



Hemophilia treatments and the role of the lab

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Disclosure

No disclosures concerning the topic of the presentation. This presentation includes own and published data for the management of haemophilia.

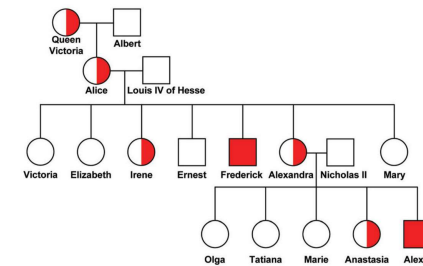
Hemophilia A and B

Similarities and differences

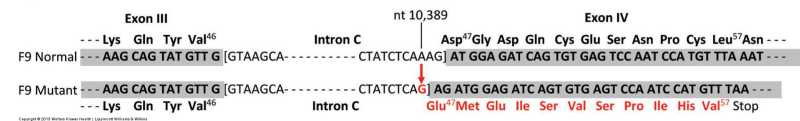


	Hemophilia A	Hemophilia B
frequency	1:5,000	1:30,000
Affected coagulation factor	FVIII (cofactor function)	FIX (serine protease function)
Half life of the factor	short	longer
Correlation of factor level with clinical severity	YES	YES
inheritance	XR	XR
Most frequent mutations	Intron 22, intron 1 inversions	missense variants
Mutation databases	https://www.factorviii-db.org/	https://www.factorix.org/
Differential dg.	vWD (2N), combined FV+FVIII deficiency	Vitamin K deficiency, VKF def.

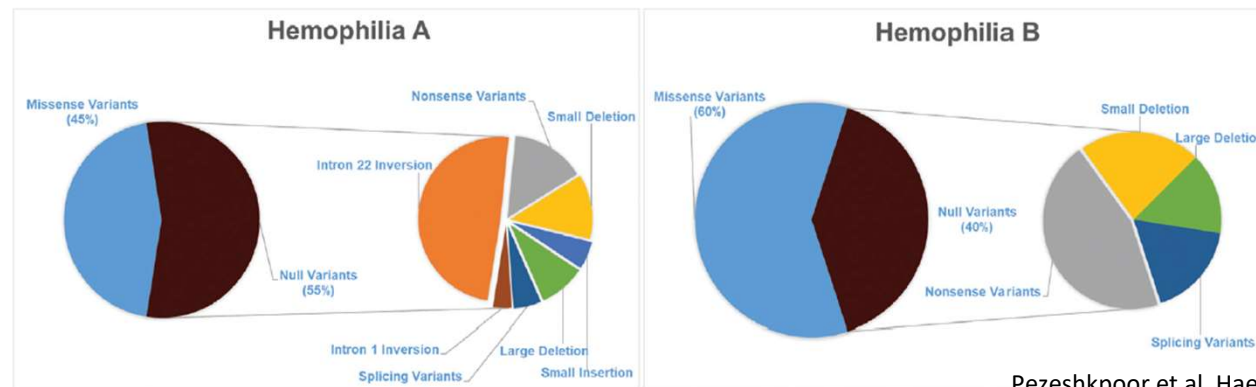
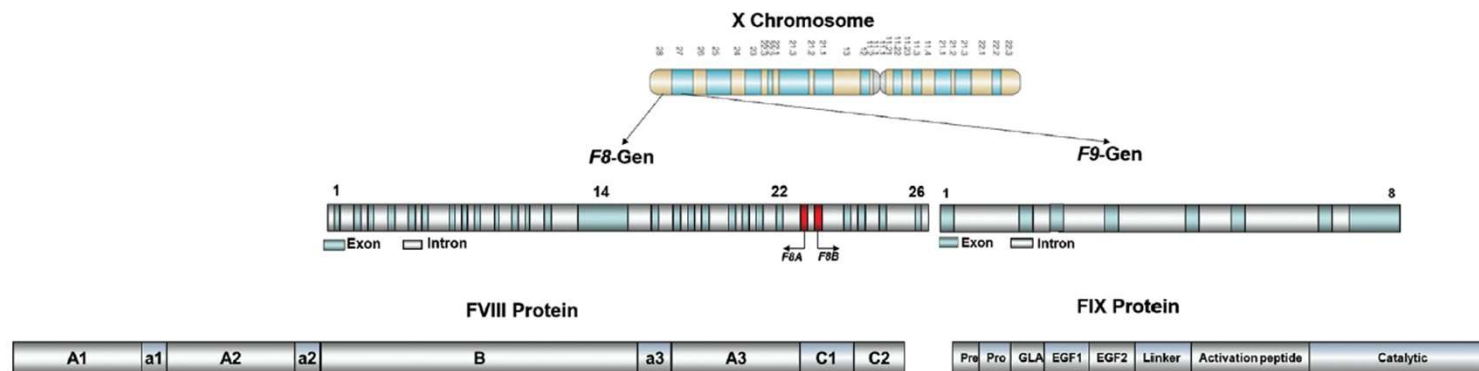
A



B

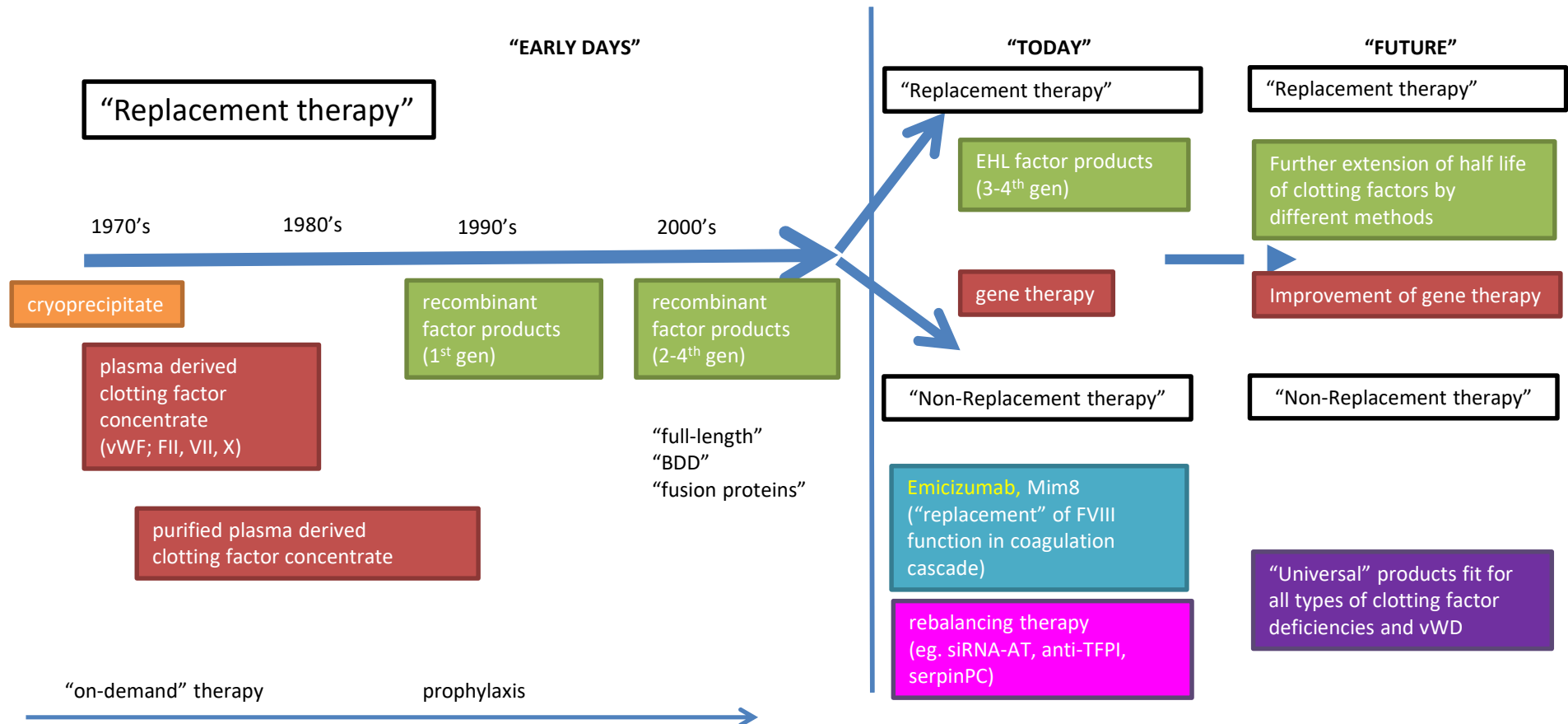


Gene and protein structure of FVIII and FIX, and the genetic background of Hemophilia A and B



Pezeshkpoor et al. Haemostaseologie 2022;42:390-9.

Evolution of hemophilia therapy

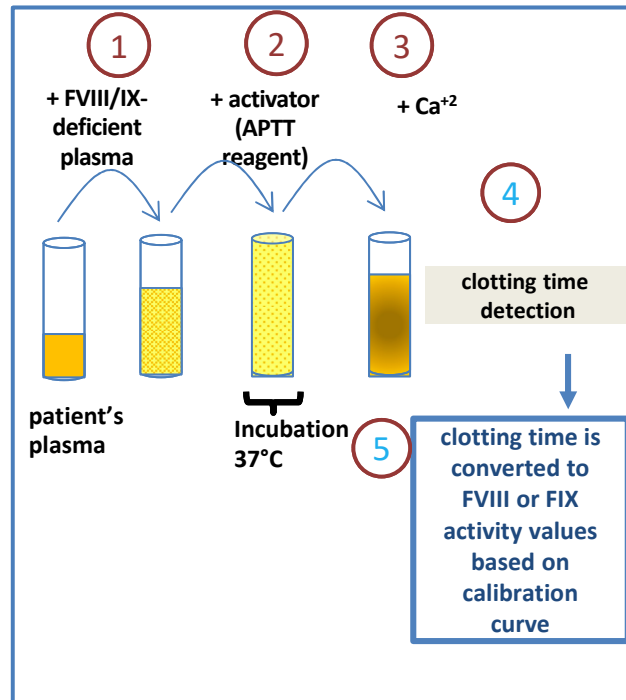


Role of the laboratory in the management of patients with hemophilia A and B

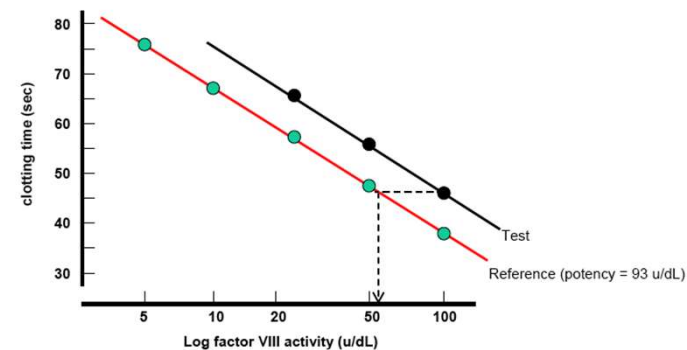
1. Diagnosis of Haemophilia A or B and determining the severity of the disease → measuring FVIII/IX levels in **plasma**
2. Monitoring of Hemophilia A and B therapy: Determining clotting factor levels of recombinant (including EHL) products and monitoring other, non-factor therapies → measuring FVIII/IX **concentrates in deficient plasma** or measuring **non-replacement drugs' levels** in plasma. In case of non-replacement products: determine the **hemostatic effect** of the drug, **measurement of FVIII/FIX levels in the presence of a certain drug**
3. Detecting and determining the concentration (amount) of **inhibitors** directed to clotting factors or to the therapeutic drug (ADA)

Difficulties: It is difficult to choose the most appropriate test for the different purposes. Clinical laboratory studies are still ongoing and more are warranted to solve the emerging problems.

