MAYO CLINIC

Laboratory Monitoring of New Hemostatic Agents (FVIII & FIX)

Rajiv K. Pruthi, M.B.B.S

Co-Director, Special Coagulation & DNA Diagnostic Laboratory
Director, Mayo Comprehensive Hemophilia Center
Department of Internal Medicine/Hematology
Dept of Laboratory Medicine & Pathology/Hematopathology
Mayo Clinic, Rochester, MN

pruthi.rajiv@mayo.edu

CP1245494-1

Disclosures

- **Relevant Financial Relationship(s)**
  - **None**
- **Off Label Usage**
  - **None**
  - **Chromogenic assays (USA attendees)**

pruthi.rajiv@mayo.edu

MAYO CLINIC

Learning Objectives: Participants will be able to

Know the newer hemostatic agents currently in clinical trials and/or (US) FDA approved

Recognize their molecular modifications

Recall the types of coagulation factor assays available to monitor hemostatic agents

Apply knowledge of assay limitations in the monitoring of the newer hemostatic agents

MAYO CLINIC

Case History: pharmacokinetics study

- 20 year old male severe hemophilia A
- Changed from Helixate FS to Afstyla
- Pharmacokinetic study (30 units/kg)

Last infusion 4 days ago	one stage
Pre infusion FVIII	<0.01 IU/mL^{-1} (<1%)
Post infusion FVIII	0.3 IU/mL^{-1} (30%)

MAYO CLINIC

Case History: pharmacokinetics study

- 20 year old male severe hemophilia A
- Changed from Helixate FS to Afstyla
- Pharmacokinetic study (30 units/kg)

Last infusion 4 days ago	one stage	chromogenic
Pre infusion FVIII	<0.01 IU/mL^{-1} (<1%)	<0.04 IU/mL^{-1} (<4%)
Post infusion FVIII	0.3 IU/mL^{-1} (30%)	0.6 IU/mL^{-1} (60%)

- 'Correction factor' applied:
  - One stage results x 2

MAYO CLINIC

New Hemostatic agents

Product (t ½ hr)	Company	Characteristics
Factor VIII		
rFVIII; (BAX 855) (14.7±3.8)	Baxter/Shire	Random peglyation
rFVIII; (Kovaltry) (14.3±3.7)	Bayer	HSP70, proper FVIII folding
rFVIII;(Afstyla) (14.2±3.7)	CSL Behring	Trunc B-domain single chain
Human-cl rhFVIII (17.1±11.2)	Octapharma	Expressed in human cell lines
rFVIII; (BAY 94-9027) (~19)	Bayer	Site specific glycopegylation
rFVIII;(N8-GP) (~19)	Novo Nordisk	Site specific glycopegylation
rFVIII (rFVIIIFc) (19.7±2.3)	Biogen Idec	IgG Fc fragment fusion
Factor IX		
Trenonacog alfa (24±6.0)	Emergent BioSolutions	rFIX (Thr-148 polymorphism)
Nonacog gamma (25.4±6.9)	Shire	CHO cell line
rFIX; (N9-GP) (~111)	Novo Nordisk	Site specific glycopegylation
rFIX (rFIXFc) (86.5±32.2)	Biogen Idec	IgG Fc fragment fusion
rFIX (r-FIX-FP) (104±18.7)	CSL Behring	Fusion with albumin

