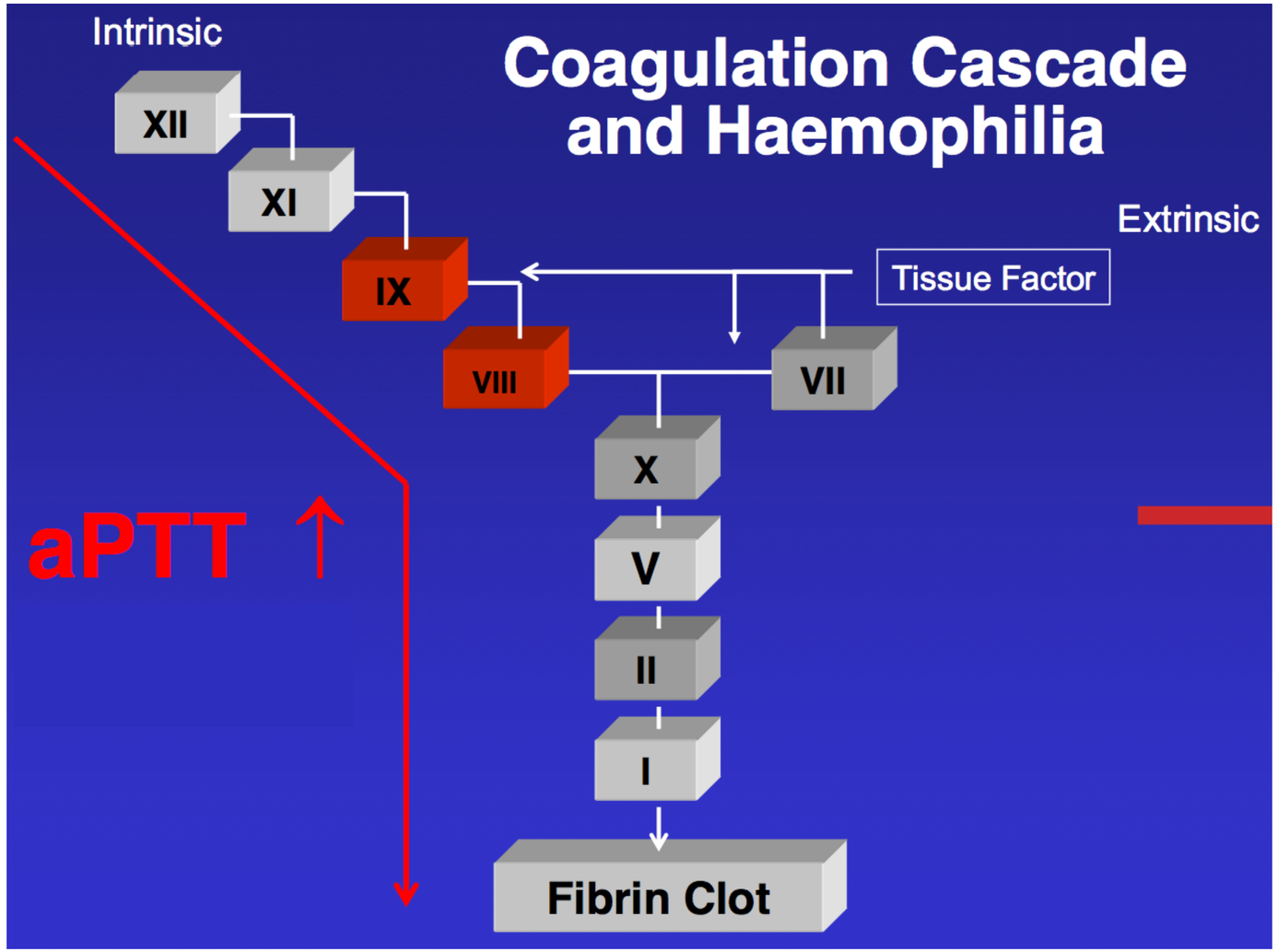
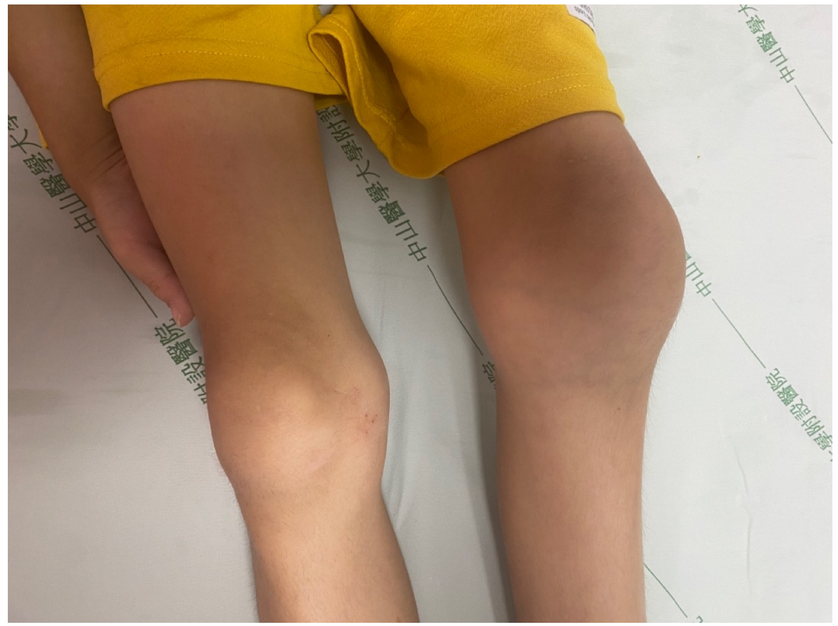
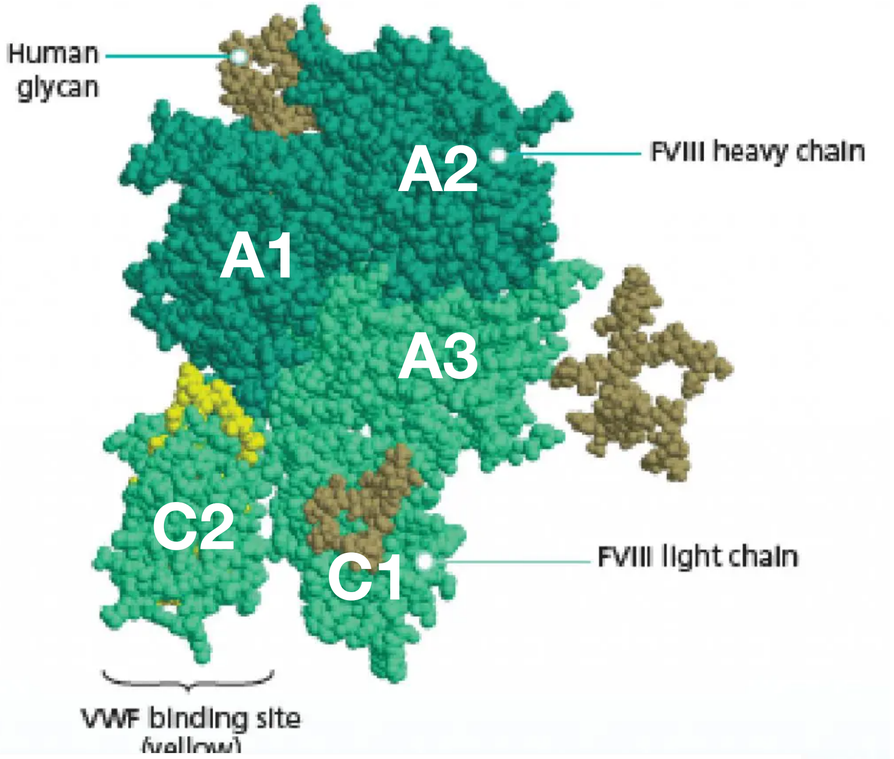
Laboratory Monitor of Factor VIII and IX in patients with EHLs therapy