One-stage clotting assay (FVIII or FIX)



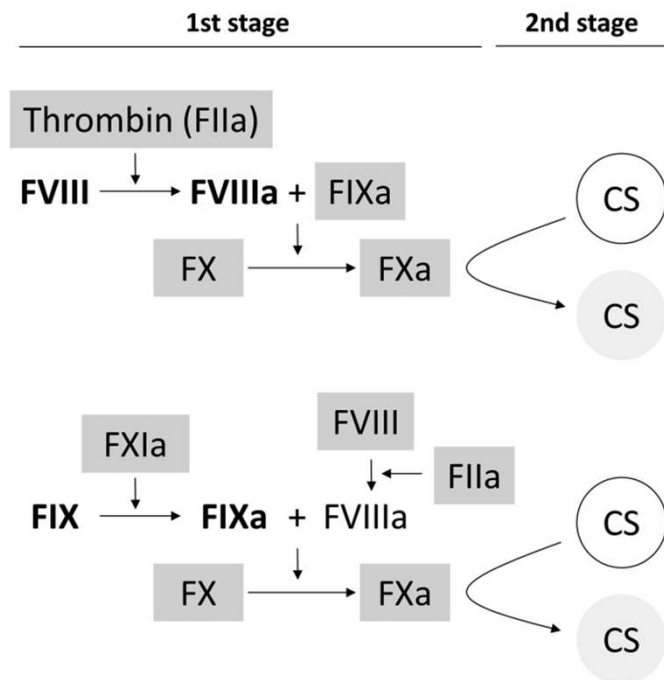
1. Moser KA, Adcock Funk DM. *Am J Hematol.* 2014;89:781-784; 2. Potgieter JJ et al. *Eur J Haematol.* 2015;94 Suppl 77:38-44; 3. Peyvandi F et al. *J Thromb Haemost.* 2016;14:248-261; 4. http://www.practical-haemostasis.com/Factor%20Assays/1_stage_aptt_assays.html. Accessed June 6, 2016.



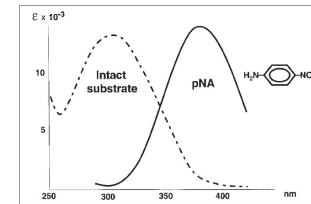
Essential components of one-stage clotting assay:

- 1, Factor deficient plasma (FVIII deficient or FIX deficient, ie. FVIII/FIX activity less than 1%)
- 2, **Activator (APTT reagent: surface activator + phospholipids)**
- 3, CaCl₂ (25 mmol/L)
- 4, **calibrator** (given potency of FVIII or FIX, calibrated against WHO reference preparation or plasma)
- +1, buffer for plasma/calibrator dilution

Chromogenic assay (FVIII or FIX)



Müller et al. Haemostaseologie, 2022



eg. Ile-Glu-Gly-Arg-pNA

The peptide mimics the FXa cleavage site in prothrombin

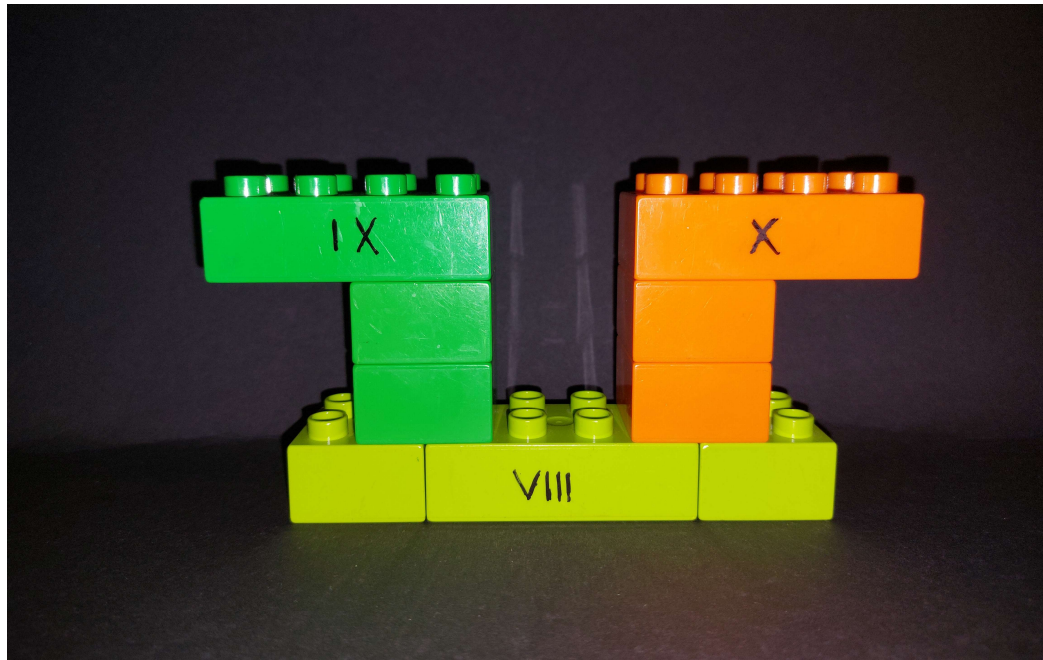
Essential components of chromogenic assay:

- 1, **purified clotting factors (FX, FIXa, thrombin, or FX, FVIII, FXIa), PL and Ca²⁺ (kit)**
- 2, chromogenic substrate
- 3, **calibrator** (given potency of FVIII or FIX, calibrated against WHO reference preparation or plasma)
- 4, phospholipids, Ca²⁺
- +1, buffer for plasma/calibrator dilution

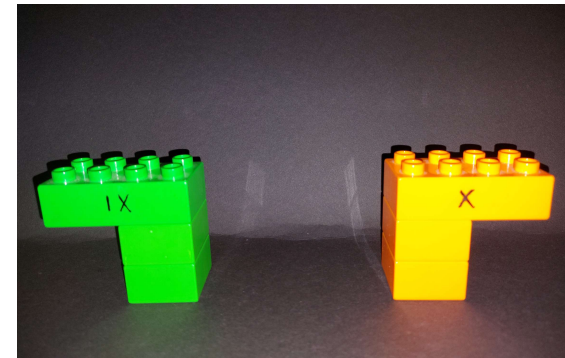
This method is recommended for potency labeling of recombinant factor products in Europe.

Factor “replacement” therapy

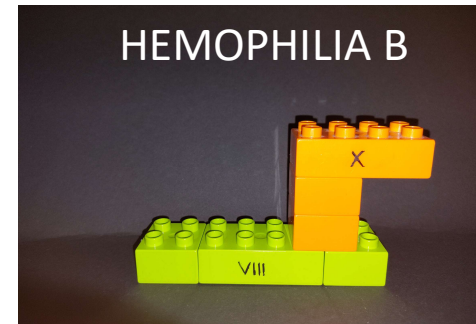
Normal TENASE (X-ase) complex



HEMOPHILIA A



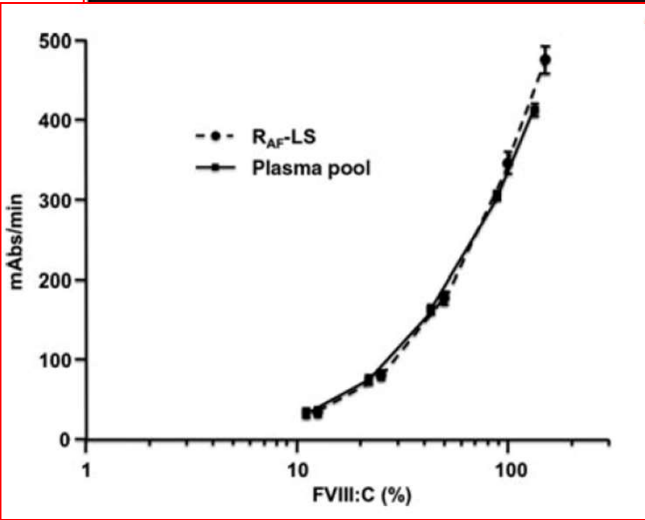
HEMOPHILIA B



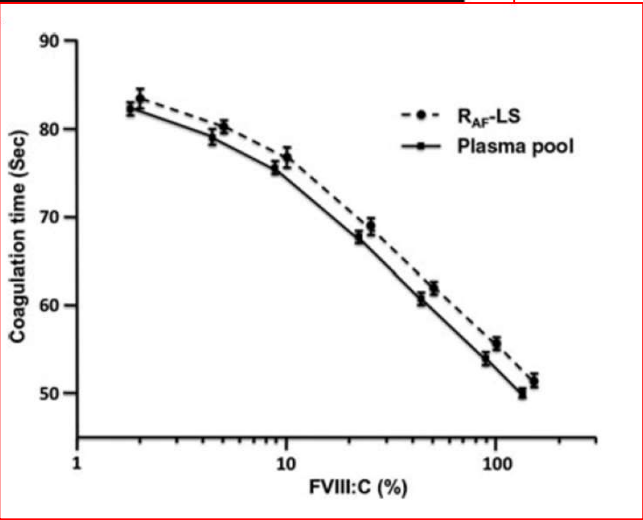
Laboratory issues in case of Moroctocog alfa (a B-domain depleted rFVIII)