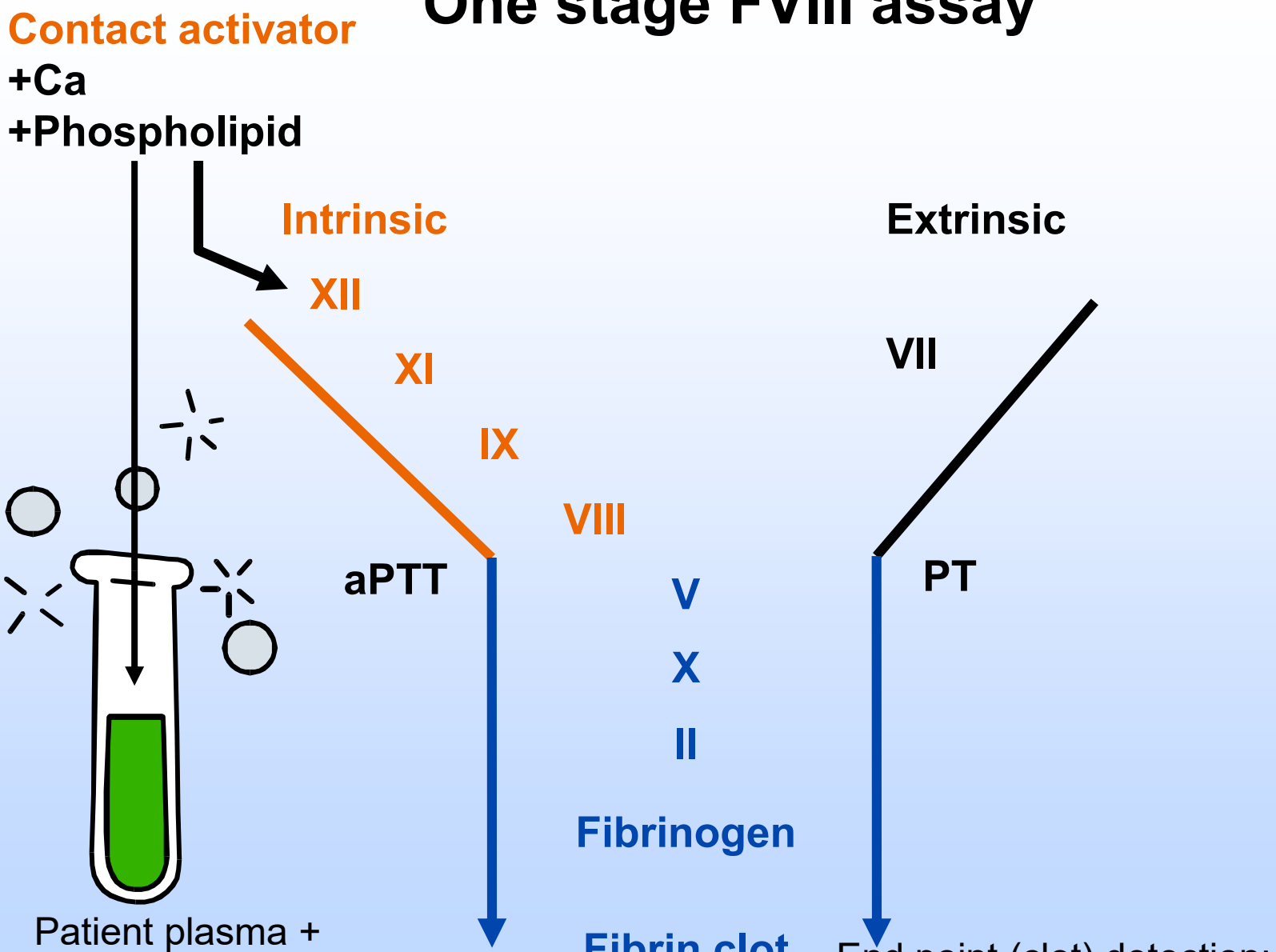
MAYO CLINIC

MAYO CLINIC

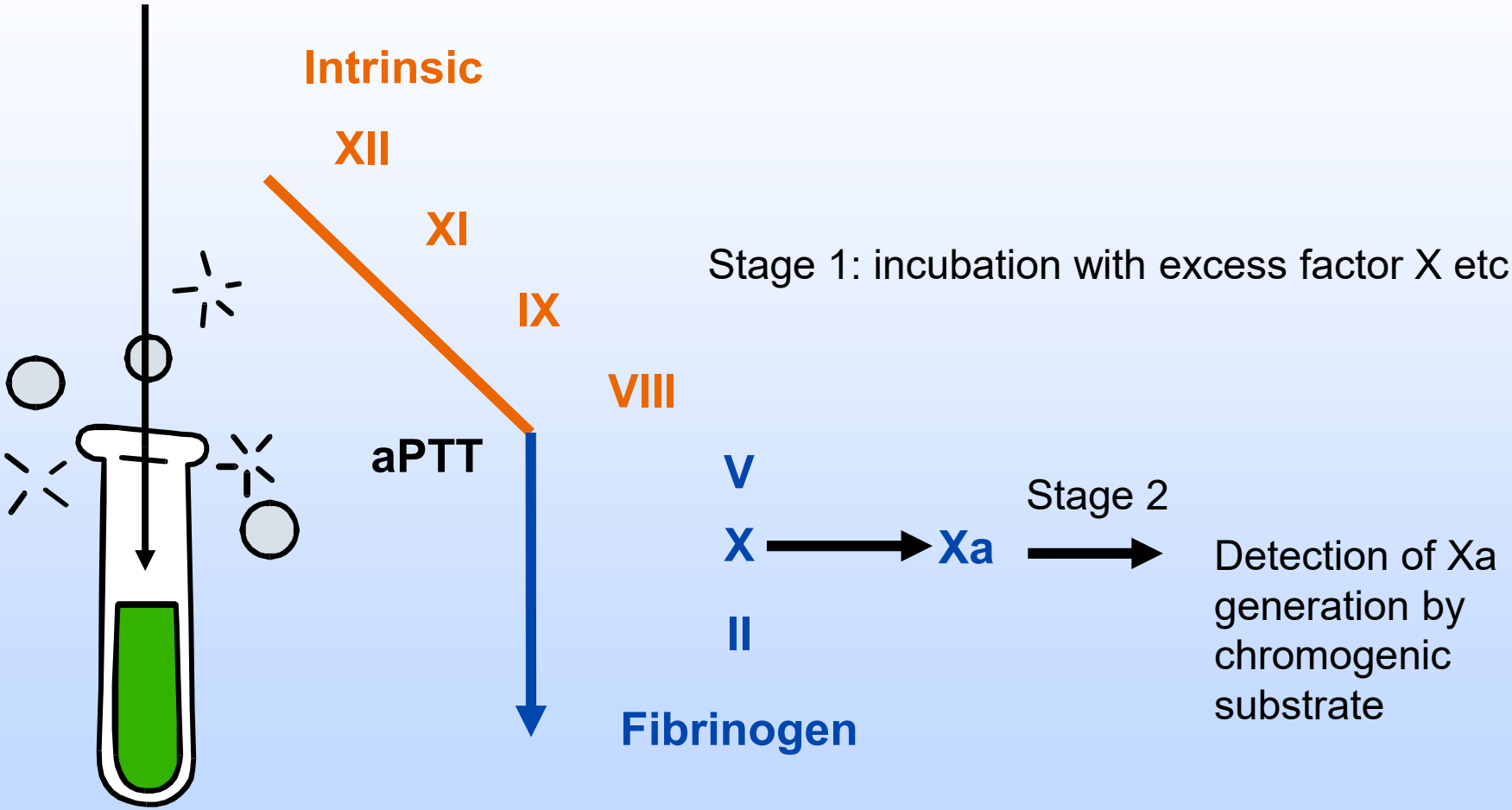
FVIII assays

One stage
Two stage
Chromogenic

One stage FVIII assay



Chromogenic FVIII assay



Factors influencing factor assays: contact activators, phospholipid & calibrators

Activator	Phospholipid (PL)	Calibrators	Instrumentation
Ellagic acid based			
Ellagic acid	Synthetic		
Polyphenolic acid (ellagic acid like)	Cephalin	Plasma derived standards	
Silica based			Mechanical methods
Colloidal silica	Soya	Chromogenic standards	
Micronized silica	Vegetable/Plant		Optical methods
Silica dioxide	Bovine	Concentrate specific standards	
Sulfatides and silica	Rabbit brain		
Kaolin	Porcine and chicken		

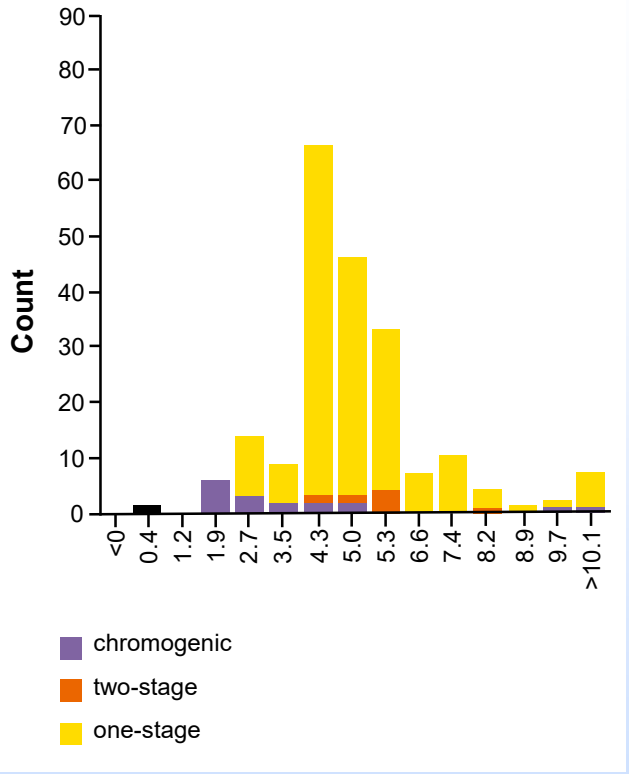
MAYO CLINIC

MAYO CLINIC

Current status of FVIII assays: data from proficiency surveys

Current Status: Variety of FVIII Assays Being Used (ECAT 2013)

	n	Assigned value	CV (%)	range	Your result	Z-score
Total Group	206	5	28.4	0 -83	4	-0.62
Chromogenic assay	18	3	50.2	0-12		
Chromogenix Coamatic Factor VIII	9	2		0-12		
Chromogenix Coatest Factor VIII	2	4		4-4		
Hyphen Biomed. Biophen Factor VIII	2	4		3-4		
Siemens Factor VIII	5	4		2-5		
two-stage clotting assay	7	6		5-8		
Il Hemosil SynthASil	2	5		5-6		
Siemens Actin FS	1	6				
Siemens Pathromtin SL	2	5		5-6		
Stago/Roche Cephalin/Kaolin/CK Prest	1	6				
Tcoag TriniClot APTT S	1	8				
one-stage clotting assay	181	5	26.2	3-83	4	-0.72
Erilyd Kaolin	1	5				
Hemoliance Synthasil APTT	1	5				
IL HemosiL APTT-SP liquid silica	12	5	19.1	4-6		
IL HemosiL SynthASil	37	6	21.7	4-9	4	-1.28
Other	2	4		4-4		
Pacific Hemostasis APTT-LS	1	3				
Siemens Actin	1	3				
Siemens Actin FS	20	5	24.0	3-10		
Siemens Actin FSL	15	5	26.1	3-83		
Siemens Pathromtin SL	22	4	25.0	3-10		
Stago/Roche Cephalin/Kaolin/CK Prest	27	4	21.5	3-7		
Stago/Roche Cephascreen	5	6		4-25		
Stago/Roche PTT (automate)/STA APTT	24	5	21.3	4-20		
Stago/Roche PTT-LA	2	5		5-5		
Tcoag Automated APTT	2	6		5-7		
Tcoag MDA Platelin LS	1	3				