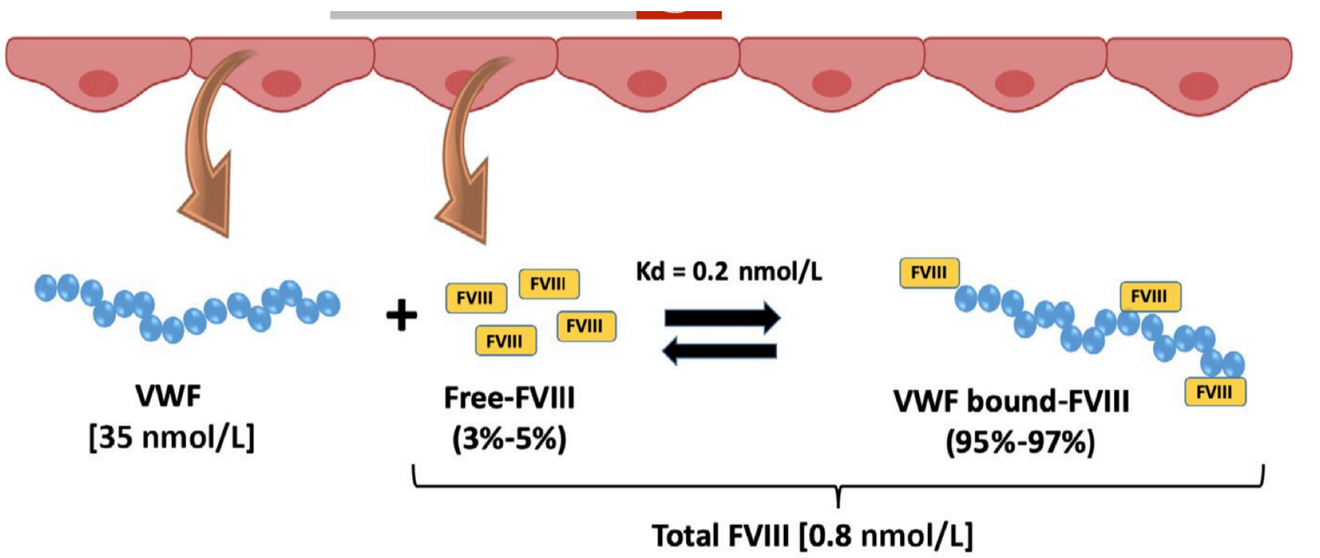
中山醫學大學附設醫院
血液病中心 翁德甫醫師

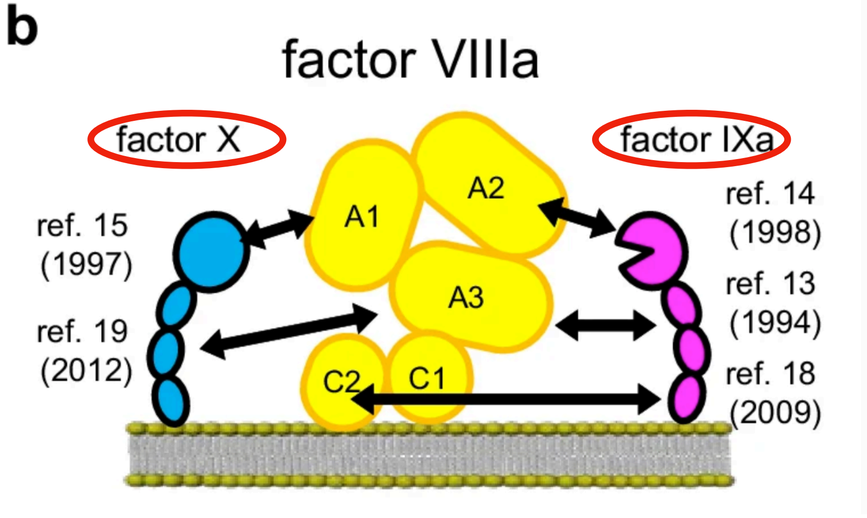
Coagulation and Hemophilia

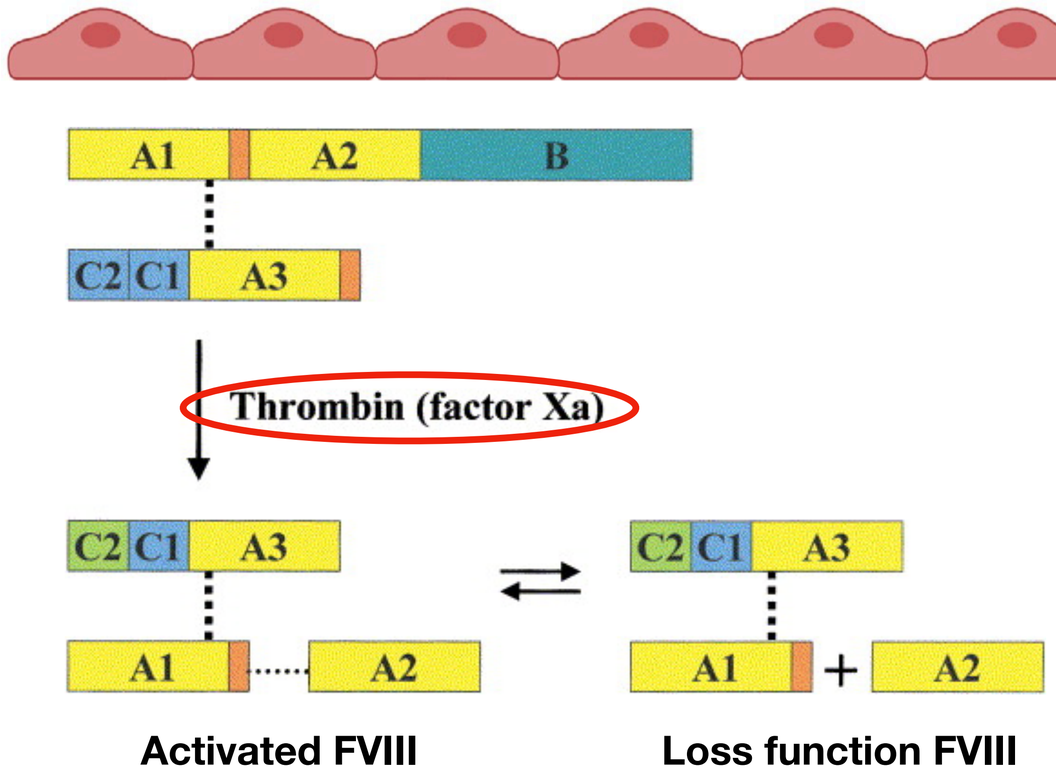




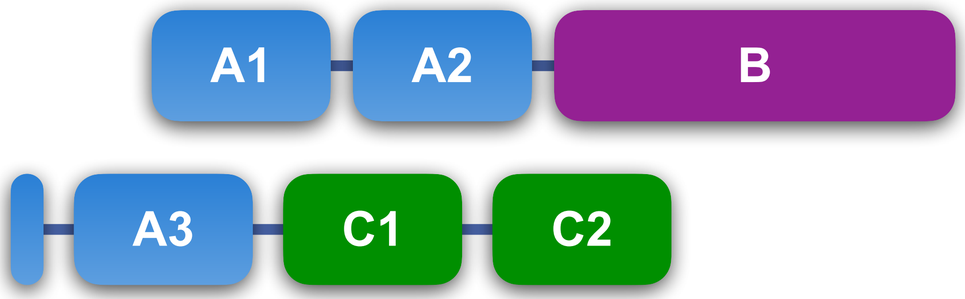

Human glycan
A2
FVIII heavy chain
A1
A3
C2
C1
FVIII light chain
VWF binding site (yellow)


Kd = 0.2 nmol/L
FVIII
VWF [35 nmol/L]
Free-FVIII (3%-5%)
VWF bound-FVIII (95%-97%)
Total FVIII [0.8 nmol/L]

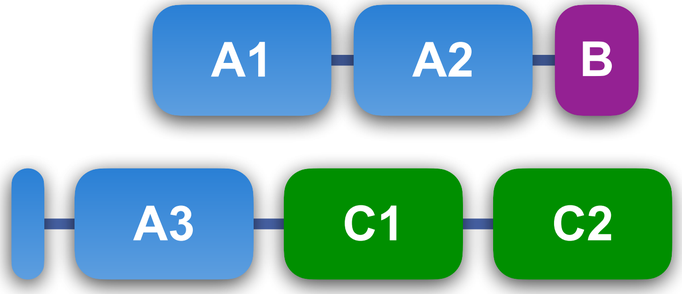

b
factor VIIIa
factor X
factor IXa
A1
A2
A3
C2
C1
ref. 15 (1997)
ref. 19 (2012)
ref. 14 (1998)
ref. 13 (1994)
ref. 18 (2009)


A1
A2
B
C2
C1
A3
Thrombin (factor Xa)
Activated FVIII
Loss function FVIII

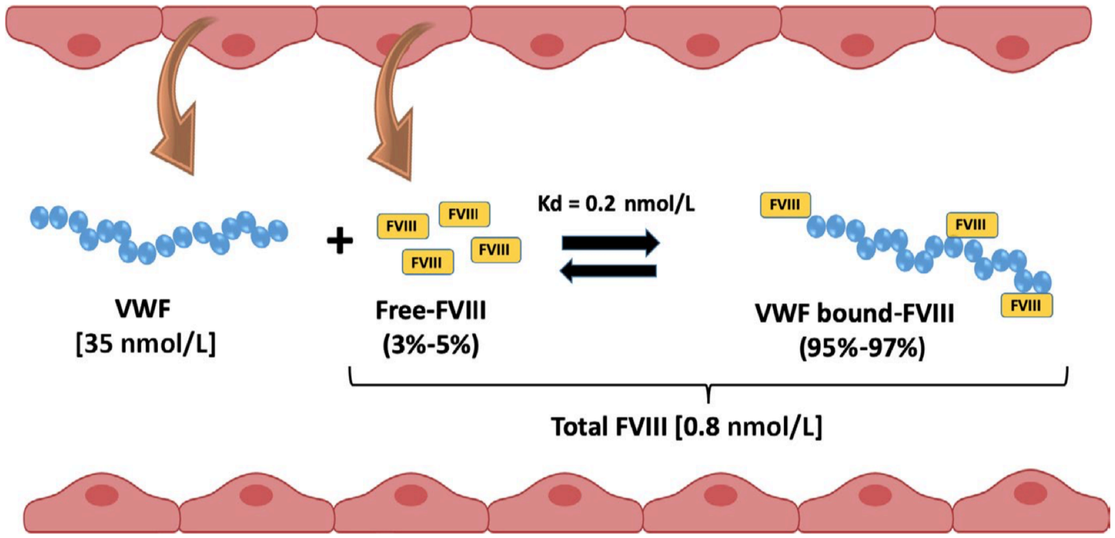
B domain deletion and Single chain rFVIII



Native FVIII or Full-length FVIII



BDD-FVIII

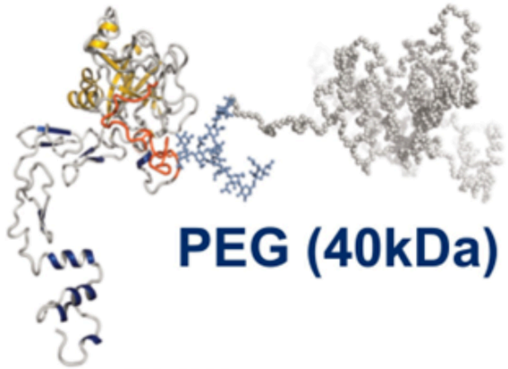


Single chain construct: Afstyla®

單鏈結構第八因子增強與VWF結合力而更穩穩定

Introduction of EHL

PEGylated



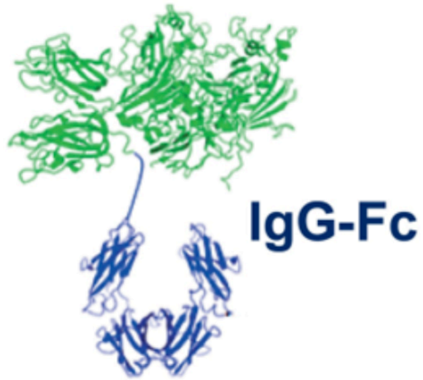
FVIII

- Jivi (60kD)
- N8-GP (40kD)
- Adynovate (20kD)

FIX

- N9-GP (40kD)

Fc-fusion



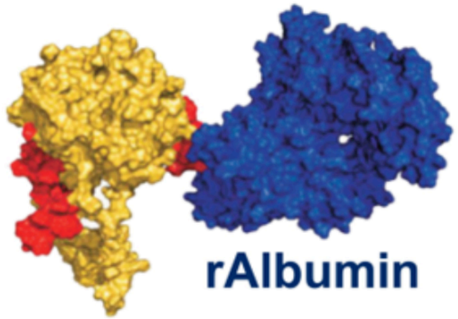
FVIII

- Eloctate

FIX

- Alprolix

Albumin-fusion



FIX

- Idelvion

Measuring Factor level has been, and will remain, a complex issue

Why are we measuring FVIII activity

1. Potency labeling of factor concentrates

- This can then be randomly checked by national quality control centers in some countries
  - Potency of factor, use chromogenic assay for most EHLs
  - 1 IU Xyntha (One-stage labeling, USA) = 1.37 IU Refacto AF (Chromogenic labeling, UK)

2. Quantifying FVIII levels in plasma

- **Diagnosis of hemophilia A**
  - To determine FVIII activity and clinical phenotype (severe, moderate, mild)
- **Following FVIII infusion to determine:**
  - Pharmacokinetics (PK) /in vivo recovery (IVR)
  - Detect development of inhibitors
  - FVIII levels at times of interventions
    - Is FVIII activity sufficiently high for planned activity (e.g.. surgery?)