Coagulation time (s)*	FVIII:C (%) calibration curves generated with		Difference (%) [§]
	Plasma [†]	R _{AF} LS [‡]	
55	80.9	106.8	−32.0
60	48.7	64.2	−31.6
65	28.7	37.8	−31.5
70	16.3	21.5	−31.7
75	8.7	11.6	−32.7

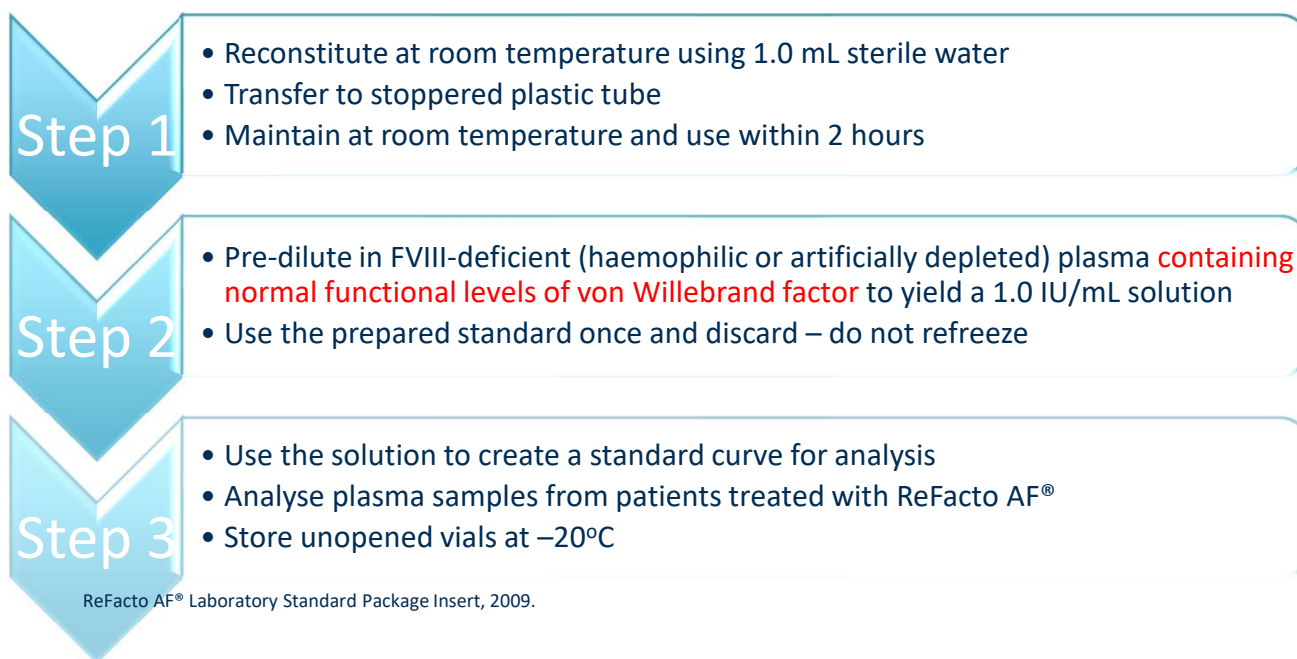
CSA



OSA



Use of ReFacto AF® Laboratory Standard



Complete description can be found in the ReFacto AF® Laboratory Standard package insert

EHL-products in Haemophilia A and B

Haemophilia A	Haemophilia B
PEGylated	
N8-GP (Turoctogog alfa pegol) BDD	N9-GP (nonacog beta pegol) FL
BAY94-9027 (Damoctocog alfa pegol) BDD	
BAX855 (Rurioctocog alfa pegol) FL	
Fc-fusion	
rFVIII-Fc (Efamoroctocog alfa) BDD	rFIX-Fc (eftrenonacog alfa) FL
Single chain FVIII	
rFVIII-SingleChain (Lonoctocog alfa) BDD	
Albumin-fusion	
	(albutrepenonacog alfa) FL

Factor VIII assay results in the one-stage clotting assay using different reagents for EHL-FVIII products

	rFVIII Fe ⁶	rFVIII-PEG ⁷	rFVIII-SC ^{10,11}	N&GP ⁸	BAY-94 ⁹
Silica reagents					
SynthaSIL	<u>Correct</u>	<u>Correct</u>	Underestimated	<u>Correct</u>	<u>Correct</u>
STA-PIT Automate	<u>Correct</u>	<u>Correct</u>	Underestimated	Underestimated	Underestimated
PTT-SP	<u>Unknown</u>	<u>Unknown</u>	Underestimated	Underestimated	Underestimated
Pathromtin SL	<u>Correct</u>	<u>Correct</u>	Underestimated	<u>Correct</u>	<u>Correct</u>
Trinicot Auto	<u>Correct</u>	<u>Correct</u>	Underestimated	Unknown	Unknown
Trinicot HS	<u>Correct</u>	<u>Correct</u>	Underestimated	Unknown	Unknown
Ellagic acid reagents					
Actin FS	<u>Correct</u>	<u>Correct</u>	Underestimated	<u>Correct</u>	<u>Correct</u>
Actin FSL	<u>Correct</u>	<u>Correct</u>	Underestimated	<u>Correct</u>	<u>Correct</u>
Synthafax	<u>Unknown</u>	<u>Correct</u>	Underestimated	Decreased	<u>Correct</u>
DG Synth	<u>Unknown</u>	<u>Unknown</u>	Unknown	<u>Correct</u>	Unknown
Kaolin reagents					
CK Prest	<u>Correct</u>	<u>Correct</u>	Underestimated	<u>Correct</u>	Unknown
Polyphenolic acid reagents					
Cephascreen	<u>Correct</u>	<u>Correct</u>	Underestimated	<u>Correct</u>	<u>Correct</u>

Incorrect results were underestimations in most of the cases.

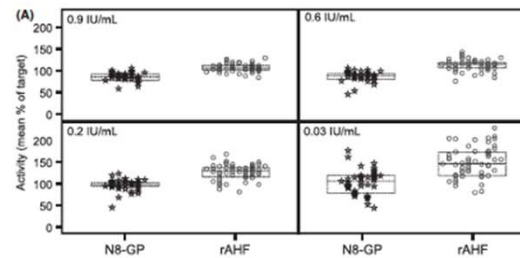
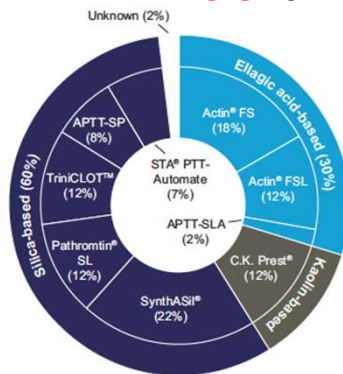
Use of correction factor of 2?

Method: the respective factor replacement products were spiked into severe congenital hemophilia A samples at different cc., and factor levels were reported as % recovery of the label potency.

ECAT study-OSA vs CSA

n=67 laboratories (n=60 OSA, n=36 CHR, n=29 both)

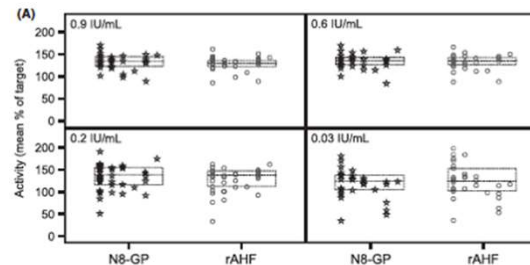
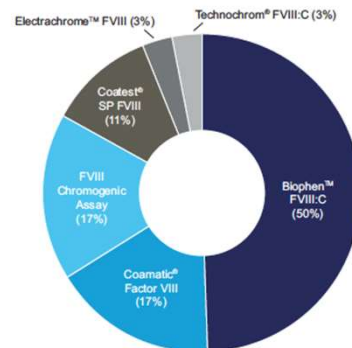
N8-GP or rAHF (Advate) spiked samples with 3%, 20%, 60% and 90% FVIII activity values



aPTT reagent	N8-GP target concentration (IU/mL)	Mean estimate (% of target)	95% CI (% target)
APTT-SP	0.03	56.9	45.1; 68.6
	0.2	59.1	48.5; 69.8
	0.6	58.6	47.9; 69.1
	0.9	56.9	46.3; 67.4
STA® PTT-Automate	0.03	59.8	40.0; 79.6
	0.2	43.1	22.1; 64.1
	0.6	43.8	22.7; 65.0
	0.9	44.1	23.7; 65.5
TriniCLOT™	0.03	82.9	69.8; 96.0
	0.2	47.7	40.4; 55.1
	0.6	42.6	35.4; 49.9
	0.9	39.7	32.4; 47.0

Abbreviation: CI, confidence interval.

Mean recovery: N8-GP 86-102% (inter-lab CV:11-26%); rAHF 109-146% (inter-lab CV:8-22%)



Mean recovery: N8-GP 119-135% (inter-lab CV: 12-27%); rAHF 123-135% (inter-lab CV: 12-29%)

Tiefenbacher et al. Haemophilia 2019;25:893-901.

Disease	Molecule name	Brand/generic name	SPC recommendations for laboratory monitoring
Hemophilia A	rFVIII-Fc	Eloctate [®] / Elocta ⁶⁷	When using an in vitro aPTT-based one-stage clotting assay for determining FVIII activity in patients' blood samples, plasma FVIII activity results can be significantly affected by both the <u>type of the aPTT reagent and the reference standard</u> used in the assay.
	BAX-855	Adynovate [®] / Adynovi ⁴⁰	A field study has indicated that plasma factor VIII levels can be monitored using either a <u>chromogenic substrate assay or a one-stage clotting assay</u> routinely used in clinical laboratories.
	rFVIII-SC	Afstyla ^{®45}	- When using an in vitro aPTT-based one-stage clotting assay for determining FVIII activity in patients' blood samples, plasma FVIII activity results can be significantly affected by both the <u>type of aPTT reagent and the reference standard</u> used in the assay. - Also there can be <u>significant discrepancies between assay results</u> obtained by aPTT-based one-stage clotting assay and the chromogenic assay according to Ph. Eur. - Plasma FVIII activity in patients receiving Afstyla using either the chromogenic assay or the one-stage clotting assay should be monitored to guide the dose administered and the frequency of repeat injections. - The <u>chromogenic assay result most accurately reflects the clinical hemostatic potential of Afstyla</u> and is preferred. - The <u>one-stage clotting assay result underestimates the FVIII activity level</u> compared to the chromogenic assay result by approximately 45%. - If the one-stage clotting assay is used, multiply the result by a <u>conversion factor of 2</u> to determine the patient's FVIII activity level.
	N8-GP	Esperoct ^{®42} (Turoctocog alfa pegol)	- The FVIII activity of Esperoct can be measured using the conventional FVIII assays, the chromogenic assay, and the one-stage assay. - When using an in vitro aPTT-based one-stage clotting assay for determining FVIII activity in patients' blood samples, plasma FVIII activity results can be significantly affected by both the <u>type of aPTT reagent and the reference standard</u> used in the assay. - When using a one-stage clotting assay <u>some silica-based reagents should be avoided</u> as they cause underestimation. - There can be significant discrepancies between assay results obtained by aPTT-based one-stage clotting assay and the chromogenic assay according to Ph. Eur.