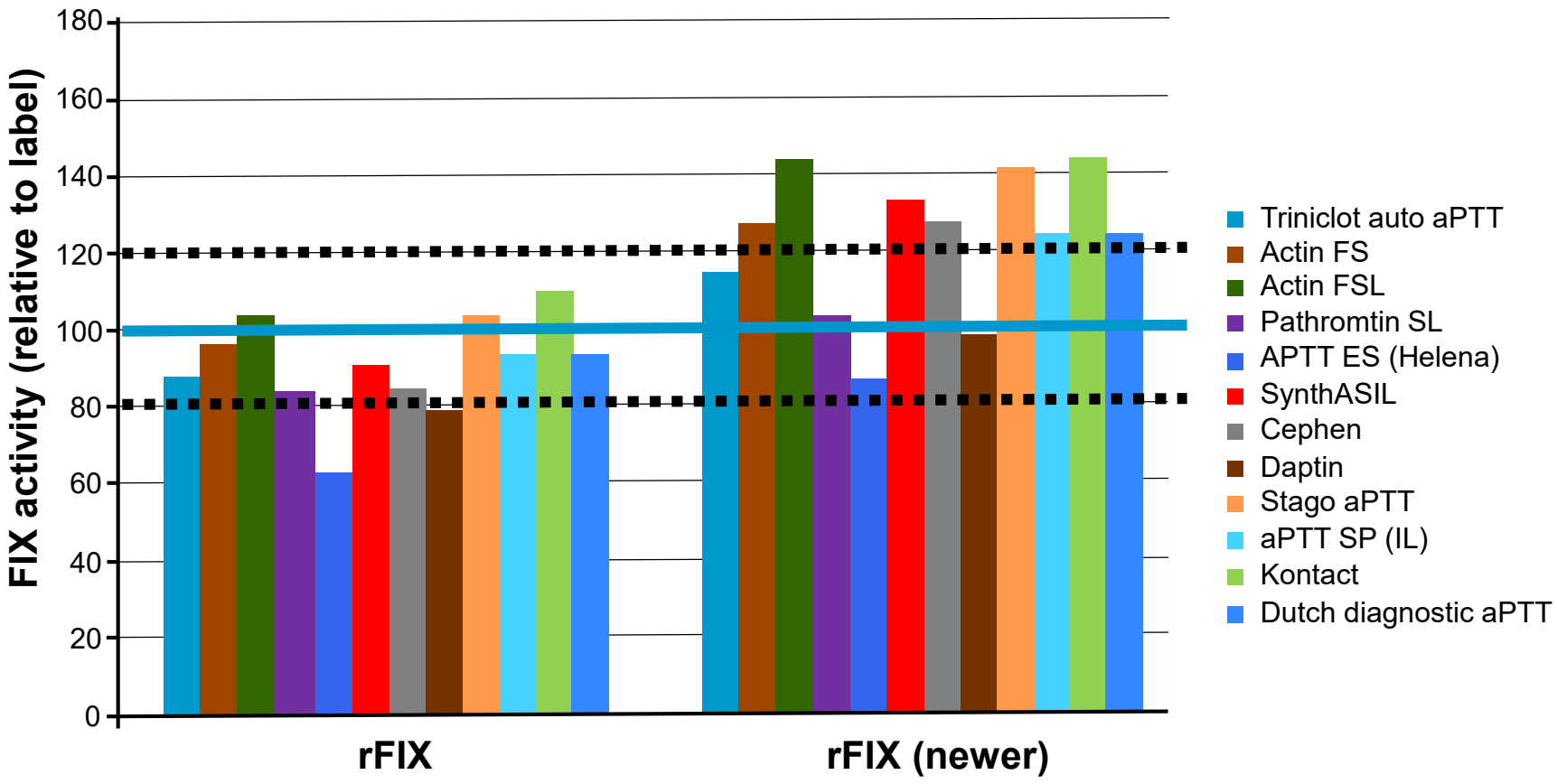
ECAT 2013 proficiency testing sample

MAYO CLINIC

MAYO CLINIC

Influence of reagent on non-modified concentrates

Assignment of rFIX Potency Varies with aPTT Reagent Used (No Modification)



Gritsch H, et al. Poster. GTH 2014.

MAYO CLINIC

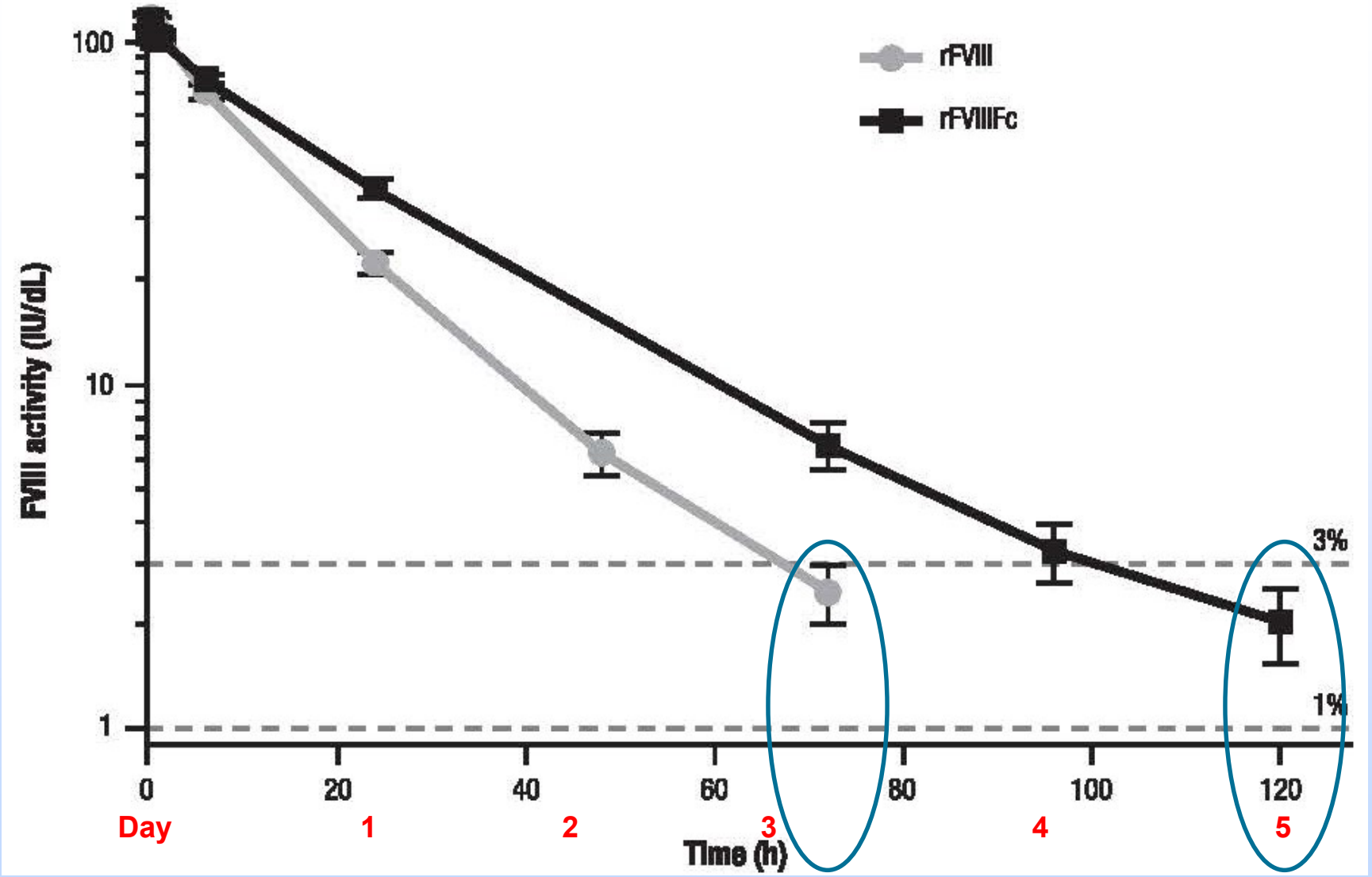
Take home message #1:

Variable reagent/instrument combinations & individual laboratory practices result in variable results of native factor assays and assays of (non-modified) concentrates

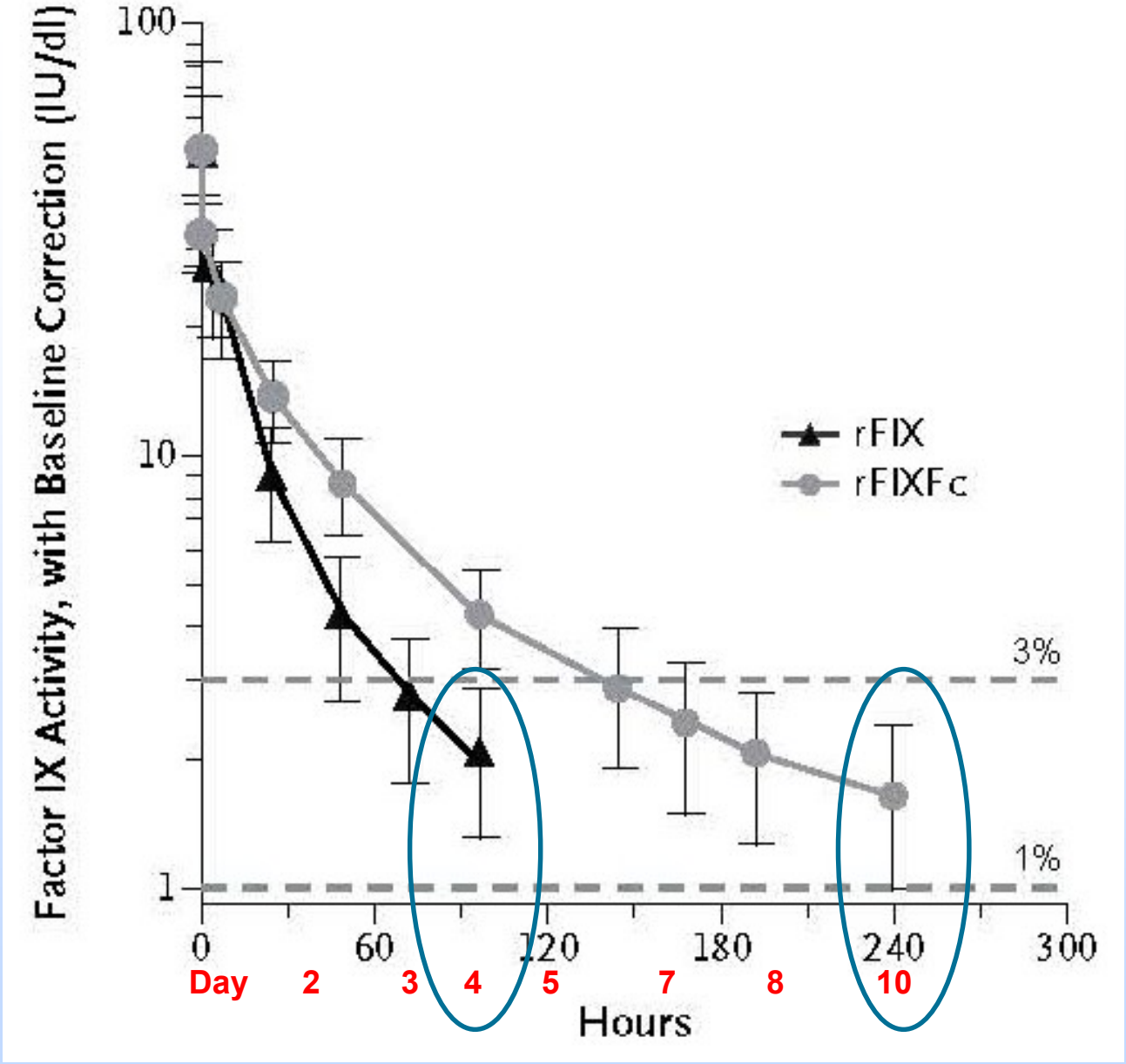
New Hemostatic agents

Product (t ½ hr)	Company	Characteristics
Factor VIII		
rFVIII; (BAX 855) (14.7±3.8)	Baxter/Shire	Random peglyation
rFVIII; (Kovaltry) (14.3±3.7)	Bayer	HSP70, proper FVIII folding
rFVIII;(Afstyla) (14.2±3.7)	CSL Behring	Trunc B-domain single chain
Human-cl rhFVIII (17.1±11.2)	Octapharma	Expressed in human cell lines
rFVIII; (BAY 94-9027) (~19)	Bayer	Site specific glycopegylation
rFVIII;(N8-GP) (~19)	Novo Nordisk	Site specific glycopegylation
rFVIII (rFVIIIFc) (19.7±2.3)	Biogen Idec	IgG Fc fragment fusion
Factor IX		
Trenonacog alfa (24±6.0)	Emergent BioSolutions	rFIX (Thr-148 polymorphism)
Nonacog gamma (25.4±6.9)	Shire	CHO cell line
rFIX; (N9-GP) (~111)	Novo Nordisk	Site specific glycopegylation
rFIX (rFIXFc) (86.5±32.2)	Biogen Idec	IgG Fc fragment fusion
rFIX (r-FIX-FP) (104±18.7)	CSL Behring	Fusion with albumin

MAYO CLINIC



Mahlangu et al Blood 2014



Powell et al NEJM 2014

MAYO CLINIC

New Hemostatic agents

Product (t ½ hr)		Assays	
Factor VIII			
rFVIII; (BAX 855)	(14.7±3.8)	One stage (any activator).	
rFVIII; (BAY 94-9027)	(~19)	One stage: Ellagic acid based activators. Chromogenic	
rFVIII;(N8-GP)	(~19)		
rFVIII (rFVIIIFc)	(19.7±2.3)	One stage and/or chromogenic	
rFVIII (Kovaltry)	(14.3±3.7)	One stage or chromogenic	
rFVIII (Afstyla)	(14.2±3.7)	Chromogenic. For one stage assay x 2	
Human-cl rhFVIII	(17.1±11.2)	One stage and/or chromogenic	
Factor IX			
rFIX; (N9-GP)	(~111)	One stage: Ellagic acid based activators. Chromogenic	
rFIX (rFIXFc)	(86.5±32.2)	One stage assay (kaolin activators under estimates)	
Trenonacog alfa	(24±6.0)	Emergent BioSolutions	rFIX (Thr-148 polymorphism)
Nonacog gamma	(25.4±6.9)	Shire	CHO cell line
rFIX (r-FIX-FP)	(104±18.7)	One stage assay (kaolin activators under estimates)	

Long acting factor concentrates

Site specific glycopegylation	Random peglyation	Fusion with Fc fragment of IgG	Fusion with albumin
rFVIII (N8-GP) [Novo Nordisk]	rFVIII (BAX 855) [Baxter] Adynovate	rFIX (rFIXFc) [Biogen Idec]	rFIX (r-FIX-FP) [CSL Behring]
rFVIII (BAY 94-9027) Bayer		rFVIII (rFVIIIFc) [Biogen Idec]	
rFIX (N9-GP) [Novo Nordisk]			

Reasonable recovery of non-PEGylated FVIII with 1-stage assays (100±25%)

