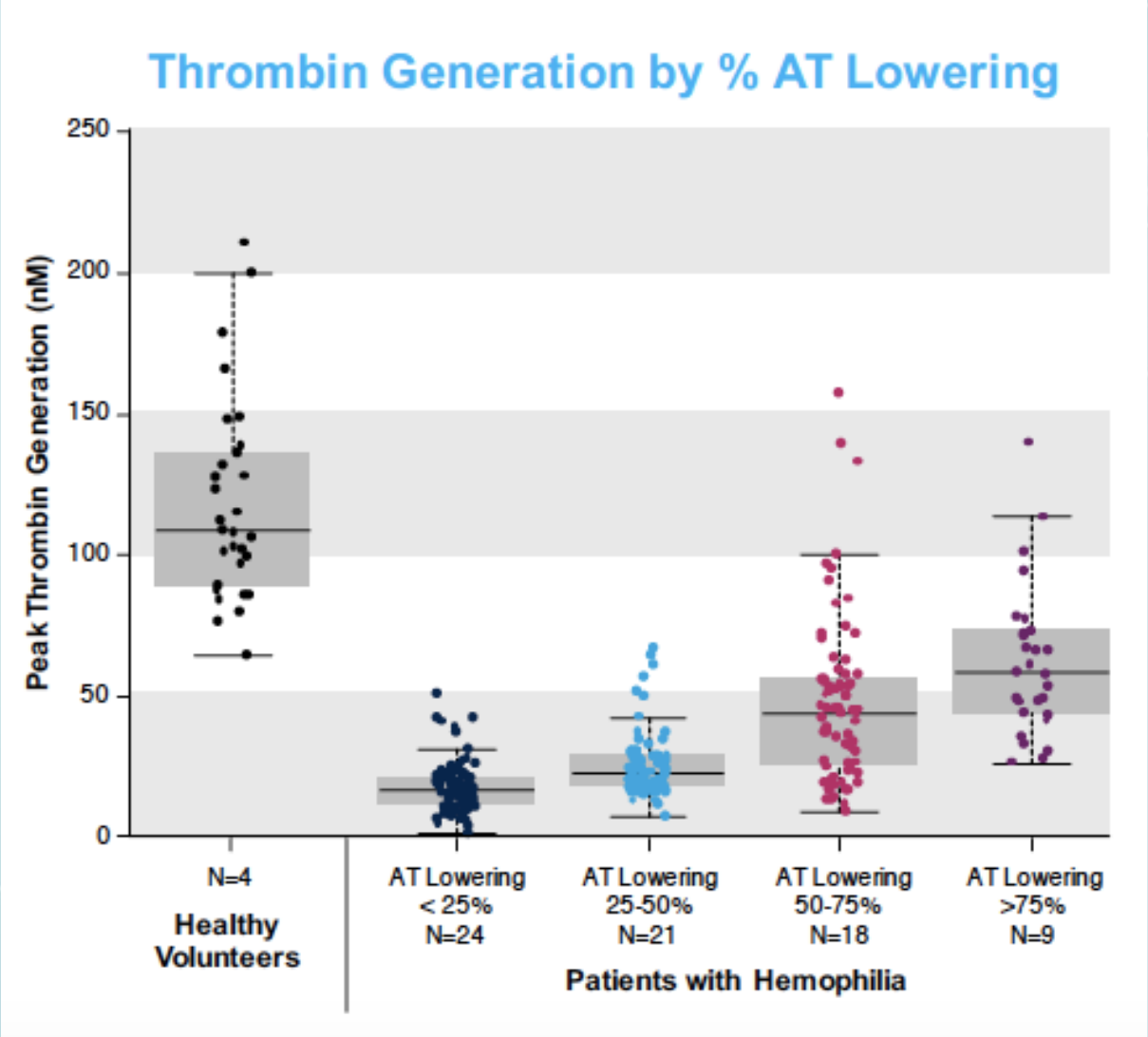


Figure courtesy of
Margaret G. Fritsma

siRNA AT3 in Primates

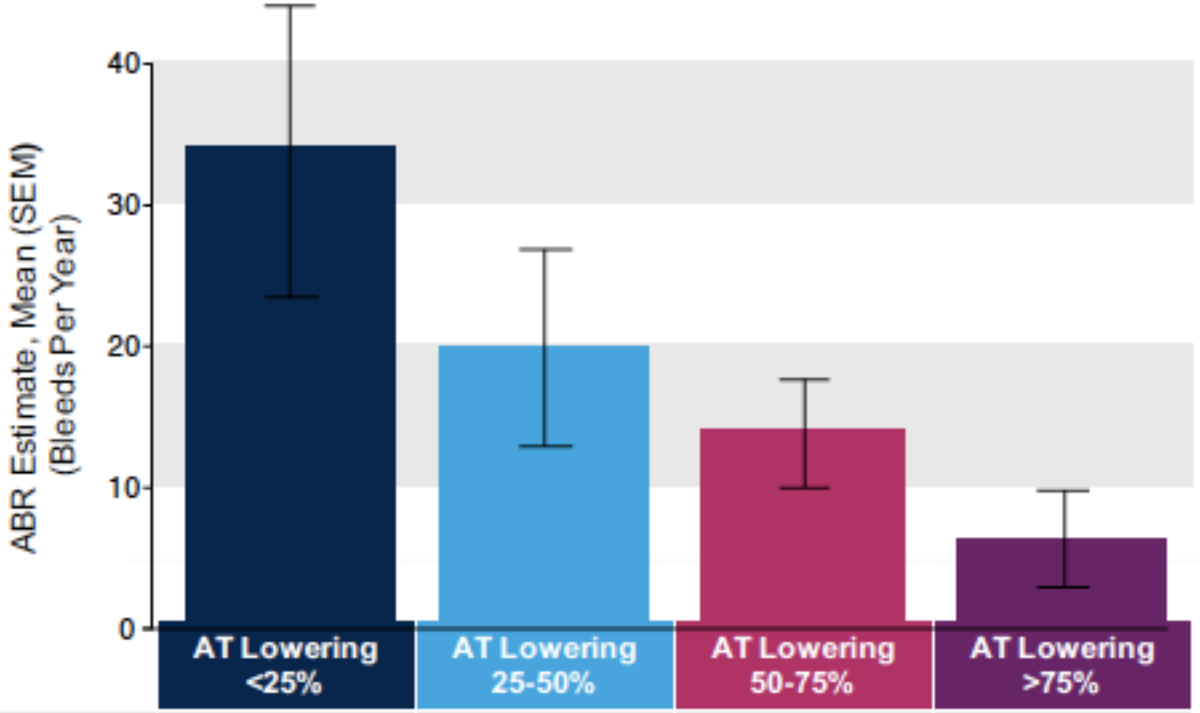


siRNA AT3 in Humans



Annual Bleed Rate in Humans Treated with siRNA AT3

Bleed Events by % AT Lowering



	AT Lowering <25%	AT Lowering 25-50%	AT Lowering 50-75%	AT Lowering >75%	
Patients	N=24	N=21	N=18	N=9	
ABR, Mean (SEM)	34 ± 10	20 ± 7	14 ± 4	6 ± 3	
ABR, Median	13	11	10	0	p<0.05

Conclusions: With AT lowering by quartile, <25% to >75%, there is reduction in ABR.

siRNA AT3 Phase I Safety

- No discontinuation
- Mild adverse events
  - Transient erythema and pain at injection site, resolved at 24 hours
  - Headache
- Bleeds treated with standard therapy
- No antibody formation
- Normal LFTs, CBC, PLTs, FG, EKG

Sehgal A et al, Nat Med 2015

Coagulation Factor Inhibitors

Bottom Line at the End (BLATE); The Participant…

- Explains the origin of anti-factor VIII (FVIII inhibitor)
- Detects FVIII inhibitors using factor assays and mixing studies
- Measures FVIII inhibitors using the Bethesda titer, Nijmegen Bethesda assay, chromogenic Bethesda assay, enzyme immunoassay and fluorescence immunoassay
- Describes coagulation factor bypass therapy to resolve bleeding in inhibitor patients
- Describes immune tolerance induction therapy
- Lists new factor concentrates designed to prevent inhibitor formation

PrecisionBioLogic

Thanks for listening!

QUESTIONS?

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