FVIII product	Actin FS (Siemens)	Pathromtin SL (Siemens)	SynthasIL (Werfen)	SynthaFax (Werfen)	APTT-SP (Werfen)	PTT-Automate (Stago)	Cephasreen (Stago)
PEGylated							
N8-GP (Turoctogog alfa pegol)							?
BAY94-9027 (Damoctocog alfa pegol)							
BAX855 (Rurioctocog alfa pegol)					?		
Fc-fusion							
rFVIII-Fc (Efmoctocog alfa)				?	?		
Single chain FVIII							
rFVIII- SingleChain (Lonoctocog alfa)							

OSA with N8-GP specific calibration

N8-GP Reference Standard
Handling Instructions

Date: 24-Nov-2017
Version: 1.0
Status: Final
Page: 1 of 2



Preparation of a standard curve with the N8-GP standard

N8GP_RS2015 (Expiry Date: 30-May-2021)

After reconstitution the solution has a concentration of 581.5 U/mL

N8GP_RS2015
green cap

Reconstitution (dissolving the powder)

The solution will have a concentration of 581.5 U/mL

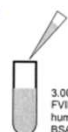
1. Remove the plastic cap and rubber stopper from the N8-GP vial. If the cap is loose or missing, do not use the vial.
2. Dispense slowly 4.3 mL solvent (0.9% sodium chloride solvent) into the powder vial. The pipette should be slightly tilted to allow for the solvent to be dispensed at the edge of the vial, just above the powder surface.
3. Gently swirl the vial until all powder is dissolved. Do not shake the vial, as this may cause 'foaming'. No mechanical whirl-mixing is allowed.
4. If the solution is discoloured or if it has bits or fragments in it, the solution should be discarded.
5. Use within 4 hours. Keep at room temperature (max 30°C or 86°F). Do not freeze.



4.3 mL reconstituted N8-GP standard; 581.5 U/mL

Dilution 1. This first dilution will have a concentration of 10.5 U/mL

6. Take 55 µL of the reconstituted reference standard into 3.00 mL of either FVIII deficient plasma or a buffer containing 1% human albumin or 1% bovine serum albumin (BSA). The human albumin should preferably be clinical grade and the BSA as pure as possible. A suitable buffer would be 50 mM of TRIS, Imidazole or HEPES, and 150 mM sodium chloride.



IMPORTANT: If using FVIII deficient plasma this must contain at least 40% (40 IU/dL) von Willebrand Factor (vWF).

Mix gently to avoid foaming; mix by aspirating and dispensing the liquid 10 times with a clean pipette tip. Do not whirl-mix.

7. Pipette 50 µL of the diluted reference standard into 0.500 mL FVIII deficient plasma containing ≥ 40% vWF.

IMPORTANT: It is mandatory to use a FVIII deficient plasma containing ≥ 40% vWF.

Mix gently to avoid foaming; mix by aspirating and dispensing the liquid 10 times with a clean pipette. Do not whirl-mix.

2. This second dilution will have a concentration of 1.0 U/mL

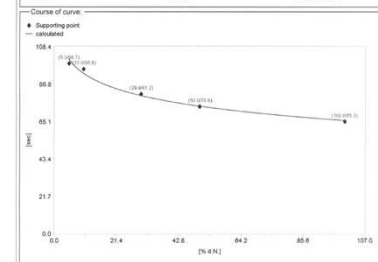
8. The reference standard will now have a concentration of 1.0 U/mL (100 IU/dL; 100%), and can be used to generate a standard curve.



Note: N8-GP diluted in FVIII deficient plasma w. ≥ 40% vWF can be aliquoted and stored at <-70°C for future use.

BCS XP Coagulation System		Reference curve	BCS XP1
DEDEC KBMP1		LabManager	
General information			
Name of reference curve:		FVIII.PSLN8GP_N8GP_2019-11-21_1328	
Creation date:		11/21/2019 / 14:05 PM	
Assay measured:		FVIII.PSLN8GP V	
Evaluation method:		Regression, loglin	
Expiration date:		Standard (measured)	
Curve type:		Standard (measured)	
Correlation coefficient:		-0.99	

Reagent lots used:	Lot number	Ref. value
NaCl	000101	
Path SL	536709	
FVIII EP	541691	
CaCl2	503851	
N8GP	820001	100.00 % d.N.



Checklist of reagents

N8-GP reference standard

0.9% sodium chloride

FVIII deficient plasma with ≥ 40% vWF

(e.g. "Factor VIII deficient plasma" from Siemens, product code OTXW17)

1% albumin buffer:

Clinical grade human albumin or high purity bovine serum albumin (BSA)

TRIS, Imidazole or HEPES

Sodium chloride

N8-GP QC samples mad in-house or provided by Novo Nordisk

Case 1

FVIII activity measurement in a patient treated by N8-GP turoctocog alpha-pegol

FVIII was measured by OSA using N8-GP or plasma standard for calibration and by CSA using plasma standard for calibration.

Factor VIII activity (%)

OSA Cal. N8-GP standard	OSA Cal. Plasma standard	CSA Cal. Plasma standard	% recovery (OSA plasma std/ N8-GP std)	% recovery (CSA plasma std/ N8-GP std)
100	86	97	86	97
66	60	86	91	130
154	142	206	92	134
15	14	19	93	127

Case 2

Adult patient with hemophilia A. He has been on plasma-derived FVIII for prophylaxis and now 5000 U lonoctocog alpha (scFVIII) is introduced.

In order to determine PK for him, blood samples were collected at different time points and FVIII was measured.

Results (OSA-Pathromtin as activator; CSA-Berichrom FVIII on BCS-XP)

Time	FVIII:C% (OSA)	FVIII:C% (CSA)	FVIII BE
30 min	82 (164)	116	Conversion factor of 2???
4 h	44 (88)	86	
24 h	14 (28)	19	
48 h	1 (2)	3,3	
5 days	<1	<1	<0,5
30 min	69 (138)	117	
30 min plasma-derived product	127	120	

Factor IX assay results in the one-stage clotting assay using different reagents

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

overestimation was 4-fold!

Young GA. J Thromb Haemost; 2019.17:1-7.

Measurement of all three concentrates is problematic and there is no consistency in results from one product to another.

Chromogenic FIX assays

Components: purified FXIa, (thrombin), FVIII and FX , CHR substrate, calcium, PL



**HYPHEN**
BioMed
A Sysmex Group Company

BIOPHEN™ FIX
Hemophilia B | FIX Assay

	R1, R2, R3: 2 x 1 mL, R4: 2 x 15 mL	221801
CE-IVD	R1, R2, R3: 2 x 2.5 mL, R4: 2 x 25 mL	221802
	R1, R2, R3: 2 x 6 mL, R4: 4 x 25 mL	221806

**HemosIL Chromogenic Factor IX Receives
510(k)-Clearance from US FDA and CE Mark in
Europe**

 Tuesday, 04 June 2024 00:00



 **Get in touch**
with the EEMEA team:
eemea@werfen.com

werfen

Disease	Molecule name	Brand/generic name	SPC recommendations for laboratory monitoring
Hemophilia B	rFIX-Fc	Alprolix ^{®43}	- When using an in vitro aPTT-based one-stage clotting assay for determining FIX activity in patients' blood samples, plasma FIX activity results can be significantly affected by both the type of aPTT reagent and the reference standard used in the assay. - Measurements with a one-stage clotting assay utilizing a kaolin-based aPTT reagent will likely result in an underestimation of activity level.
	rFIX-FP (CSL654)	Idelvion ^{®44}	- When using an in vitro aPTT-based one-stage clotting assay for determining FIX activity in patients' blood samples, plasma FIX activity results can be significantly affected by both the type of aPTT reagent and the reference standard used in the assay. - Measurement with a one-stage clotting assay using a kaolin-based aPTT reagent or Actin FS aPTT reagent will likely result in an underestimation of activity level.
	N9-GP	Refixia ^{®41} / Rebinyn [®]	- Due to the interference of polyethylene glycol (PEG) in the one-stage clotting assay with various aPTT reagents, it is recommended to use a chromogenic assay (eg, Rox Factor IX or Biophen) when monitoring is needed. - If a chromogenic assay is not available, it is recommended to use a one-stage clotting assay with an aPTT reagent (eg, Cephascreen) qualified for use with Refixia. - For modified long-acting factor products it is known that the one-stage clotting assay results are highly dependent on the aPTT reagent and reference standard used. - For Refixia some reagents will cause underestimation (30-50%), while most silica-containing reagents will cause severe overestimation of the factor IX activity (more than 400%). Therefore, silica-based reagents should be avoided. - Use of a reference laboratory is recommended when a chromogenic assay or a qualified one-stage clotting assay is not available locally.

FVIII product	Actin FS (Siemens)	Pathromtin SL (Siemens)	SynthasIL (Werfen)	SynthaFax (Werfen)	PTT- Automate (Stago)	Cephasreen (Stago)
PEGylated						
N9-GP, (nonacog beta pegol)						
Fc-fusion						
(eftrenonacog alfa)						
albumin fusion						
(albutrepenona cog alfa)						?