APTT reagent	Result in % of target			
	Advate®	Turoctocog alfa	Haemate®	Kogenate®
SythAFAX	89±10	86±7.3	83±6.4	112±6.6
Actin FSL	104±6.7	95±4.2	96±8.5	128±13
STA Cephascreen	114±11	103±7.7	101±8.6	133±13
STA CK Prest	94±8.1	95±11	84±10	106±9.5
SythASIL	100±8.1	97±10	94±8.2	106±6.8
Pathromtin SL	101±6.4	94±6.0	93±4.4	115±10
APTT-SP liquid	111±9.5	103±6.9	101±7.2	128±16
STA PTT automate	114±11	96±4.0	111±7.6	133±12

MAYO CLINIC

Ellagic acid
Kaolin
Silica based

Krogh-Meibom et al ISTH 2013

With one stage FVIII assay apparent N8-GP recovery is dependent on reagent used

Krogh-Meibom et al ISTH 2013

APTT regent	Contact activator	Phospholipid	Result in % of target
Actin FSL	Ellagic acid	Soya & rabbit brain	100±25%
Actin FS	Ellagic acid	Soya	100±25%
SynthAFAX	Ellagic acid	Synthetic	100±25%
Cephascreen	Polyphenolic acid	Cephalin	100±25%
Phospholin FS	Ellagic acid	Soya	100±25%
Dia PTT liquid	Ellagic acid	Rabbit brain	100±25%
CK-Prest	Kaolin	Rabbit brain	100±25%
Dapptin TC	Sulphatides & silica	Purified mix	100±25%
SynthASIL	Colloidal silica	Synthetic	100±25%
aPTT HS	Silica dioxide	Porcine & chicken	<50%
Pathromtin SL	Silica dioxide	Vegetable	<50%
APTT-SP liquid	Colloidal silica	Synthetic	<50%
STA-PTT-A	Colloidal silica	Rabbit brain	<50%
Triniclot Aut PTT	Micronized silica	Rabbit brain	<50%
APTT lyoph.	Micronized silica	Bovine brain	<50%
Triniclot S	Silica	Rabbit brain	<50%

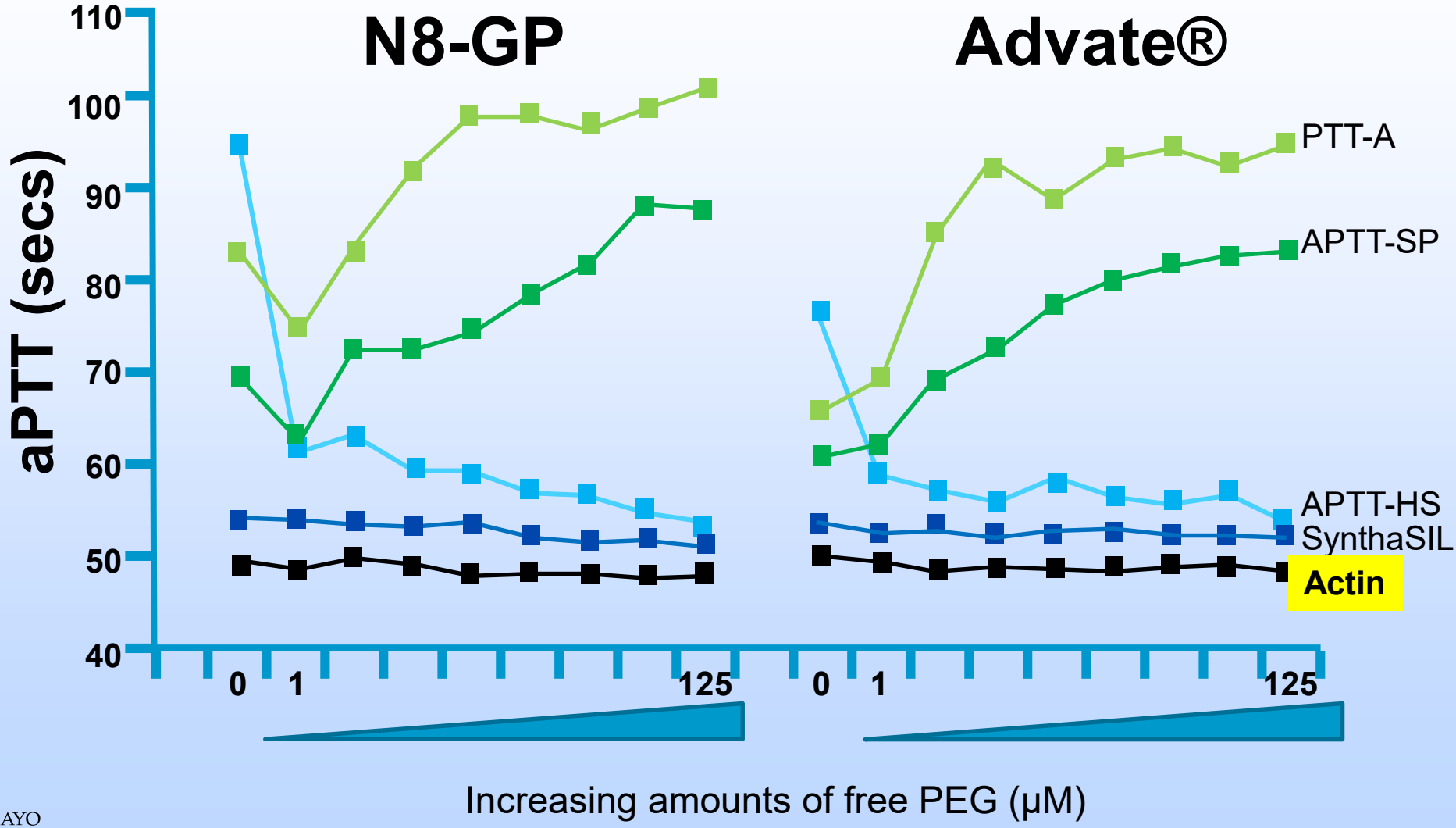
MAYO CLINIC

PEGyltation does not influence FVIII:C recovery in two-stage chromogenic assay

FVIII Conjugate	Specific activity
Turoctocog alfa	9600±740
N8-GP	11200±1050

Krogh-Meibom et al ISTH 2013

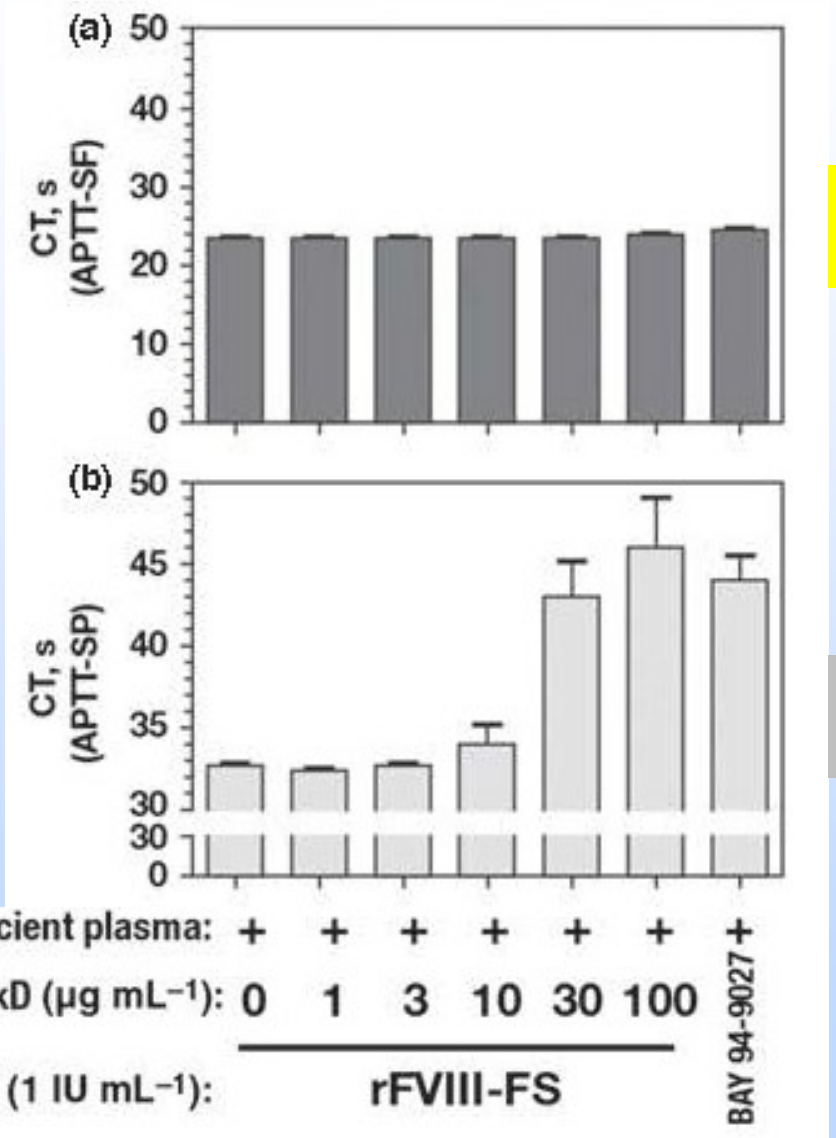
Free PEG affects APTT results in a reagent dependent manner