Recommendations on the potency labelling of factor VIII and factor IX concentrates

A. R. HUBBARD,* J. DODT,† T. LEE,‡ K. MERTENS,§ R. SEITZ,† A. SRIVASTAVA,¶ M. WEINSTEIN‡

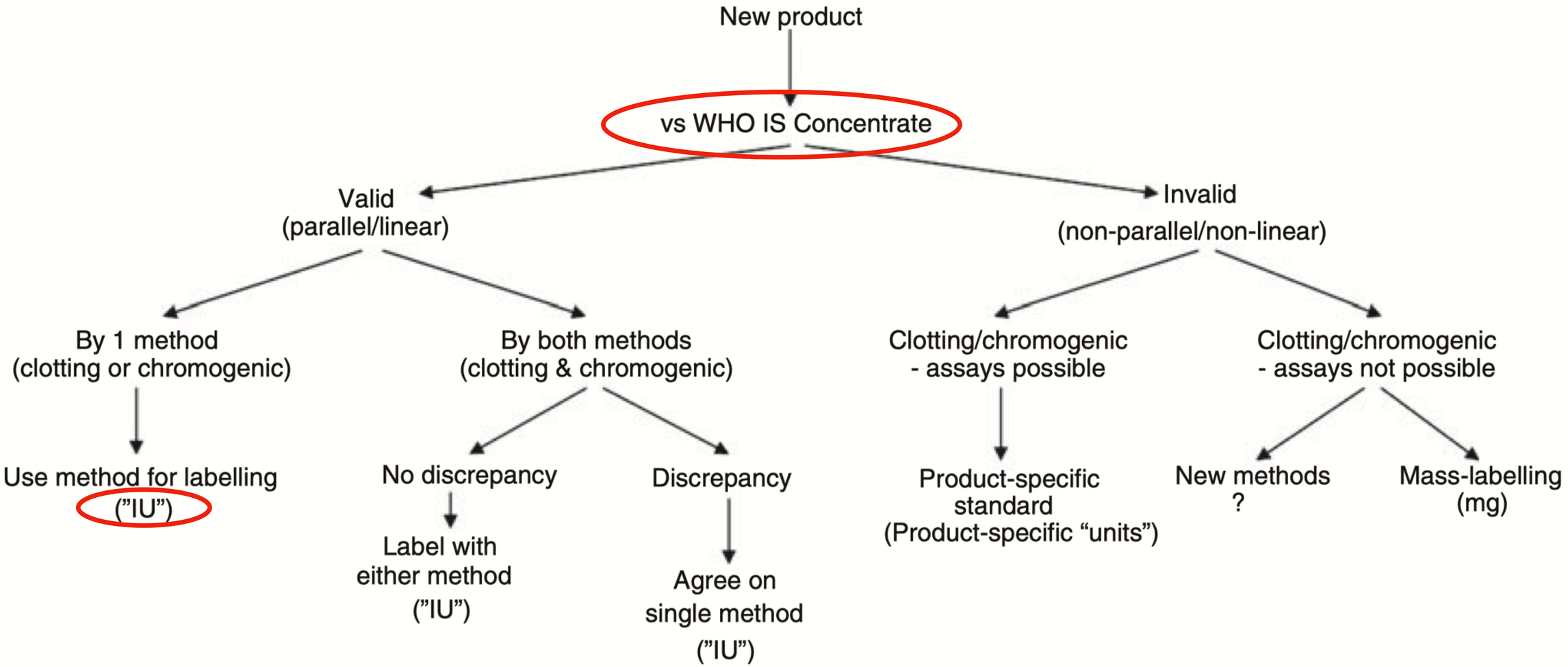
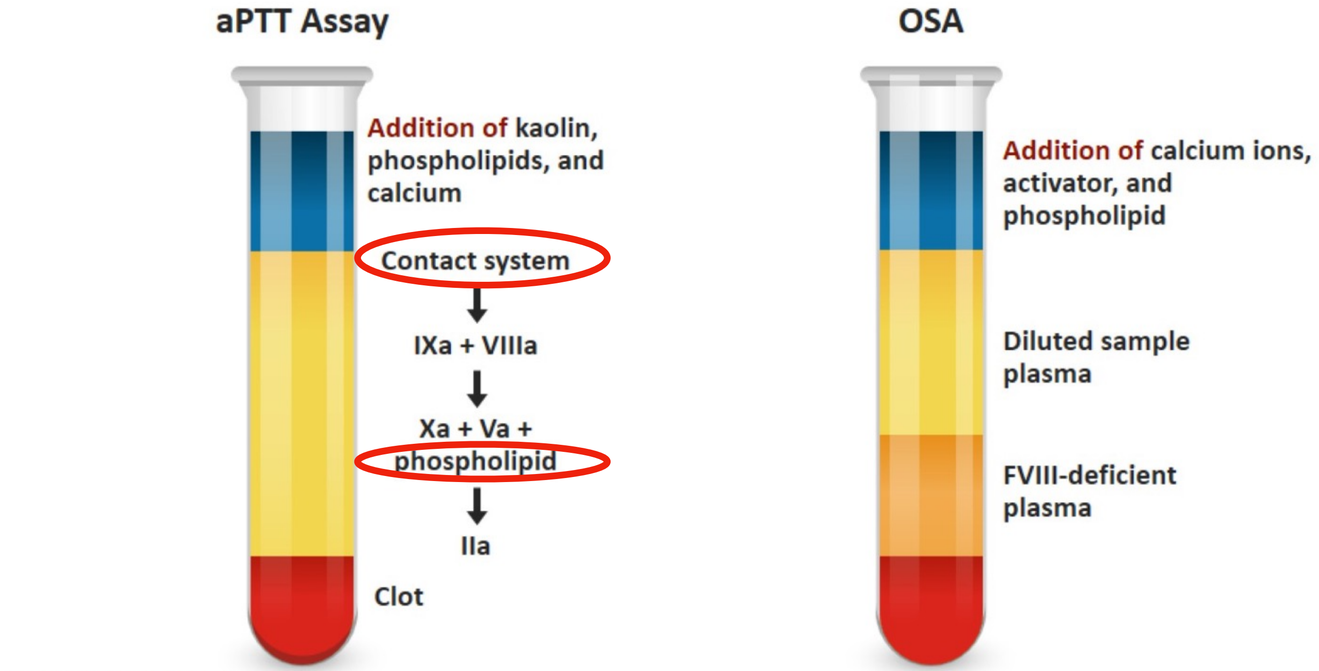


Fig. 1. Decision tree for potency labelling of new factor VIII products.

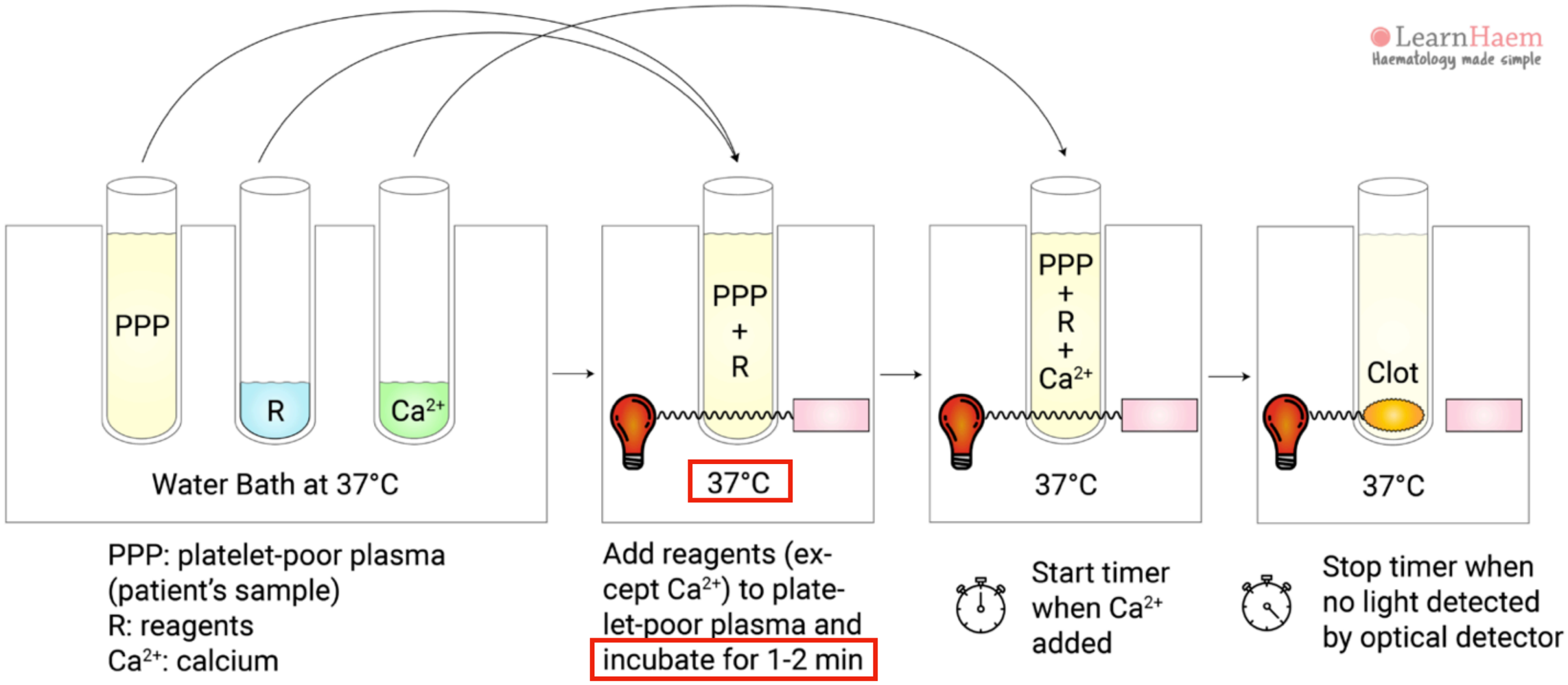
One-stage assay

Measures the ability of FVIII-deficient plasma to shorten the aPTT of diluted sample plasma



Practical-Haemostasis website.
Kitchen S, et al. *Semin Thromb Hemost.* 2017;43:331-337.

APTT	Sysmex			ACL	Stago
Reagents	**Actin FSL**	**Actin FS**	**Pathromtin SL**	**SynthAsil**	**STA-Cephascreen**
Activator	Ellagic acid	Ellagic acid	Silica	Silica	Polyphenolic component
Phospholipid	Rabbit brain, Soy bean	Soy bean	Vegetable	Synthetic	Rabbit brain



PPP、Reagents、incubation time：under or over-estimated aPTT

EHL FVIII concentrates and lab assays

Table 1 Factor VIII assay results with the one-stage clotting assay (OSCA) according to concentrate and APTT reagent used, expressed qualitatively regarding whether the results will be correct, underestimated, overestimated or unknown

	rFVIII Fc[6]	rFVIII-PEG[7]	rFVIII-SC[10,11]	N8-GP[8]	BAY-94[9]
Silica reagents					
SynthaSIL	Correct	Correct	Underestimated	Correct	Correct
STA-PTT Automate	Correct	Correct	Underestimated	Underestimated	Underestimated
PTT-SP	Unknown	Unknown	Underestimated	Underestimated	Underestimated
Pathromtin SL	Correct	Correct	Underestimated	Correct	Correct
Triniclot Auto	Correct	Correct	Underestimated	Unknown	Unknown
Triniclot HS	Correct	Correct	Underestimated	Unknown	Unknown
Ellagic acid reagents					
Actin FS	Correct	Correct	Underestimated	Correct	Correct
Actin FSL	Correct	Correct	Underestimated	Correct	Correct
Synthafax	Unknown	Correct	Underestimated	Decreased	Correct
DG Synth	Unknown	Unknown	Unknown	Correct	Unknown
Kaolin reagents					
CK Prest	Correct	Correct	Underestimated	Correct	Unknown
Polyphenolic acid reagents					
Cephascreen	Correct	Correct	Underestimated	Correct	Correct