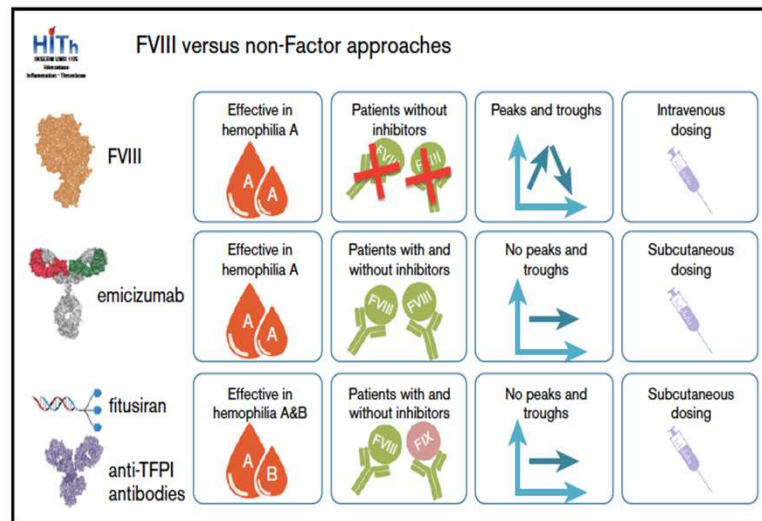
CSA is NOT RECOMMENDED

Conversion factors (?)
(Irsara et al. Hemostaseologie
2025,
DOI [https://doi.org/ 10.1055/a-2599-9908](https://doi.org/10.1055/a-2599-9908))

Drug-specific calibration for EHL-rFIX reduces reagent-related variability in OSA FIX assays, ensuring reliable and standardised results. (Grand et al. Haemophilia 2025; <https://doi.org/10.1111/hae.70115>.)

The role of the laboratory in case of non-replacement therapy

Laboratory management may include



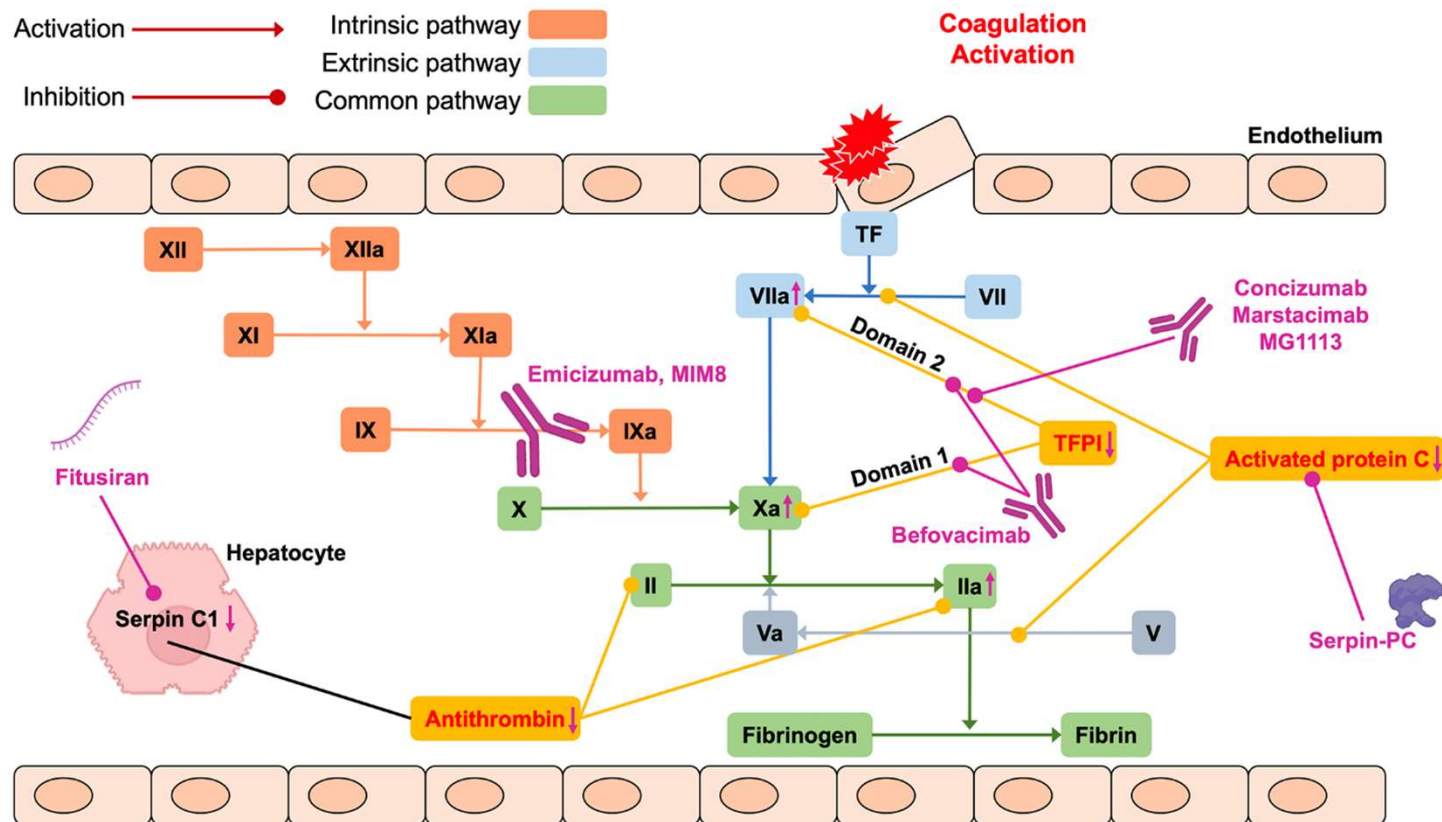
From Lentig PJ. Blood advances 2020;4:9.

1, Monitoring levels of the therapeutic agent. (?) - no PK, weak/undetermined correlation between drug level and its hemostatic effect.

2, Monitoring the hemostatic effect of the therapy. (?) - which test(s) to choose

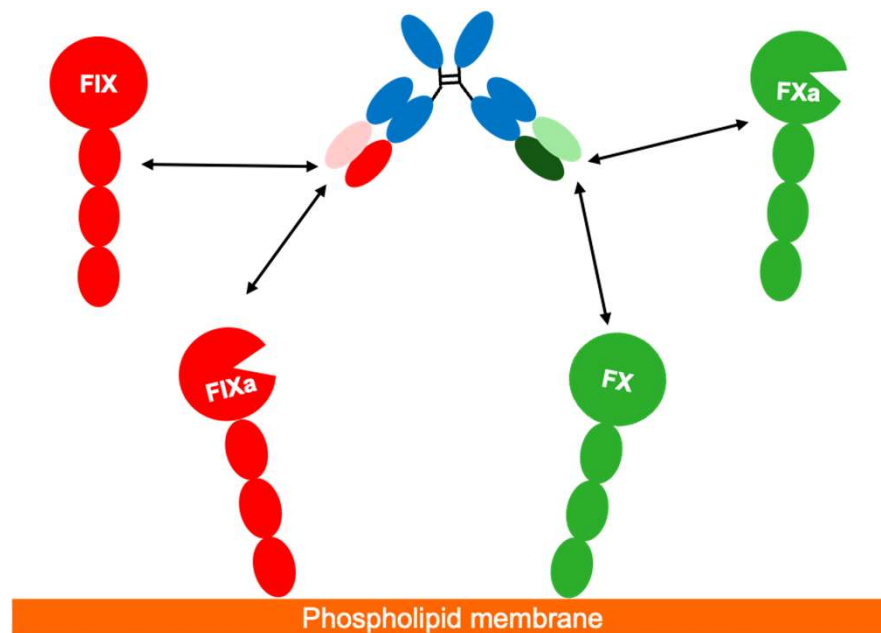
3, Monitoring the FVIII-equivalence of the therapy. (?) - does it make any sense?

4, Detecting and determining inhibitors. - anti-FVIII/FIX and ADA



Gualtierotti et al. Pharmaceuticals 2022; 15:1183.

Features of emicizumab, comparison with FVIII



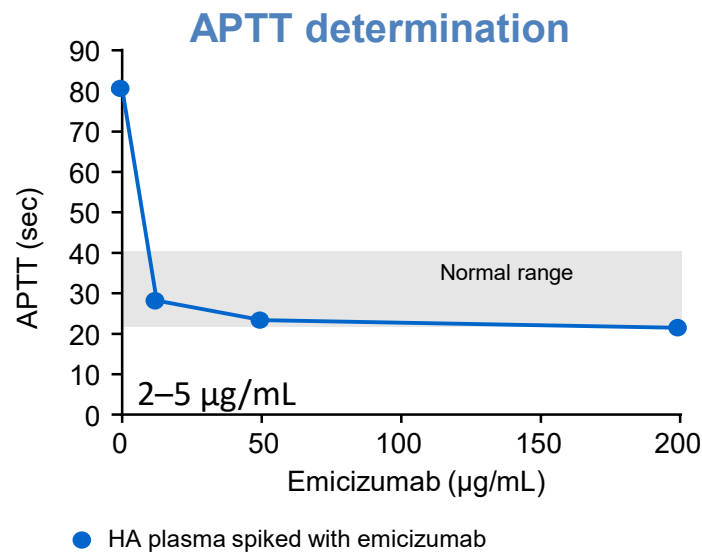
FVIIIa	Emicizumab
Specific binding to FIXa and FX (no binding to FIX and FXa)	Equally strong binding to FIX and FIXa / FX and FXa
High affinity to FIXa	Less preferable in the presence of FVIII
Full cofactor activity - Increases PL-binding of coagulation complexes - Stabilizes active center of FIXa - Binds FIXa to FX	- Partial cofactor activity - Binds FIXa to FX - Cofactor activity depends on the presence of FIXa and PL
Requires activation by thrombin	Does not require activation by thrombin
on/off mechanism	Np on/off mechanism, but the effect is dependent on the presence of FIXa

Since emicizumab effect is strongly dependent on FIXa, presence and amount of FIXa influence the results of the PL-dependent assays.

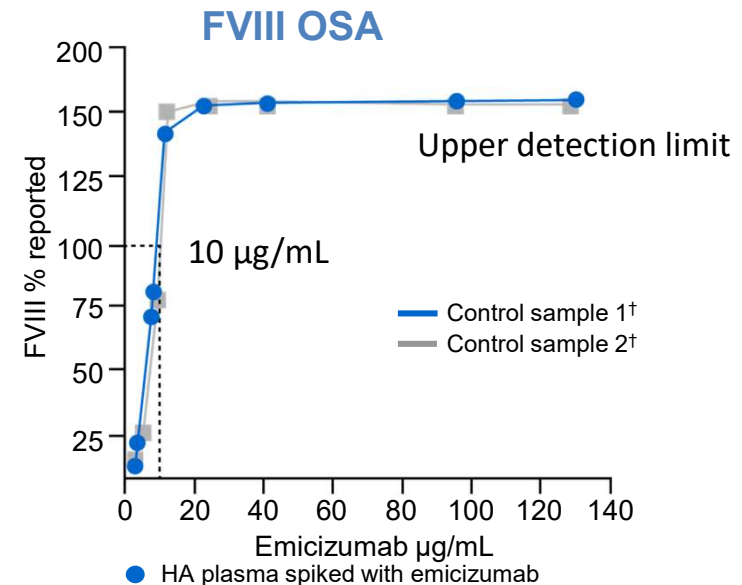
1. Kitazawa T, et al. Thromb Haemost 2017;117:1348–57;
2. Lenting PJ, et al. Blood 2017;130(23):2463–8.