MAYO CLINIC

Krogh-Meibom et al ISTH 2013

Increasing free PEG affects silica based APTT regents (BAY 94-9027)



Ellagic acid

Silica

Gu, J-M et al Haemophilia 2014:1-8

MAYO CLINIC

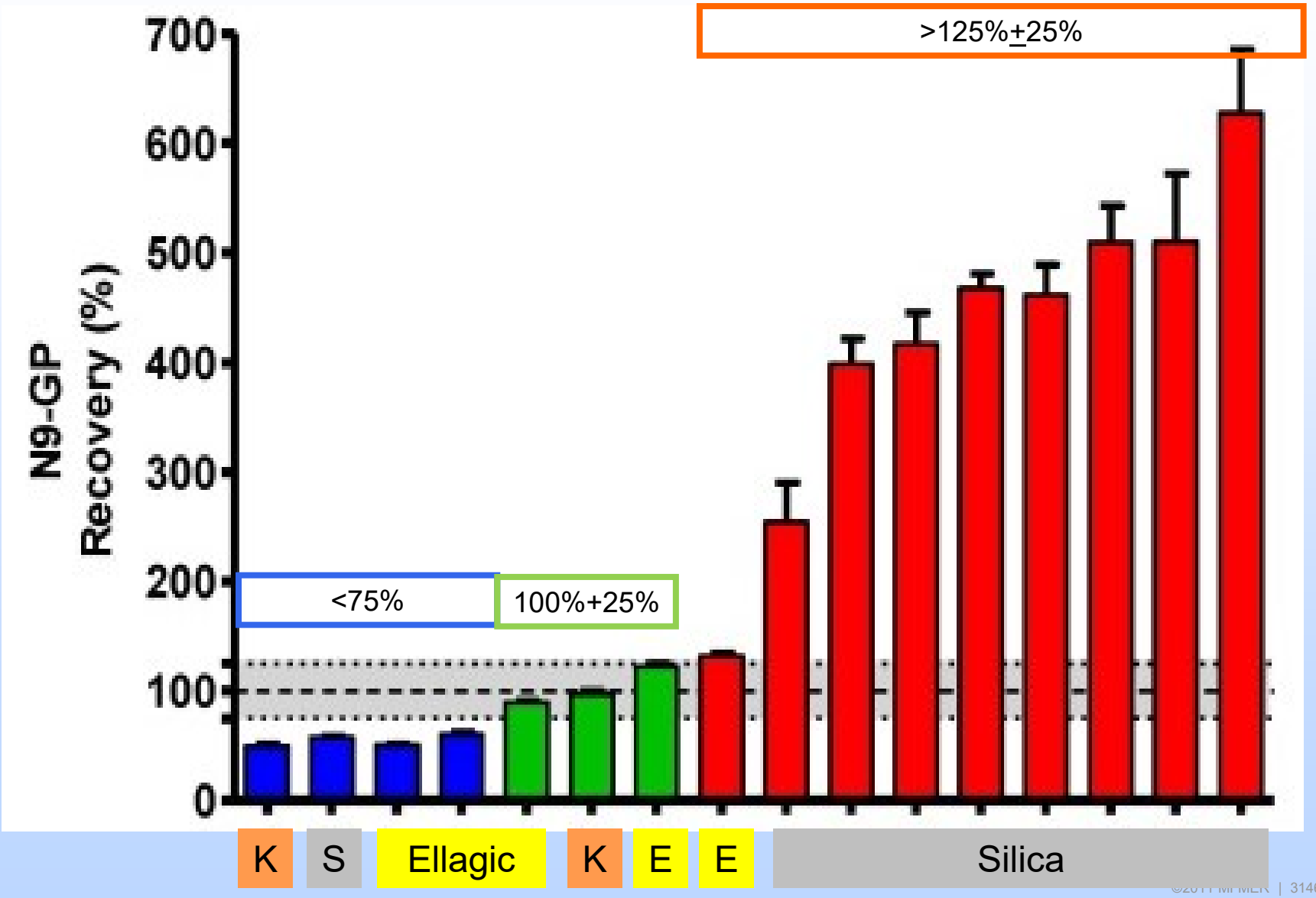
Take home message #2

Most ellagic acid & (selected silica) based reagents accurately estimate PEGylated FVIII levels.