Journal of Thrombosis and Haemostasis, 17: 567–573

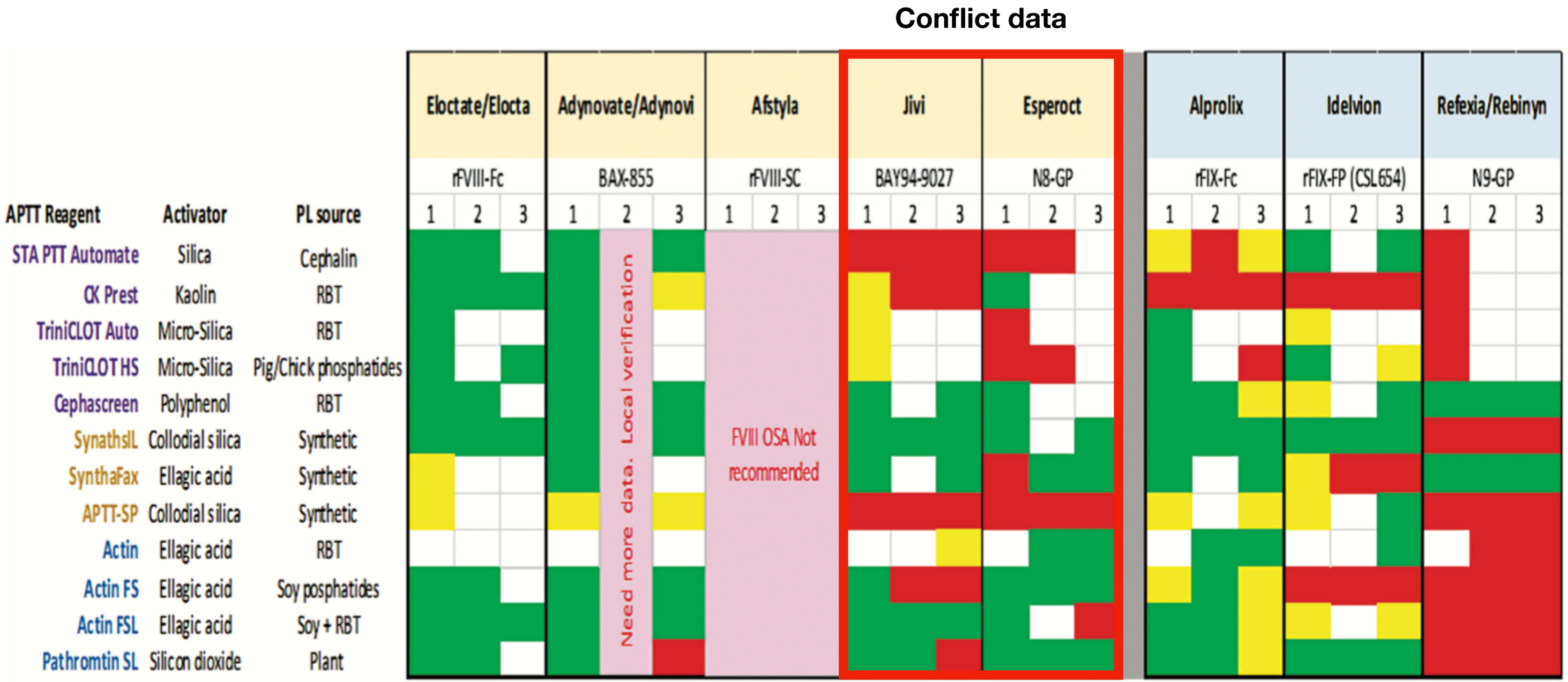
EHL FIX concentrates and Lab Assays

Table 2 Factor IX assay results with the one stage clotting assay (OSCA) according to concentrate and APTT reagent used, expressed qualitatively regarding whether the results will be correct, underestimated, overestimated or unknown

	rFIX Fc[16]	rFIX FP[17]	N9-GP[18–21]
Silica reagents			
SynthaSIL	Correct	Correct	Underestimated
STA-PTT Automate	Unknown	Correct	Overestimated
PTT-SP	Unknown	Unknown	Overestimated
Pathromtin SL	Correct	Correct	Overestimated
Triniclot Auto	Correct	Unknown	Overestimated
Triniclot HS	Correct	Correct	Overestimated
Ellagic acid reagents			
Actin FS	Correct at normal levels but too high at FIX levels of 5–20%	Underestimated	Underestimated
Actin FSL	Correct	Unknown	Underestimated
Synthafax	Correct	Unknown	Correct
DG Synth	Correct	Unknown	Correct
Kaolin reagents			
CK Prest	Underestimated	Underestimated	Underestimated
Polyphenolic acid reagents			
Cephascreen	Correct	Unknown	Correct

Journal of Thrombosis and Haemostasis, 17: 567–573

Recommended or rejected OSA methods for measuring FVIII and FIX EHL products from recent publications[1-3]



Church N, et al Haemophilia. 2018 Sep;24(5):823-832.
Bowyer AE, et al. Semin Thromb Hemost. 2022 Dec 6
Stefan Tiefenbacher, et al. Haemophilia. 2019;00:1–9.

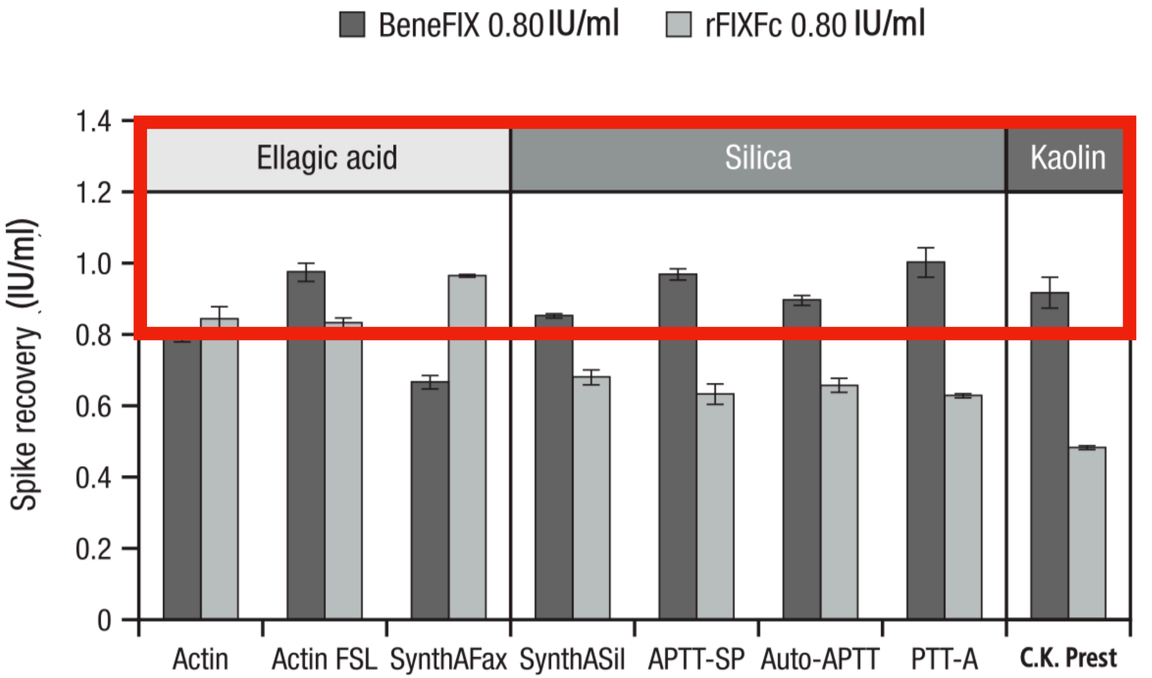
rFIX product	Label activity (IU/ml)	Mean spike recovery (IU/ml)	% of expected (label activity)	Intra-laboratory CV (n=3)		Inter-laboratory CV (n=30)
				Median	Range	
BeneFIX	0.80	0.966	121	5.6 %	0 %-25.0 %	12.1 %
	0.20	0.289	144	3.6 %	0 %-21.4 %	19.7 %
	0.05	0.084	168	7.3 %	0 %-39.8 %	29.8 %
rFIXFc	0.80	0.707	88	6.7 %	0 %-18.4 %	26.3 %
	0.20	0.214	107	6.7 %	1.4 %-20.9 %	35.5 %
	0.05	0.066	132	8.4 %	0 %-36.7 %	44.1 %

rFIX, recombinant factor IX; IU, International Units; CV, coefficient of variation; rFIXFc, recombinant factor IX Fc fusion protein.