Effect of emicizumab on APTT and on the classical one-stage clotting assay for FVIII

Monitoring



There is no need for “FVIII activation” for emicizumab effect, which shortens clotting time.
APTT is not reflecting to the patient’s coagulation state.



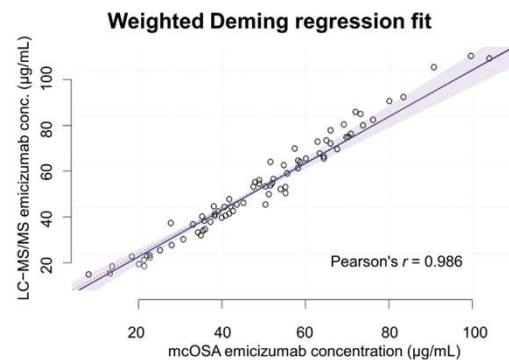
Not suitable for emicizumab measurement, measured “FVIII activity” of patients on emicizumab is above 150%.
Not suitable for FVIII inhibitor detection (underestimation).

1. Adamkewicz J, et al. Thromb Haemost 2019;119(7):1084–93;
2. Kitchen S, et al. WFH Laboratory Manual 2010. Available at: <http://www1.wfh.org/publications/files/pdf-1283.pdf> [Accessed September 2021];
3. Tagariello G, et al. Blood Transfus. 2017;15:557–561.

Determination of emicizumab “level” in modified one-stage clotting assay

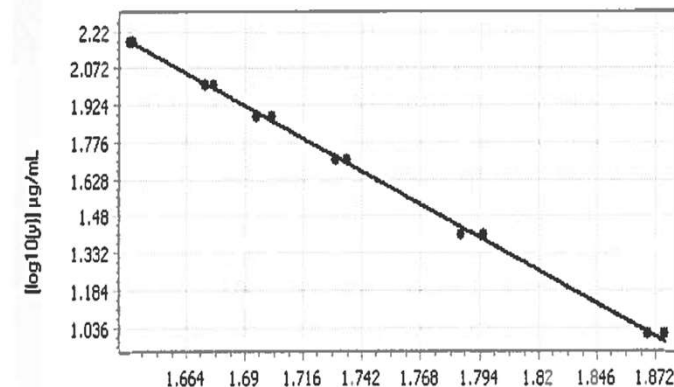
Monitoring

- r2 Diagnostica (Emicizumab calibrator and 2 level control plasma)
- Internationally approved protocol for ACL TOP (Werfen) and BCS-XP/Sysmex (Siemens) coagulometers, 2-150 µg/mL measuring range



Donners AAMT, et al. RPTH 2022;6:e12725.

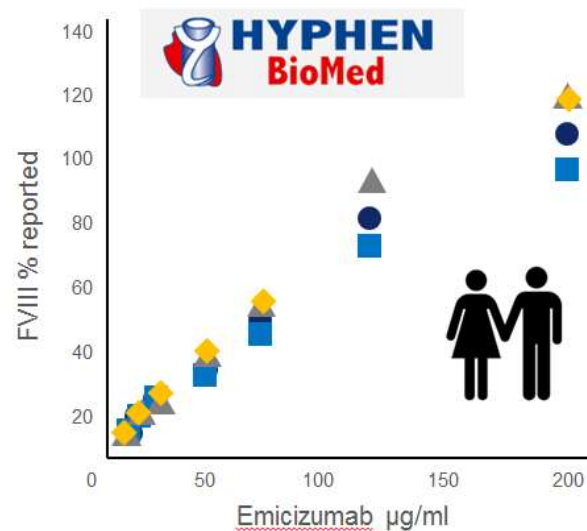
Results obtained by modified OSA and LC-MS/MS method show satisfactory correlation.



Concentration	Target	%CV	Mean Value		
150.00 %	150.000		43.7	43.6	43.7
100.00 %	100.000		47.3	47.1	47.5
75.00 %	75.000		50.0	49.6	50.4
50.00 %	50.000		54.1	53.8	54.4
25.00 %	25.000		61.8	61.1	62.5
10.00 %	10.000		74.6	73.9	75.2

Behavior of emicizumab in human and bovine FIXa-FX based chromogenic assays

Monitoring/inhibitor testing



Sensitive to both emicizumab and FVIII
FVIII value obtained by this assay is not representative for the patient's own FVIII level.

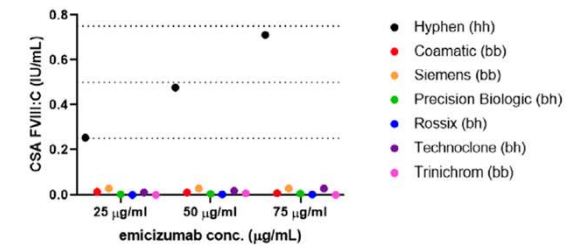
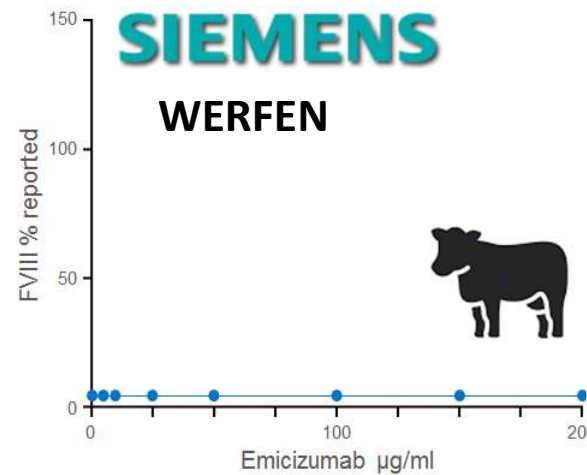


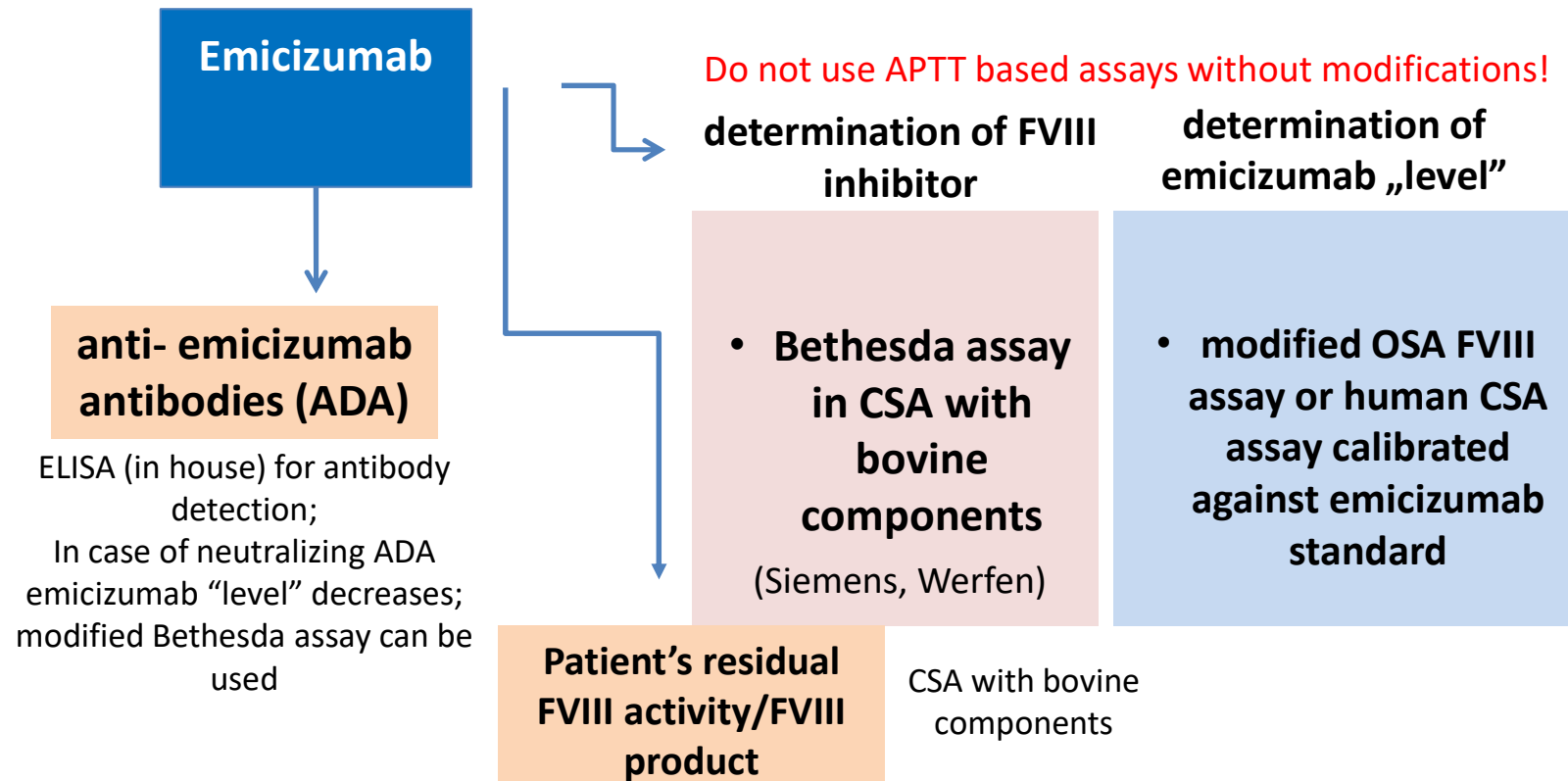
FIGURE 1 Chromogenic (CSA) FVIII:C of SHA plasma spiked with emicizumab 25–75 µg/ml using 7 CSA reagents. Coamatic (bb), Precision Biologic (bh), Rosix (bh), Technoclone (bh), Hyphen (hh), Trinichrom (bb), Siemens (bb) where bb is bovine FX/bovine FIXa, bh is bovine FX/human FIXa and hh is human FX/human FIXa. The dashed lines indicate 25, 50 and 75 µg/ml

Bowyer AE, et al. Int J Lab Hematol 2022;45:368–76

- **NOT sensitive to emicizumab**
- sensitive to FVIII
 - even in the presence of emicizumab the true FVIII level of the patient is measured (useful for inhibitor testing or measuring residual FVIII level of the patient)

Emicizumab-related methods, according to clinical questions-recommendations

Monitoring/inhibitor testing



Classical OSA FVIII determination does not reflect to the patient's real FVIII activity and emicizumab effect, therefore FVIII determination in OSA is absolutely useless and may be misleading.