Most silica based reagents underestimate PEGylated FVIII levels

N9-GP recovery varies with reagent

Holm PK et al ISTH 2013



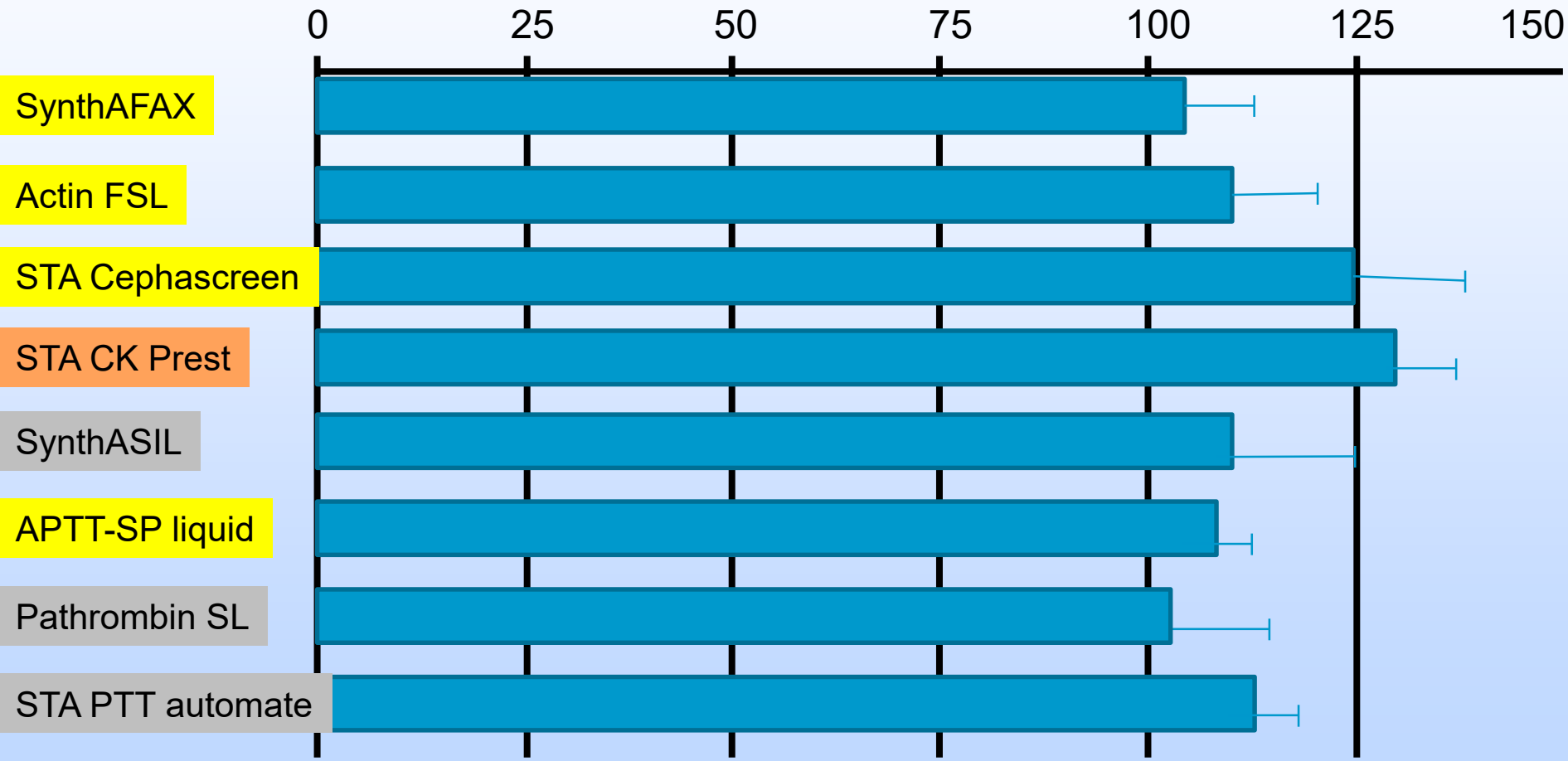
MAYO CLINIC

Take home message #3

Most ellagic acid, silica and kaolin based reagents resulted in under/over estimation of FIX

Chromogenic assays resulted in expected FVIII and FIX recovery (data not shown)

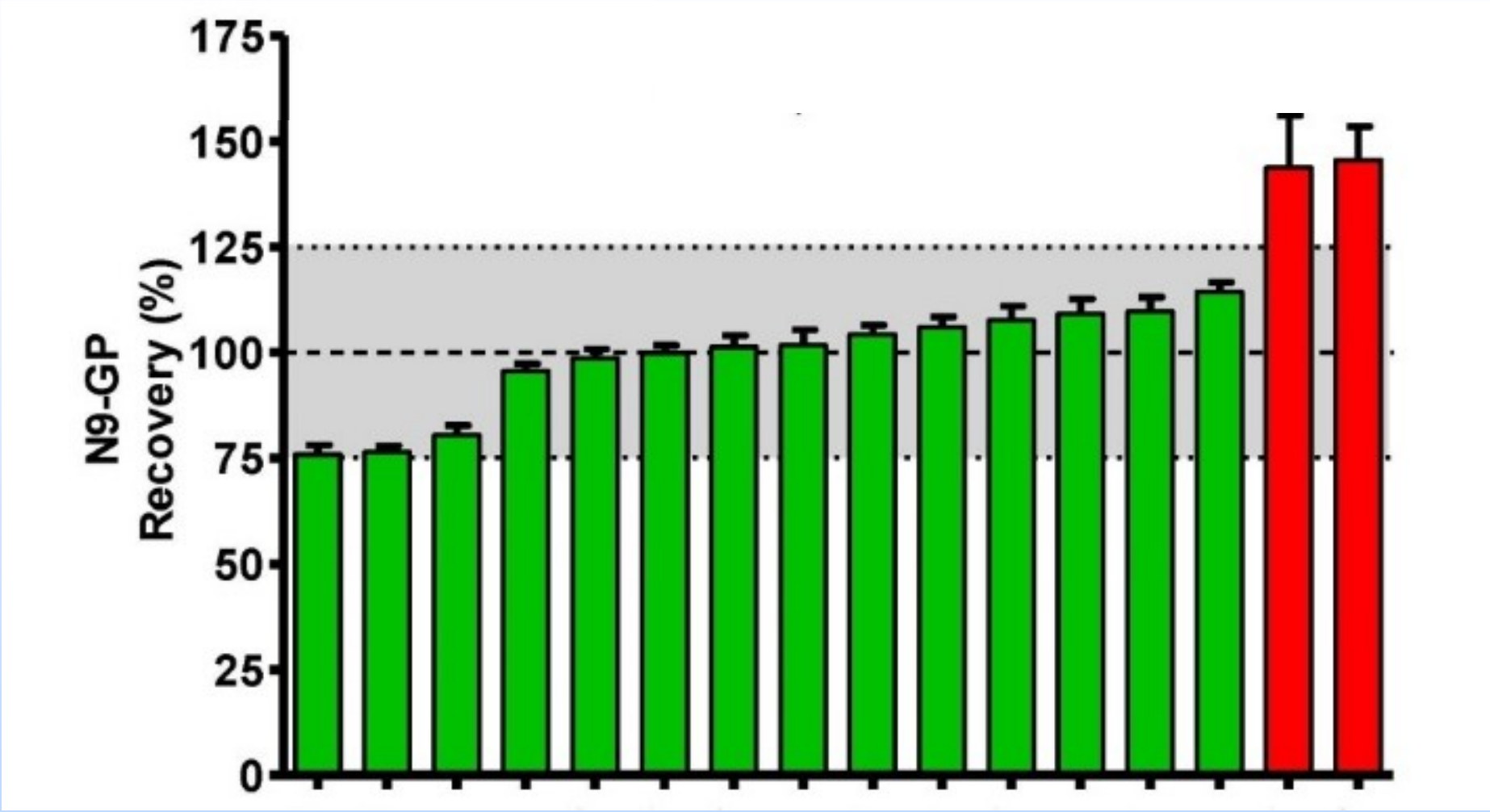
Use of N8-GP product as calibrator reduces reagent dependent variability



MAYO CLINIC

Krogh-Meibom et al ISTH 2013

Use of N9-GP standard corrects for reagent variability



Holm PK et al ISTH 2013

MAYO CLINIC

Data on other coagulation factor concentrates:

Also in response to a request by FDA, CSL reported the results of a field trial comparing the ChS and OS assays in the BLA application, section 5.3.1.4. (b) (4) laboratories (of (b) (4) requested) completed data entry (13 from the United States). Laboratories were supplied with spiked samples of (b) (4) for both Afstyla and a (b) (4) , to be assayed in multiple aliquots [(b) (4) the results from the ChS and OS assays]. The results showed a relatively (b) (4) relationship between values obtained by ChS or OS assays, with a suggested conversion factor of ChS activity = (b) (4) x OS activity.

What does the above excerpt represent?

A) Leaked email from Hillary Clinton's server

B) Donald Trumps Tax return

C) Leaked email from Edward Snowden

D) Pharma information from FDA website.