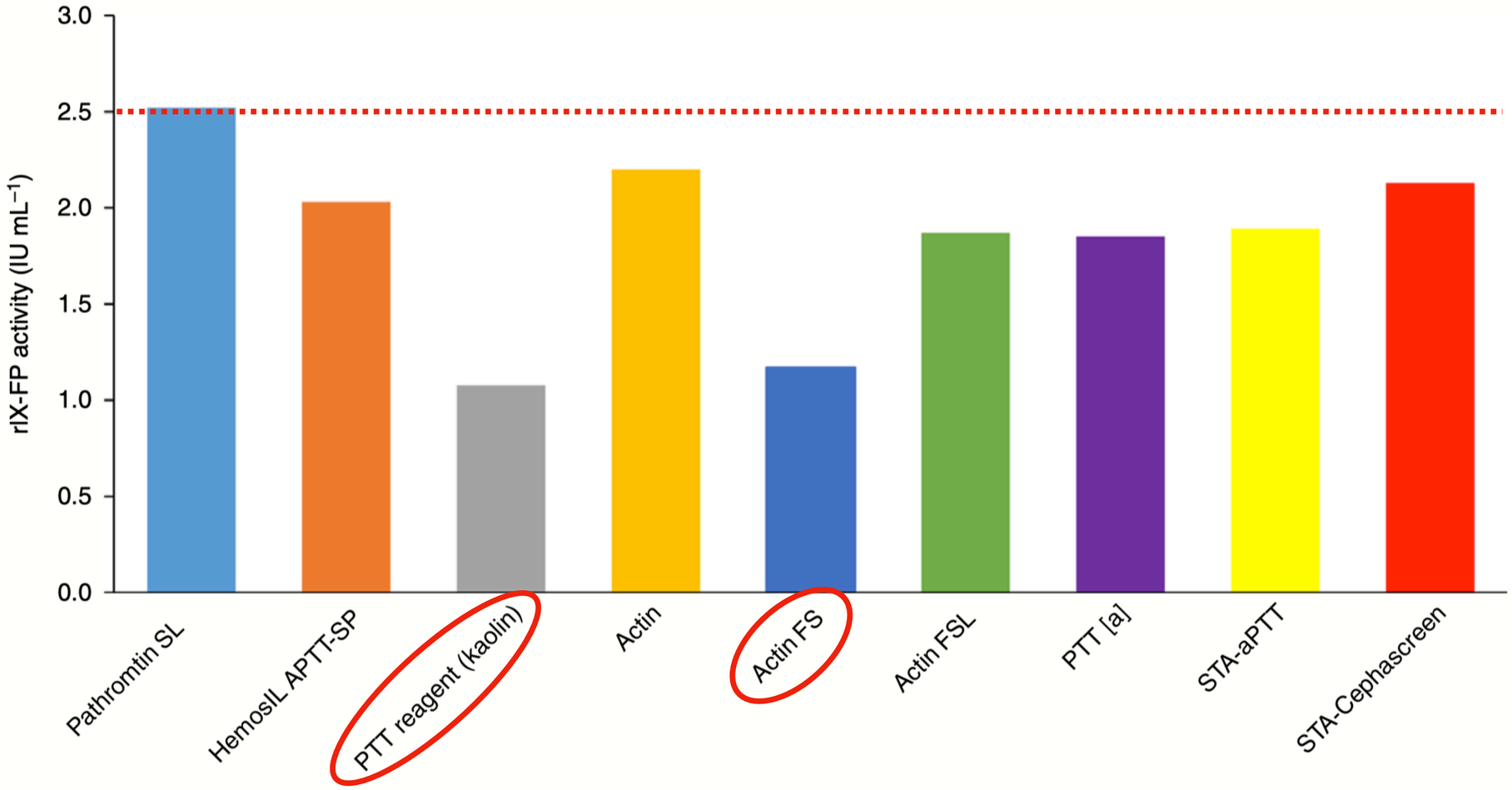
Benefix®
Pfizer

Alprolix (rFIXFc)
SANOFI

Under-estimated in Silica/Kolin reagents aPTT test



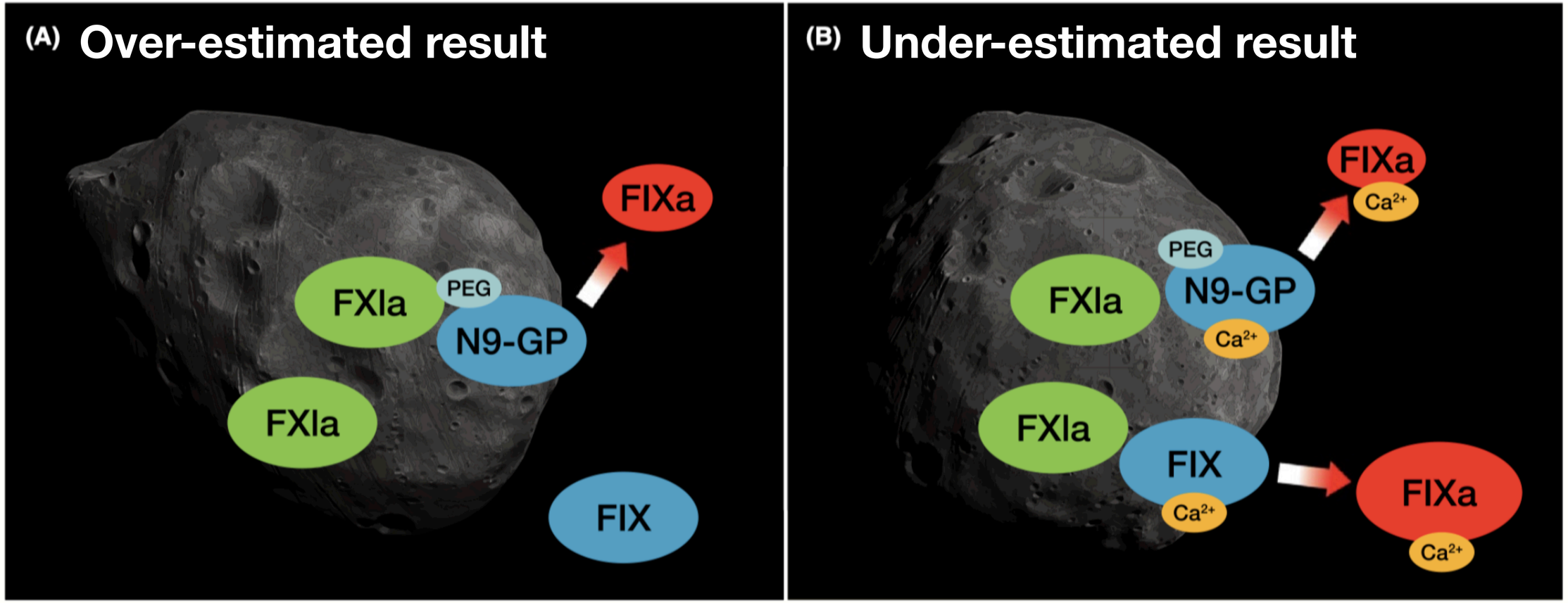
Performance of a rIX-FP in one-stage clotting assays



rIX-FP: Albumin fusion FIX

J Thromb Haemost 2019; 17: 138–48.

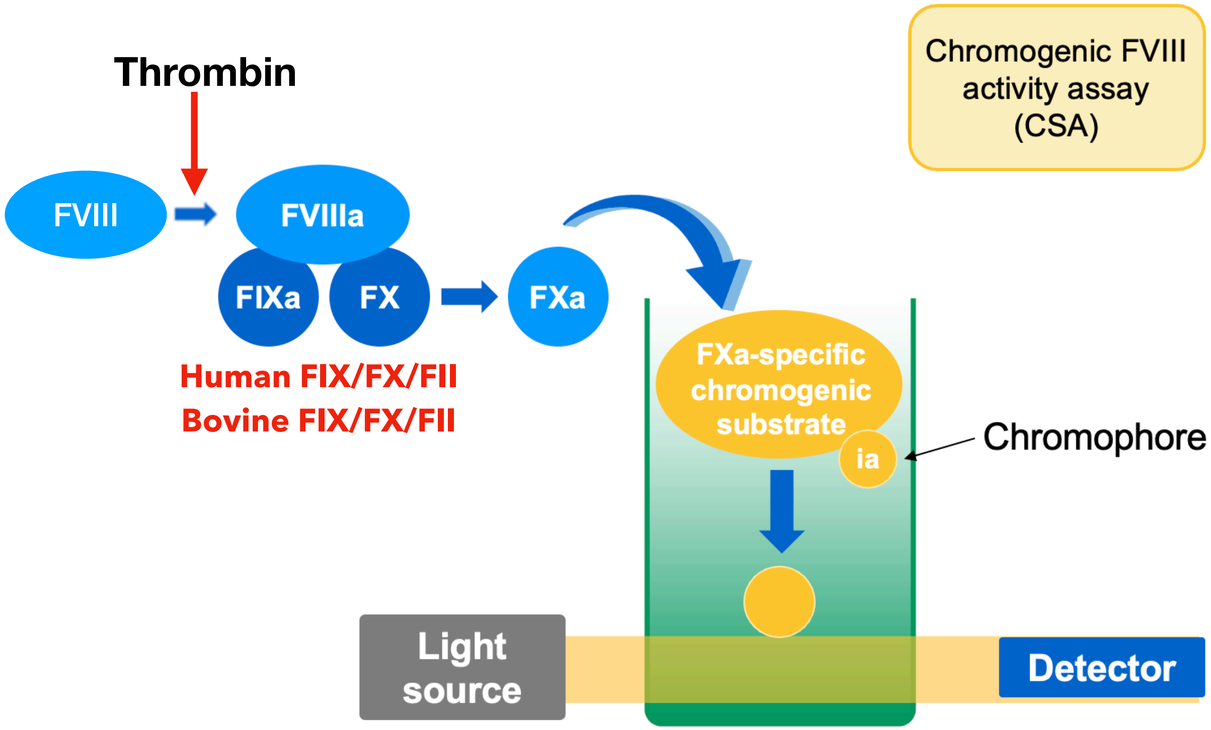
Over/under-estimated results of PEGylated FIX

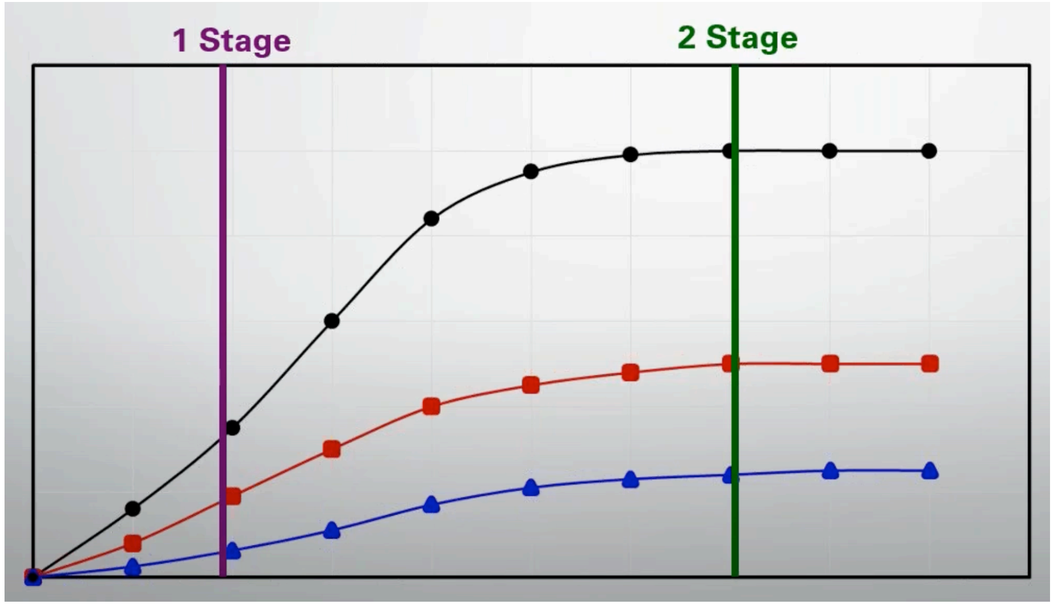


N9GP absorption by silica and activated by FXIa before recalcificartion

Contact activator (ellagic acid, kaolin or silica) in some aPTT reagents negatively affects the enzymatic activity of FXIa

Two stage: Chromogenic assay





One-stage: more sensitive to **thrombin activation**
Two stage: more sensitive to **stable condition of activated factor**