Emicizumab “efficacy” in a boy with HA with inhibitors

5-years old boy with severe HA. He was put on prophylaxis early with rFVIII, then – due to a head injury and intracerebral bleeding – high dose rFVIII was administered.

Inhibitor development was detected, and ITI was unsuccessful at 2 occasions (anti-FVIII 4000 BU, spontaneous and post-traumatic joint bleedings)

Emicizumab therapy was started with clinical success.

Questions:

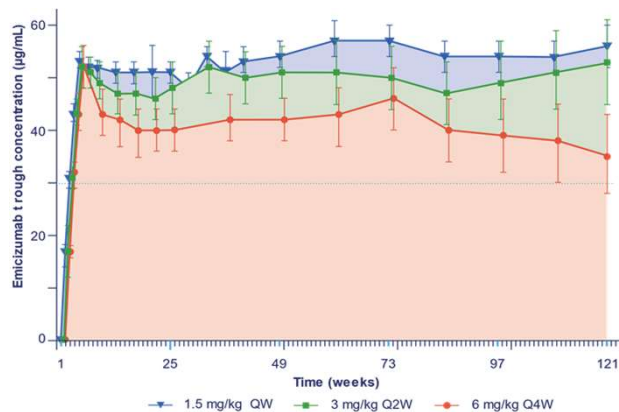
Does plasma emicizumab level meet the required concentration?

What is the anti-FVIII inhibitor level of the patient right now?

Laboratory results of the patient

Monitoring/inhibitor testing

APTT (sec)	Emicizumab (µg/mL)	FVIII activity% (CSA with bovine components)	FVIII activity % (classical OSA)	FVIII inhibitor BU (CSA with bovine components)	FVIII inhibitor BU (classical OSA)
26.6	46.5	<1	278	35.0	1.2



Which test results are correct?

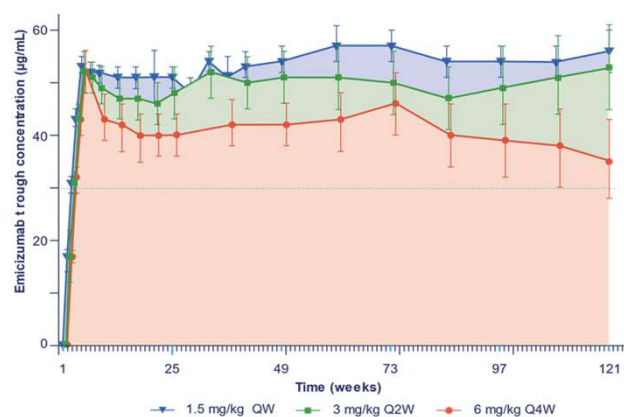
Plasma level of emicizumab is stable independently of dosing regimen.

1. Jimenez-Yuste V, et al. Blood Transfus. 2014 Jul;12(3):314-9;
2. Mahlangu. Expert Opinion on Pharmacotherapy. 2022;
3. Callaghan et al Blood. 2021 Apr 22;137(16):2231-2242

Laboratory results of the patient

Monitoring/inhibitor testing

APTT (sec)	Emicizumab (µg/mL)	FVIII activity% (CSA with bovine components)	FVIII activity % (classical OSA)	FVIII inhibitor BU (CSA with bovine components)	FVIII inhibitor BU (classical OSA)
26.6	46.5	<1	278	35.0	1.2



Plasma level of emicizumab is stable independently of dosing regimen.

date	APTT (sec)	Emicizumab (µg/mL)	FVIII activity % (bovine CSA)	FVIII inhibitor BU (bovine CSA)	FVIII activity % (OSA)
Month 1	26.6	46.5	<1	35.0	278
Month 2	28.2	25.0	<1	33.0	ND
Month 3	25.2	35.0	<1	43.0	303
Month 6	27.3	64.0	<1	17.0	381
Month 8	23.8	58.8	<1	13.5	ND
Month 12	24.2	58.8	<1	8.0	440

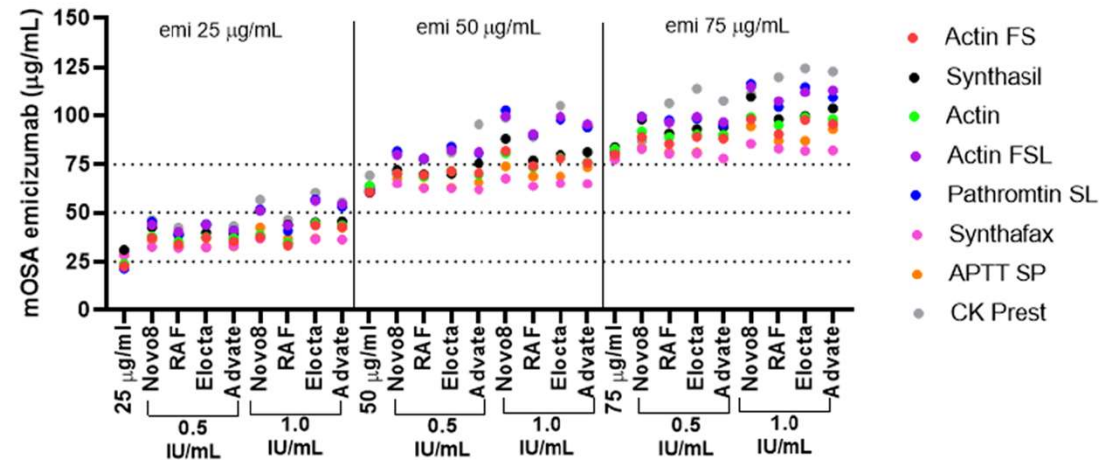
1. Jimenez-Yuste V, et al. Blood Transfus. 2014 Jul;12(3):314-9;
2. Mahlangu. Expert Opinion on Pharmacotherapy. 2022;
3. Callaghan et al Blood. 2021 Apr 22;137(16):2231-2242

Determination of emicizumab level in the presence of FVIII by modified OSA -be aware of overestimation-

Monitoring

FVIII products in the study:

- Novoeight (Novo Nordisk)
- Refacto AF (Pfizer)
- Elocta (Sobi)
- Advate (Takeda)



HA plasma was spiked with rFVIII products at 0.5 and 1.0 IU/mL final concentration and with emicizumab at 0, 25, 50 and 75 µg/mL final concentration.

Haemostasis assays which are influenced by the presence of emicizumab and Mim8

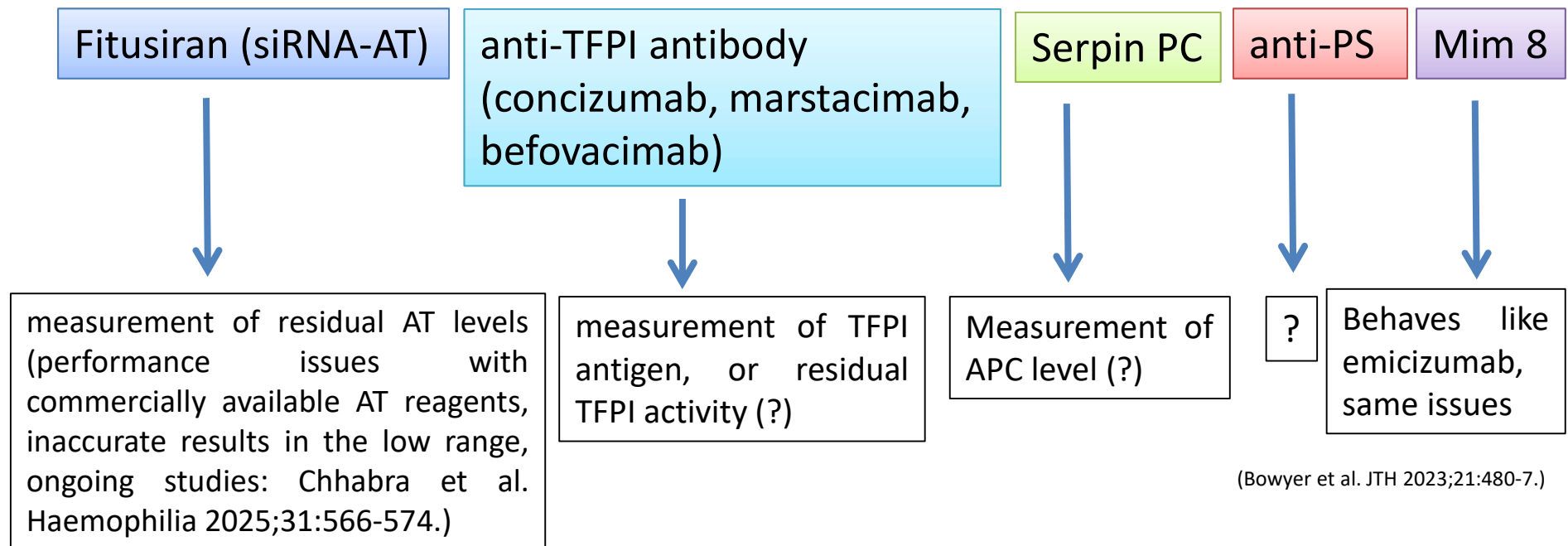
- APTT
- All assays based on APTT determination (eg. APC resistance functional assay, Protein C or Protein S activity in clotting assays, Activity assays for intrinsic clotting factors)

Haemostasis assays which are NOT influenced by the presence of emicizumab and Mim8

- PT, TT
- PT-based clotting factor activity determination (FII, FV, FX, FVII)
- Immunassays in haemostasis (eg. vWF:Ag, D-dimer, etc.)
- Genetic testing (eg. FV Leiden, FII20210G/A)
- Platelet function assays (eg. lumiaggregometry)

Monitoring of non-replacement therapeutic agents (rebalancing therapy)

Monitoring



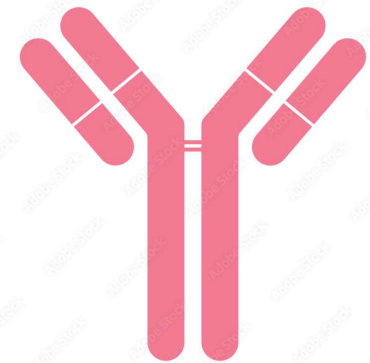
Results may be influenced by several pre-analytical and analytical issues (bypassing th, other hemophilia agents, etc.) Results may not reflect to clinical outcome and cannot be translated to FVIII activity.