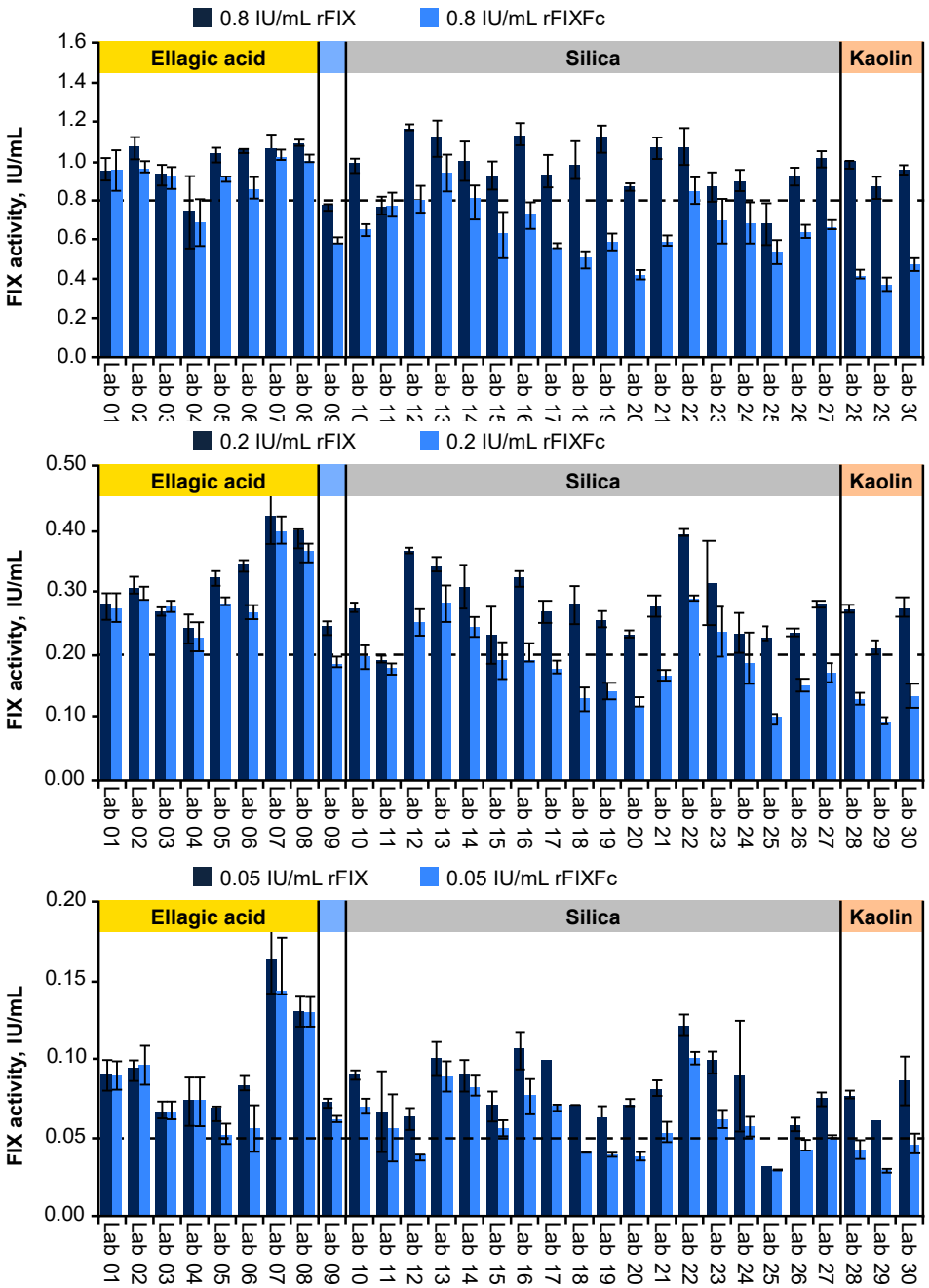
MAYO CLINIC

New Hemostatic agents

Product (t ½ hr)		Assays	
Factor VIII			
rFVIII; (BAX 855)	(14.7±3.8)	One stage (any activator).	
rFVIII; (BAY 94-9027)	(~19)	One stage: Ellagic acid based activators. Chromogenic	
rFVIII;(N8-GP)	(~19)		
rFVIII (rFVIIIFc)	(19.7±2.3)	One stage and/or chromogenic	
rFVIII (Kovaltry)	(14.3±3.7)	One stage or chromogenic	
rFVIII (Afstyla)	(14.2±3.7)	Chromogenic. For one stage assay x 2	
Human-cl rhFVIII	(17.1±11.2)	One stage and/or chromogenic	
Factor IX			
rFIX; (N9-GP)	(~111)	One stage: Ellagic acid based activators. Chromogenic	
rFIX (rFIXFc)	(86.5±32.2)	One stage assay (kaolin activators under estimates)	
Trenonacog alfa	(24±6.0)	Emergent BioSolutions	rFIX (Thr-148 polymorphism)
Nonacog gamma	(25.4±6.9)	Shire	CHO cell line
rFIX (r-FIX-FP)	(104±18.7)	One stage assay (kaolin activators under estimates)	

Reagent Effect on Various aPTT-based Assays(rFIXFc)

	Instrument	aPTT Reagent	Activator
Lab 1	Stage Evolution	Siemens Actin FS	Ellagic acid
Lab 2	Siemens CA7000	Siemens Actin FS	
Lab 3	Siemens BCS-XP	Siemens Actin FS	
Labs 4–8	Siemens BCS-XP	Siemens Actin FSL	
Lab 9	Stago Evolution	Stago Cephascreen	Polyphenols
Lab 10	Stago Destiny	Trinity Auto APTT	Silica
Lab 11	Trinity MDA-II	Trinity Auto APTT	
Lab 12	Siemens BCS-XP	Trinity Auto APTT	
Lab 13–15	IL ACL TOP	IL SynthASil	
Lab 16–21	Stago Evolution	Stago PTT-A	
Lab 22	IL ACL TOP	Stago PTT-A	
Lab 23	Siemens BCS-XP	Stago PTT-A	
Lab 24	IL ACL TOP	Pathromtin SL	
Lab 25	Siemens BCS-XP	IL Hemosil	
Lab 26	Trinity MDA-II	Tcoag Platelin L	
Lab 27	Trinity MDA 180	Tcoag Platelin L	
Labs 28–30	Stago Evolution	Stago CK-Prest	Kaolin



MAYO CLINIC

Sommer 2013

New Hemostatic agents

Product (t ½ hr)		Assays	
Factor VIII			
rFVIII; (BAX 855)	(14.7±3.8)	One stage (any activator).	
rFVIII; (BAY 94-9027)	(~19)	One stage: Ellagic acid based activators. Chromogenic	
rFVIII;(N8-GP)	(~19)		
rFVIII (rFVIIIFc)	(19.7±2.3)	One stage and/or chromogenic	
rFVIII (Kovaltry)	(14.3±3.7)	One stage or chromogenic	
rFVIII (Afstyla)	(14.2±3.7)	Chromogenic. For one stage assay x 2	
Human-cl rhFVIII	(17.1±11.2)	One stage and/or chromogenic	
Factor IX			
rFIX; (N9-GP)	(~111)	One stage: Ellagic acid based activators. Chromogenic	
rFIX (rFIXFc)	(86.5±32.2)	One stage assay (kaolin activators under estimates)	
Trenonacog alfa	(24±6.0)	Emergent BioSolutions	rFIX (Thr-148 polymorphism)
Nonacog gamma	(25.4±6.9)	Shire	CHO cell line
rFIX (r-FIX-FP)	(104±18.7)	One stage assay (kaolin activators under estimates)	

Conclusions/challenges/to do items:

- Optimal reagent(s) for monitoring of long acting factor concentrates vary with molecular modification of protein
- Hemophilia Centers
  - Be aware of assay limitations
  - Communicate with your coagulation laboratory
- Coagulation laboratories
  - Communicate with hemophilia centers
  - Validate performance characteristics of in-house reagents with newer concentrates
    - Published data, availability of spiked samples, proficiency testing samples etc
    - Introduction of newer reagents for concentrate specific assays (if feasible)
    - Participate in quality assurance surveys

MAYO CLINIC

Conclusions/challenges/to do items:

- Pharma and Diagnostics companies
  - Continue collaboration
  - Optimizing reagent choices
  - Make available spiked plasma samples for in-house optimization of assays
  -
- Organizations (NASCOLA, CLSI, CAP,ECAT others)
  - Educational/Quality assurance efforts

pruthi.rajiv@mayo.edu

MAYO CLINIC