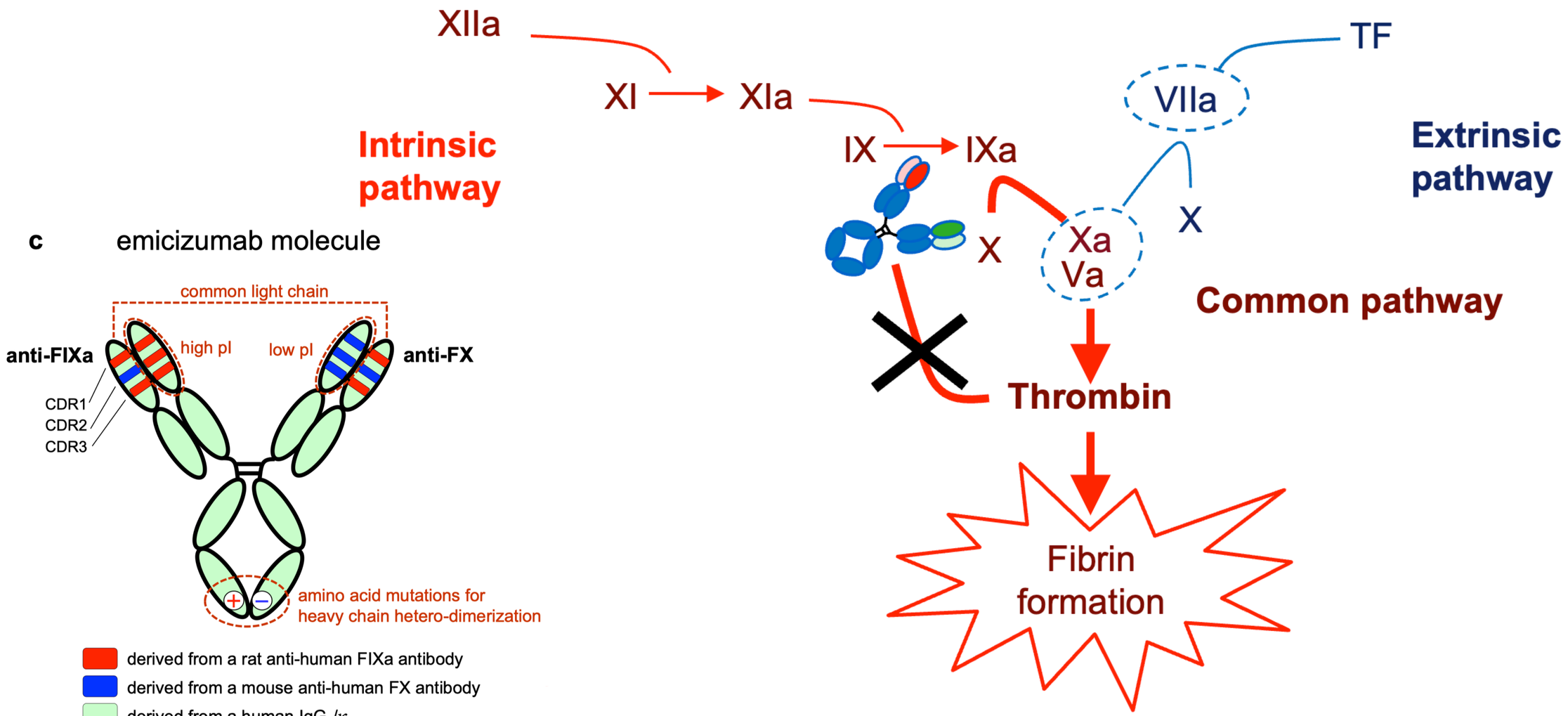
1. Potgieter J. et al. Eur J Haematol 2015;94:38-44.

Differences Between OSA and CSA

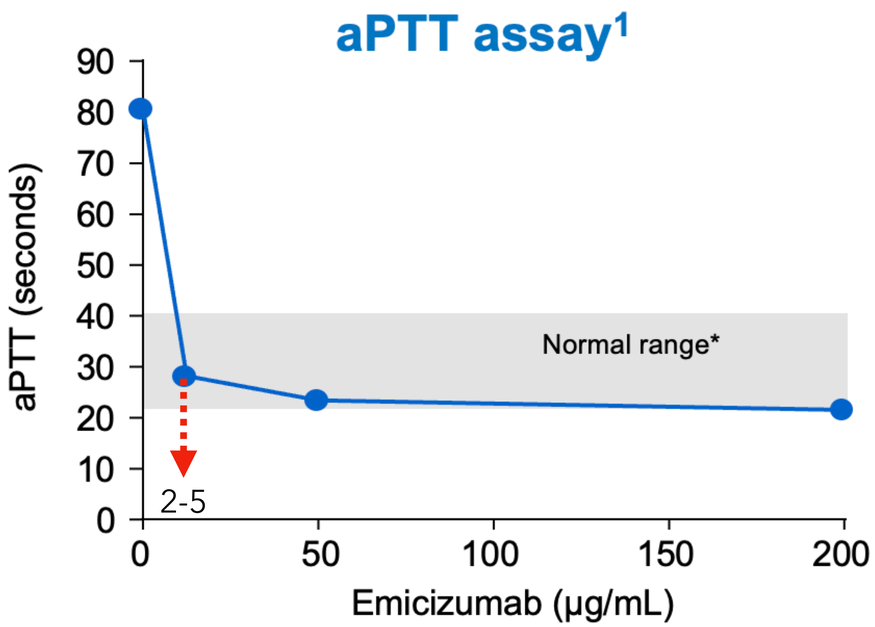
	One-staged assay	Chromogenic assay
Advantages	Simple and rapid Inexpensive Easy to automate Widely used for clinical monitoring	**Not sensitive to FVIII activation** FVIII deficient plasma not required Lower interlaboratory variability
Limitations	**Sensitive to FVIII activation** Large variety of assay kits, reagents • Activator • Phospholipid content • Congenital-deficient vs immuno-depleted plasma • Clot detection of the automated analyzers High interlaboratory variability	Perceived to be technically complex More expensive than OSA Not as widely used Perceived to be more difficult to automate
Sensitivity	Lupus anticoagulant, heparin, lipid impurities, DOAC	Very sensitive to NOAC

Peyvandi F, et al. J Thromb Haemost. 2016;14;248-261.

Emicizumab: Rule Breaker



Emicizumab has a strong effect on aPTT

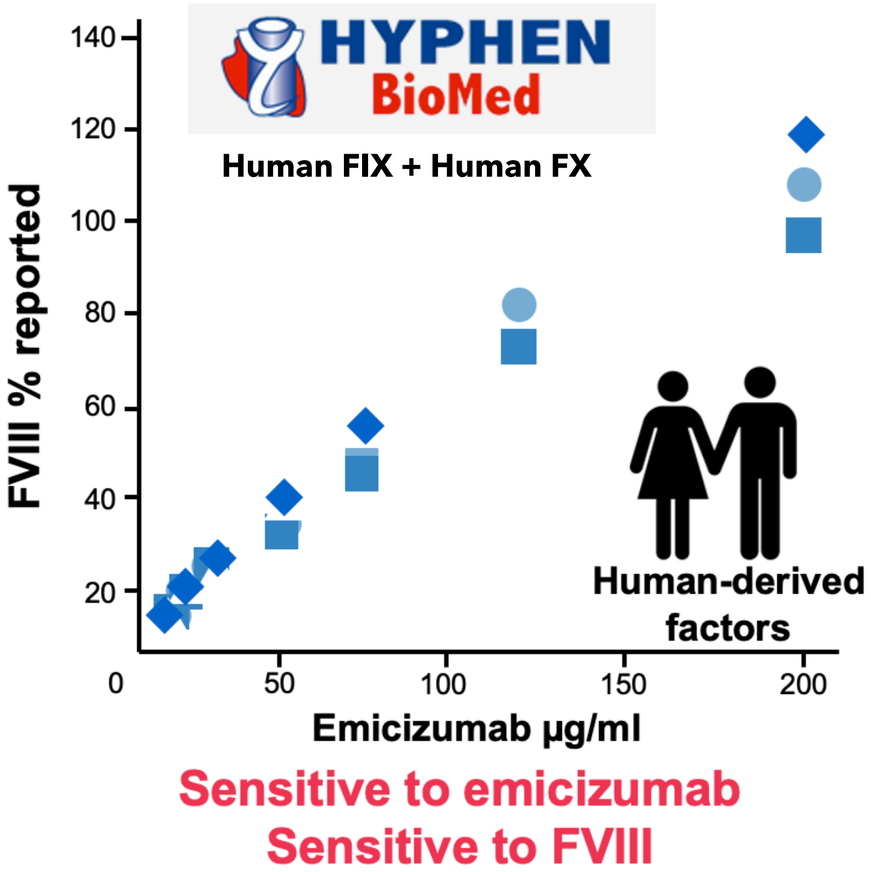


Plasma from a hemophilia patient, spiked with increasing amounts of emicizumab

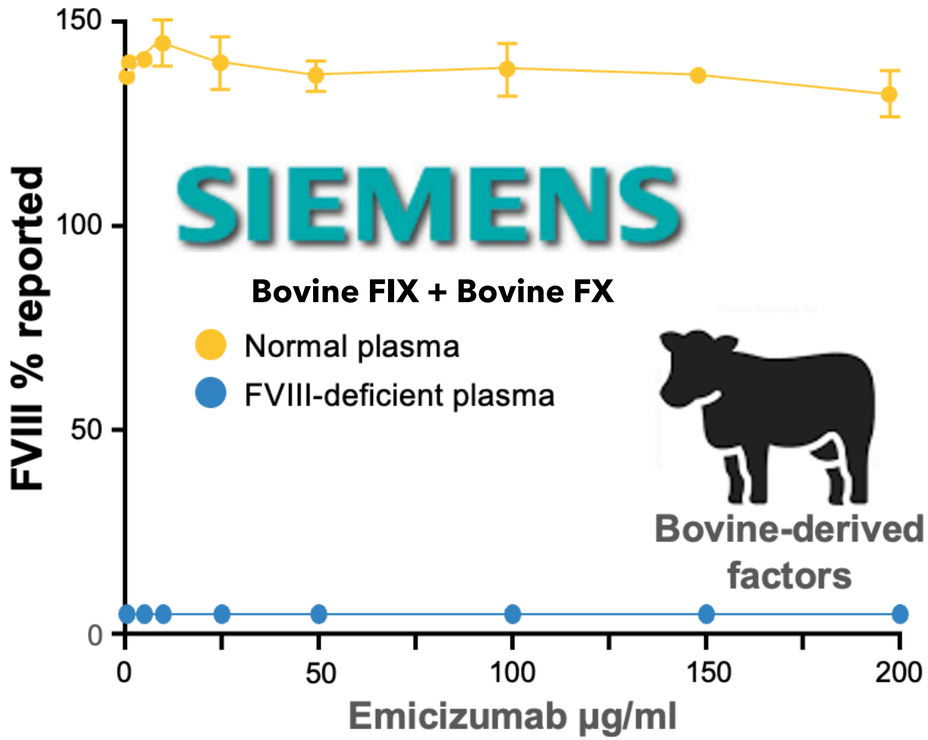
- FVIII activation is a rate-determining step of the aPTT assay[1]
- Unlike FVIII, emicizumab does not require activation for its cofactor activity, which leads to shorter clotting times with emicizumab versus FVIII[1]
- PwHA treated with emicizumab will have a normal aPTT test result with a drug level of >2–5 µg/mL[1]

Chromogenic FVIII assays using human-derived factors cannot be used to detect FVIII activity when emicizumab is present[1,2]

- Chromogenic FVIII assays* using human-derived factors are sensitive to both emicizumab and FVIII
- Emicizumab drives coagulation specifically with human FIXa and FX, but not with bovine FIXa and FX