FVIII activity determination and FVIII (FIX) inhibitor testing in the presence of non-replacement therapy

- Emicizumab, Mim8: chromogenic FVIII assay with bovin components
- Fitusiran and anti-TFPI ab: APTT-based one-stage clotting assay is OK
- SerpinPC: no experience, chromogenic assay should work

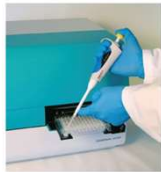
ADA testing in case of non-replacement therapy

- Special, in-house assays: ELISA, Western blotting, SPR methods



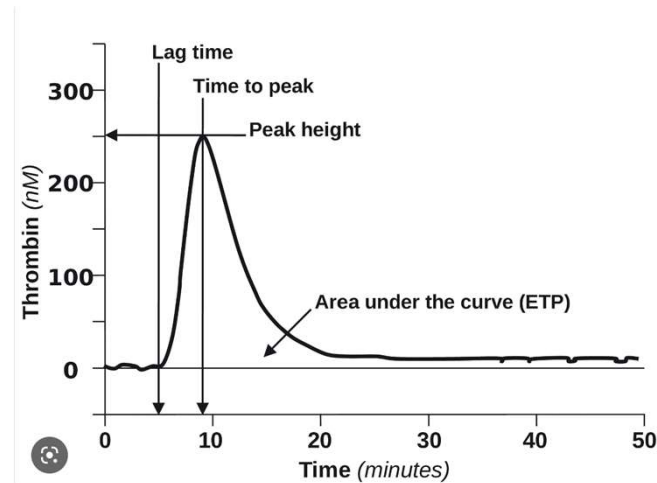
Measurement of Thrombin generation on different platforms

Hemostatic effect



CALIBRATED AUTOMATED THROMBOGRAM

The Calibrated Automated Thrombogram (CAT) is a comprehensive system to measure Thrombin Generation based on fluorescence according to the Hemker & al. method. (Research Product)



ST GENESIA SYSTEM

The ST Genesia offers a fully automated system to measure thrombin generation in plasma, with features like the once daily calibration, the reference plasma and the temperature control.

Diagnostica Stago



BCS XP System

Siemens

INNOVANCE ETP

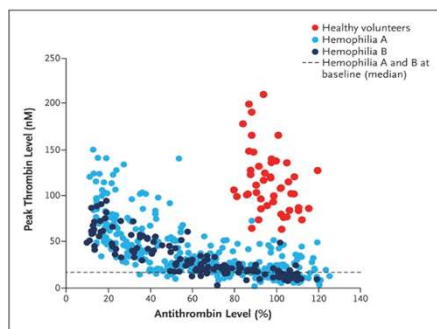
Our INNOVANCE ETP assay* is a new, innovative measure of global coagulability developed for routine clinical use.



Technoclone

Correlation of TG results with fitusiran and concizumab therapies

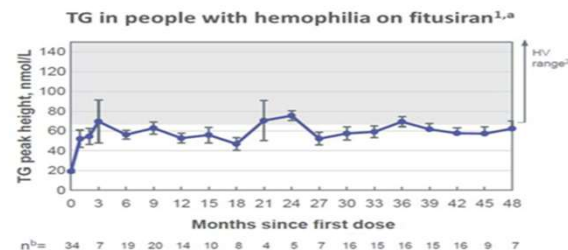
Hemostatic effect



Relationship between Antithrombin Level and Thrombin Generation.

Pasi et al. N Eng J Med 2017;377:819-28.

Figure: Longitudinal assessment of thrombin generation with monthly fitusiran from baseline to month 48

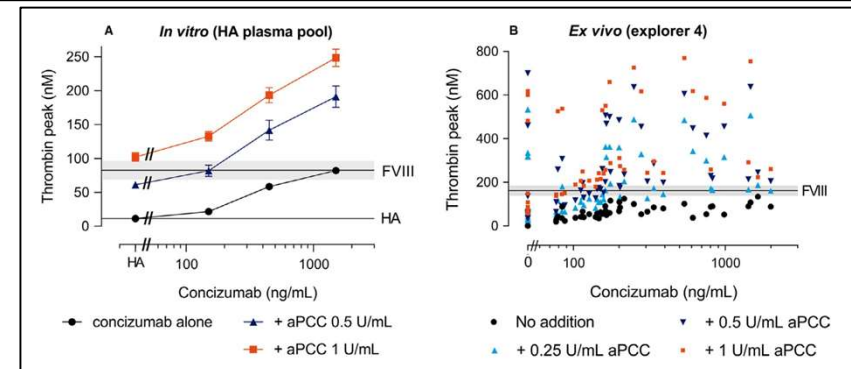
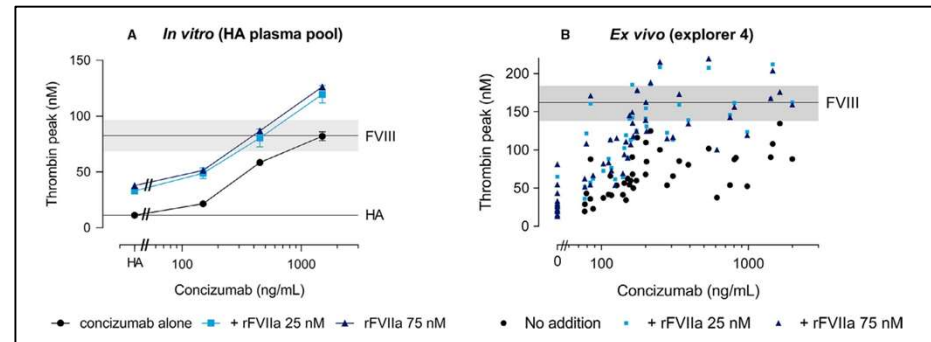


Data cutoff: March 10, 2020

*Data indicate mean TG peak height $\pm$ SE. Month 0 indicates baseline values. $n=5$ for month 1 and $n=6$ for month 2. AT, antithrombin; HV, healthy volunteer; OLE, open-label extension; TG, thrombin generation

1. Sanofi Genzyme. Data on file. 2. Pasi et al. N Engl J Med. 2017;377:819-828.

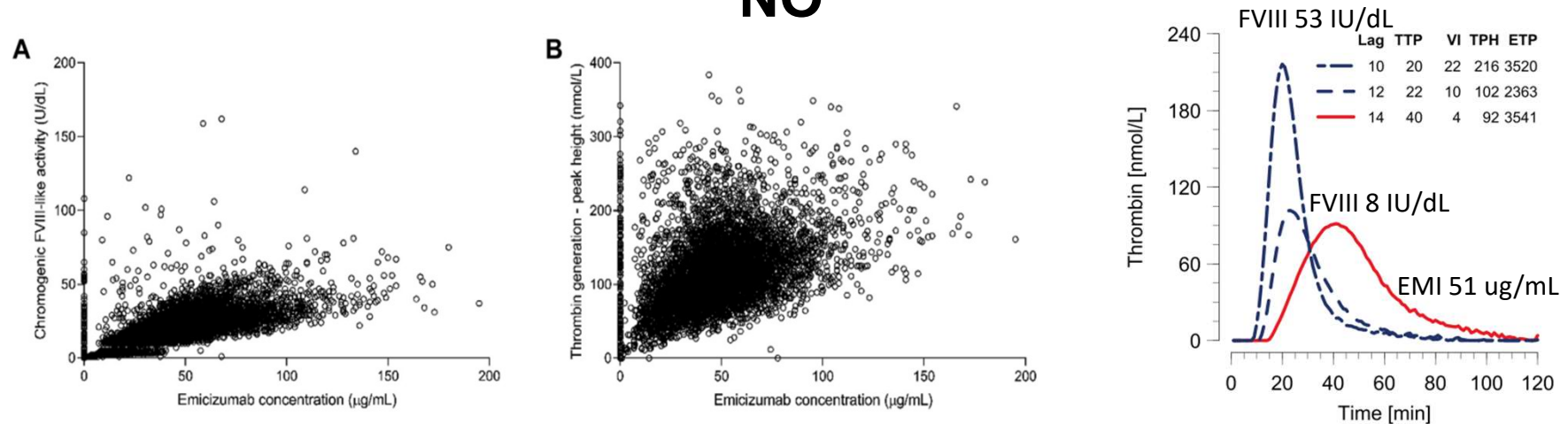
Négrier et al. Blood 2020;136:36-7.



Kjalke et al. J Thromb Haemost 2021;19:1687-96.

rFVIIa, APCC, and rFVIII were fully active in enhancing plasma TG potential in the presence of concizumab and suggested an up to approximately 25% additional effect conferred by drug-drug interaction.

Determining “FVIII-equivalence”? NO



There are important limitations when trying to estimate FVIIIeq by TGA or CSA. Estimation of FVIIIeq differs according to different TGA parameters and activators. Results show high inter-individual variability. While in the presence of FVIII the rate limiting step of tenase complex is the amount of FVIII, while in case of emicizumab the amount of FIXa is the rate limiting factor (where the presence of platelets is also critical), therefore FVIIIeq can not be estimated in plasma-based assays. Moreover, FVIIIeq does not systematically reflect a consistent hemostatic phenotype. Stating that emicizumab corresponds to a certain (in most studies 9%–20%) FVIII activity has limited clinical relevance.

Kiialainen et.al. Res Pract Thromb Haemost. 2024;8:e102306

Dargaud et al. Haemophilia 2025; 0:1-6.

Yamaguchi T, et al. Thromb Rest 2023;222:131–39.;

Kraemmer et al. Haemophilia 2025; 31:1092-1102.

Recommendations for clinical laboratories in the era of novel haemophilia therapies

1. Laboratories should follow the guidelines and before implementing an assay for a specific product an appropriate method validation should be performed. Monitoring of non-replacement therapy requires special methodology.
2. Companies should suggest a method for drug monitoring and support clinical labs.
3. Product-specific standard should be used for method calibration in certain situations (eg. emicizumab, Mim8, EHL products).
4. Standardization of global hemostasis tests requires further actions before using them in laboratory monitoring of hemophilia treatment. Correlation between TG and clinical outcome should be investigated. Results obtained from global assays can not be translated to “FVIII equivalent” values.
5. Inhibitor testing and ADA testing requires special methodology (in-house methods, novel and expensive techniques)
6. Clinical labs should be familiar with these challenges and communication between clinical and laboratory staff is crucial, in order to provide the best medical attendance for patients with hemophilia.