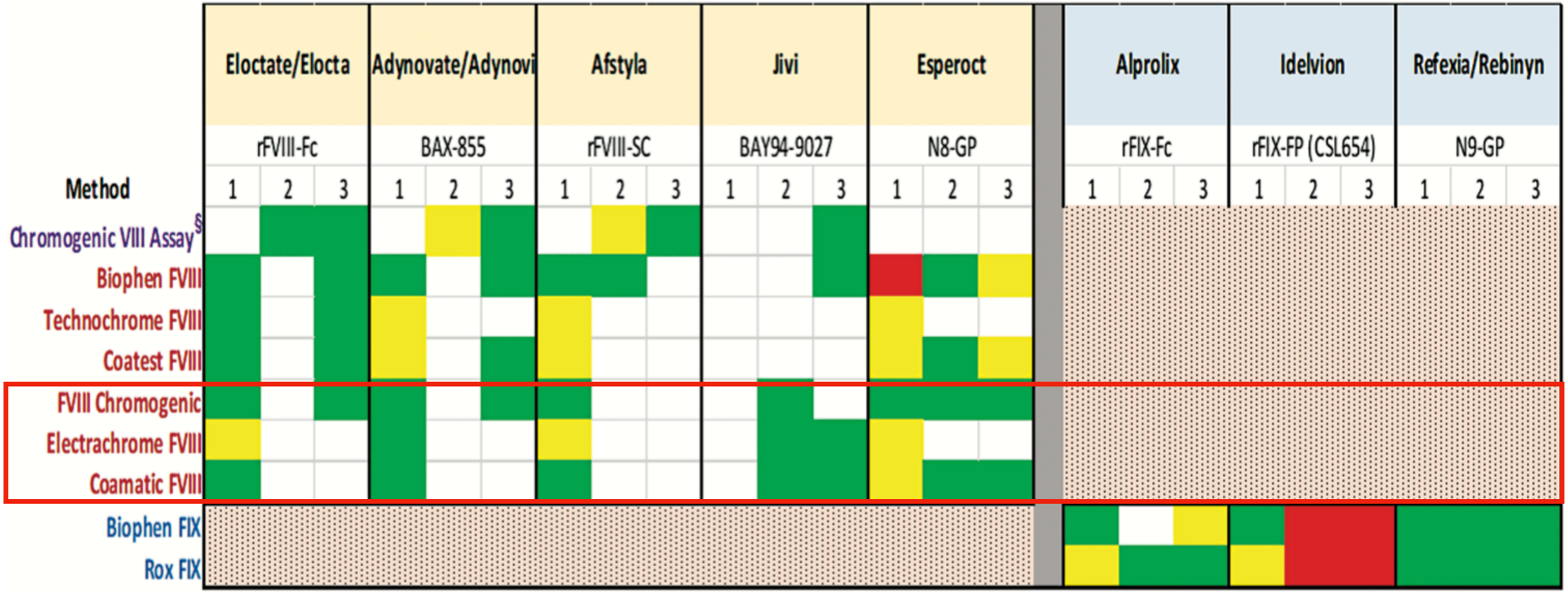
Sensitive to emicizumab
Sensitive to FVIII



Insensitive to emicizumab
Sensitive to FVIII

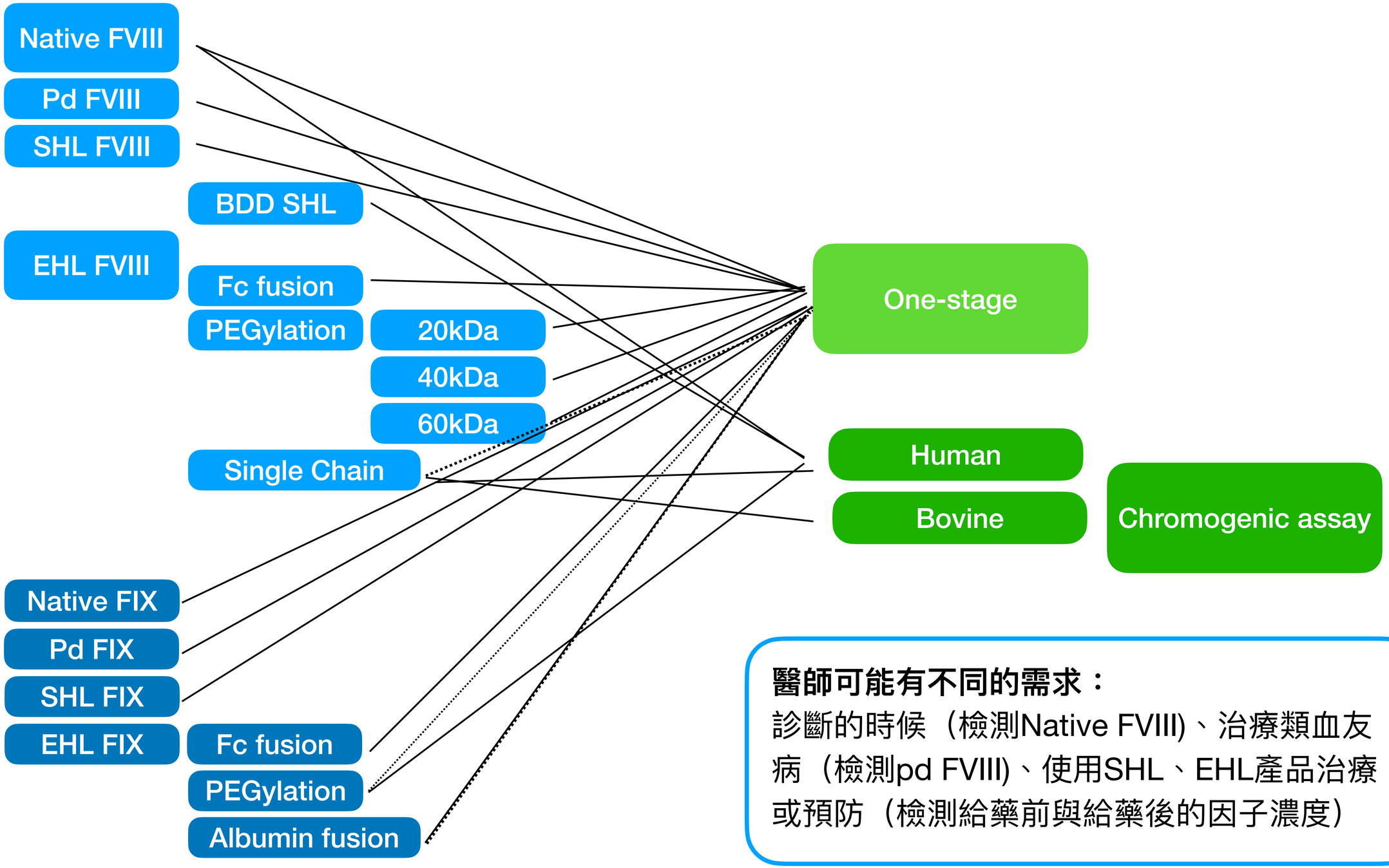
1. Calatzis A, et al. ECTH 2016; oral presentation 32;
2. Adamkewicz J.I, et al. Thromb Haemost 2019;119:1084–1093;
3. Nougier C, et al. Eur J Haematol. 2020;105:675–681.

Recommended or rejected CS methods for measuring FVIII and FIX EHL products from recent publications[1-3]



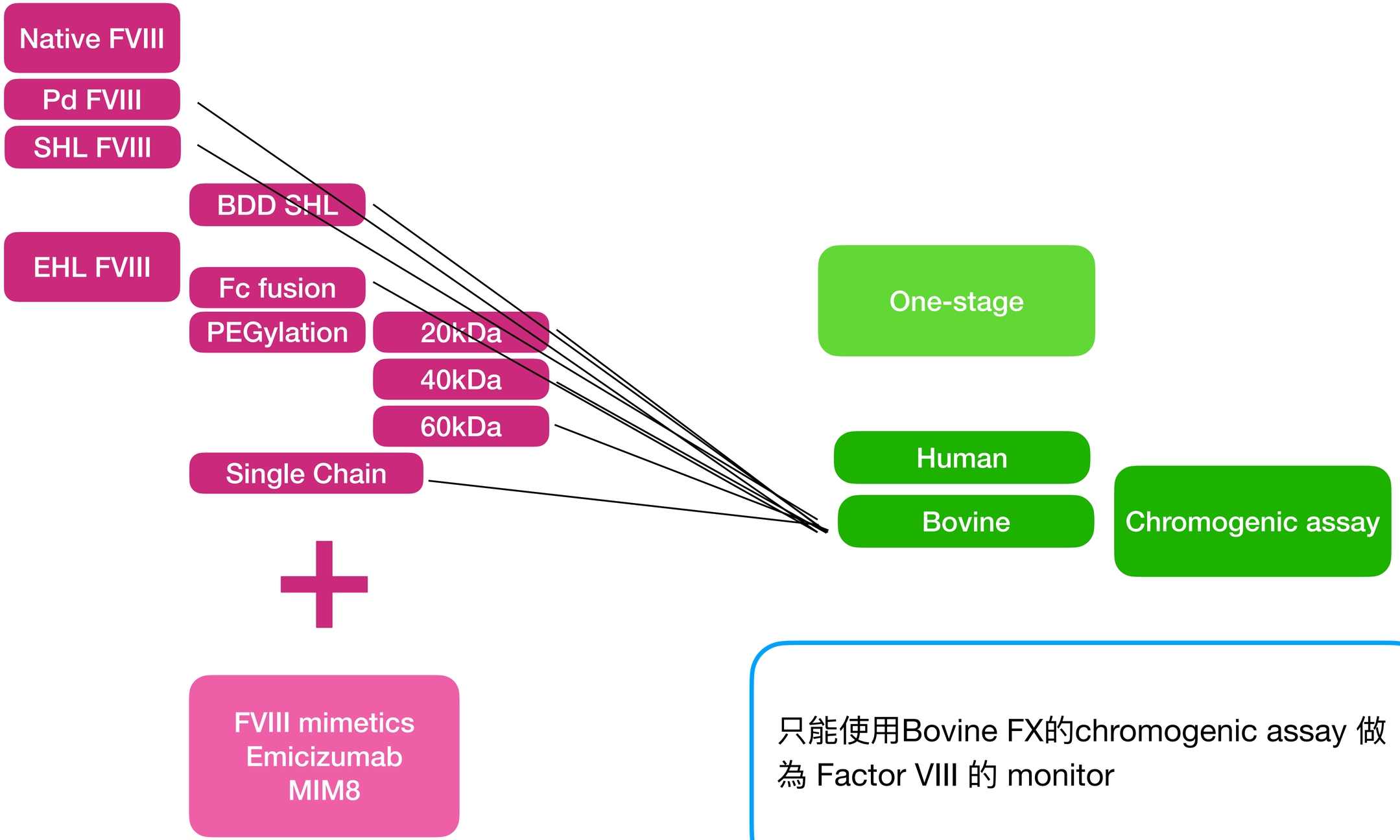
Conflict Data in Chromogenic assay

Church N, et al Haemophilia. 2018 Sep;24(5):823-832.
Bowyer AE, et al. Semin Thromb Hemost. 2022 Dec 6
Stefan Tiefenbacher, et al. Haemophilia. 2019;00:1–9.



醫師可能有不同的需求：

診斷的時候（檢測Native FVIII)、治療類血友病（檢測pd FVIII)、使用SHL、EHL產品治療或預防（檢測給藥前與給藥後的因子濃度）



只能使用Bovine FX的chromogenic assay 做為 Factor VIII 的 monitor

我想知道病人凝血因子的濃度。

醫師

我把機器檢測出來的凝血因子濃度報告發出去。

醫檢師

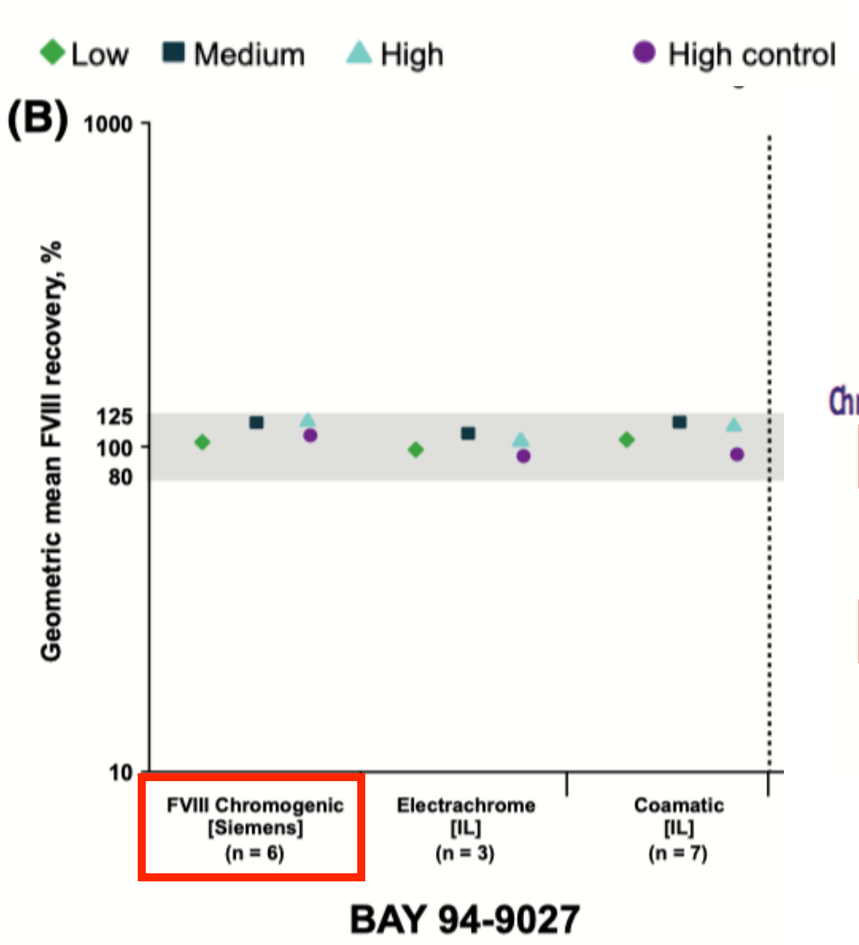
Case one

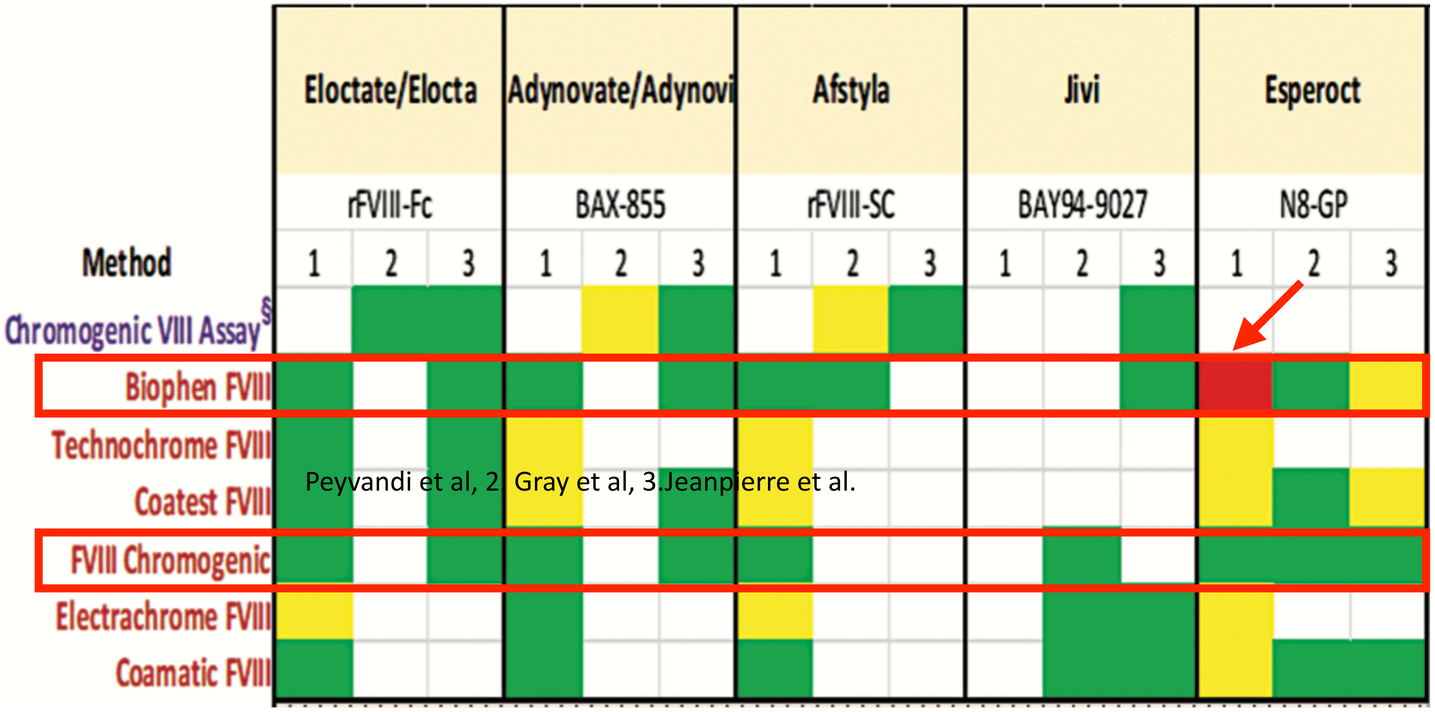
- 46YO male with hemophilia A, severe type
- Check Jivi (PEGylated rFVIII) level for PK study

Post-infusion	4hr	24hrs	48hrs	WAPPS Half-life
One stage		64.4%	17.5%	14.5 hrs
Chromogenic assay (Bovine)	27.1%		3.5%	8.5 hrs

- Why the result of bovine chromogenic assay is very low ?

Is chromogenic assay consistent with Jivi®



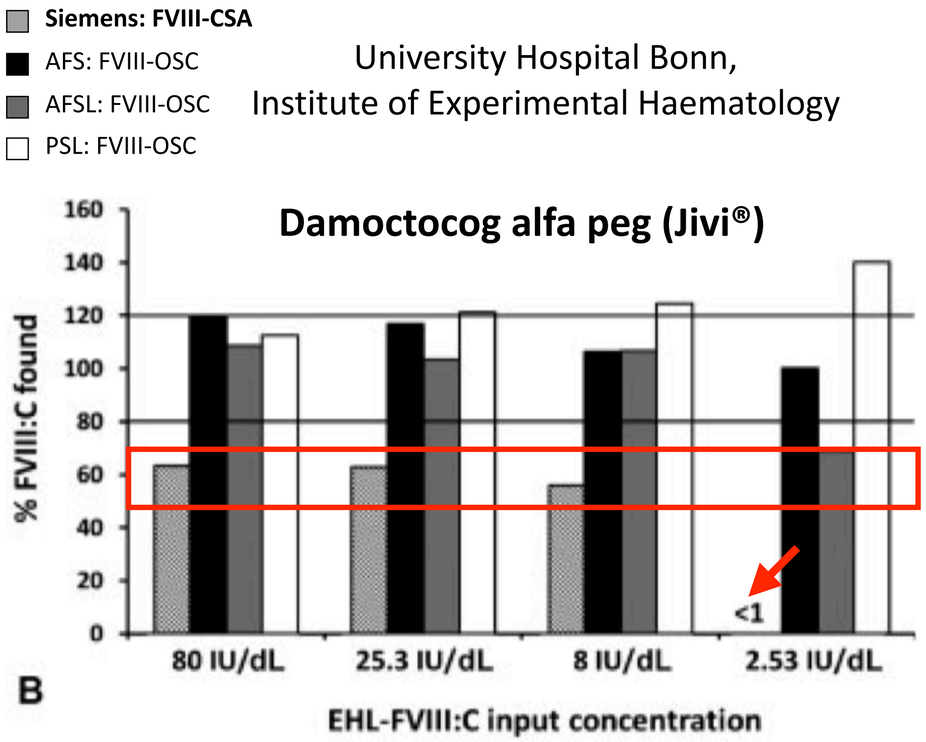


The review article suggested FVIIII Chromogenic (Bovine) is available to monitor PEGylated FVIII (Jivi)

OSA: one stage assay; BCSA: bovine chromogenic strata assay

Church N, et al Haemophilia. 2018 Sep;24(5):823-832.
Bowyer AE, et al. Semin Thromb Hemost. 2022 Dec 6
Stefan Tiefenbacher, et al. Haemophilia. 2019;00:1–9.

Recovery of Jivi® with Chromogenic assay[1,2]



Recovery of BAY 94-9027 [Jivi®] with One-stage Clotting and Chromogenic Assays: A Multicentre Study[2]

APTT reagent	Type of reagent	N	Median Recovery (range)(%)
Actin FSL	Ellagic Acid	4	99 (79-141)
Siemens	**Bovine**	**7**	**80 (56-100)**
Trinichrom	**Bovine**	**6**	**59 (54-73)**
Precision Biologics	**Himan/ Bovine**	**1**	**75 (58-95)**

BCSA (Siemens) test with Jivi® spiked test

- **Under-estimated** in all levels[1]
- The recovery decreased from 100% to 56% as the Jivi concentration decreased[2].

OSA: one stage assay; BCSA: bovine chromogenic substrate assay

1. Müller J,et al. Hamostaseologie. 2020 Nov;40(S 01):S15-S20
2. Meijer P, et al. Res Pract Thromb Haemost. 2021; 5 (Suppl 2).

Case 2

- 63YO male with hemophilia A, severe type
- Received **Afstyla® (single-chain factor VIII)** treatment for major surger7 (total hip joint replacement).
- Check factor VIII level with one-stage and bovine chromogenic assay after surgery under Afstyla treatment
    - FVIII (one stage): **75IU/dl;**
    - FVIII (Bovine chromogenic assay): **35IU/dl**

Chromogenic assay is more accurate than one-stage for “Afstyla”

Chromognic assay is 2 fold of one-stage

So, which data is right or both data is wrong?

Con't~

- Which FVIII level is right ?
  - If FVIII one stage level is correct: 75IU/dl，the patient is under enough treatment level (>80-100%)
  - If FVIII chromogenic assay is correct: 35IU/dl，the patient had high risk of post-operation bleeding.
- After discussion with laboratories:
  - Recheck Factor VIII chromogenic assay is **150% (Equipment problem)**
  - **Finally: FVIII (OS): 75% and FVIII (BCS): 150%**

Check list for EHLs

- ☐ For Diagnosis
- ☐ Under factor treatment
  - ☐ plasma-derived or standard half-life factor:
  - ☐ Extended half-life factors: ☐ Fc fusion、☐ PEGylation、☐ Albumin-fusion
  - ☐ For monitor trough level (confirmed <1%)
  - ☐ For PK/IVR study (IVR: in vivo recovery): (reported level >150% is needed)
- ☐ Under Emicizumab prophylaxis (Bovine chromogenic assay only)
- ☐ Under Emicizumab prophylaxis inhibitor monitor (Bovine chromogenic assay only)

Conclusion

- With the introduction of newer treatments, hemostasis monitor is crucial for management of the patients.
- Extended half-life factors (EHLs) and FVIII mimetic (Emicizumab) are revolutionizing hemophilia care but is a challenge to laboratories due to the shear diversity of opinions, which require specific reagents for laboratory monitor.
- It ’s no longer an option to have only single assays available for monitor FVIII and FIX treatment.
  - Diversity of treatments to the patients taking new treatments.
  - **Facilitate accurate communication between clinical and laboratory staff.!!!**