Pitfalls in de bepaling van Factor V

Marc Jacqu

UZLeuv

Patiënt met ernstige hemofilie A (FVIII < 1%)

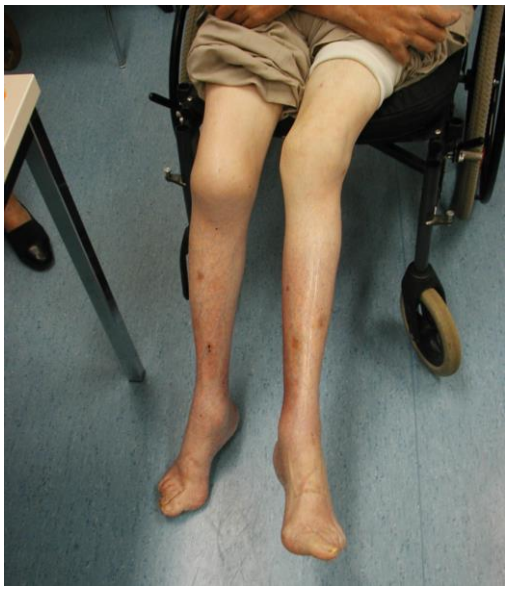
- APTT = 24.4 sec
- FVIII one stage assay: 348,6%
- FVIII chromogene test A: 25 %
- FVIII chromogene test B: <1%

misleidende informatie

! op spoed
! op de operatie kamer

Aangeboren tekort aan FVIII: hemofilie A:

- **bloedingen in zacht weefsels, spieren en gewrichten**
- **ernstige artropathie als een gevolg van terugkerende gewrichtsbloedingen**



Aangeboren tekort aan FVIII: hemofilie A:

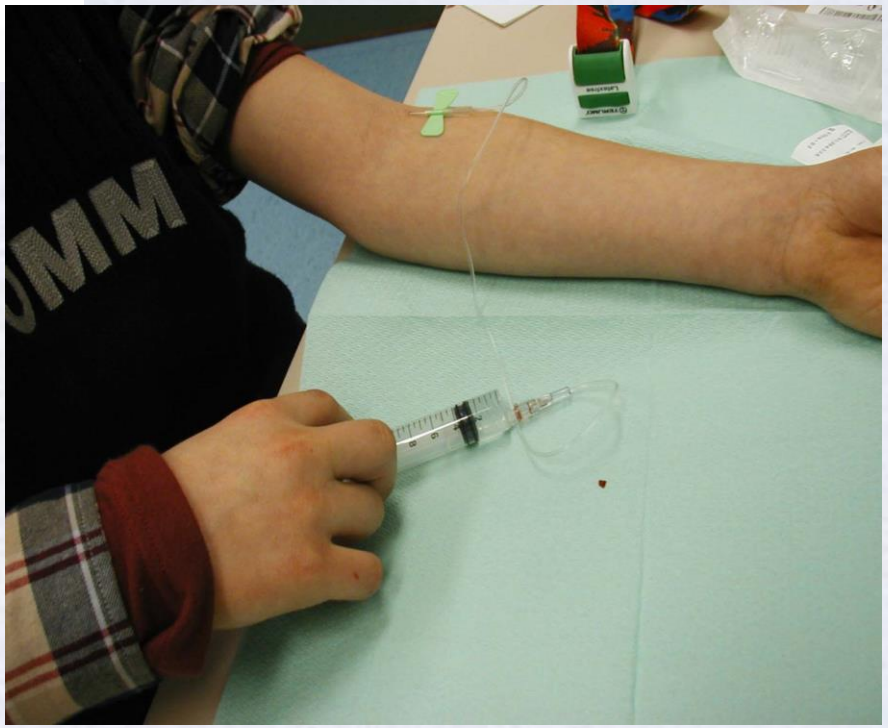
- bloedingen in zacht weefsels, spieren en gewrichten
- ernstige artropathie als een gevolg van terugkerende gewrichtsbloedingen
- **behandeld door de toediening van FVIII**

Prophylaxis

met FVIII

Lu	Ma	Me	Je	Ve	Sa	Di
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

180 i.v. injecties/jaar



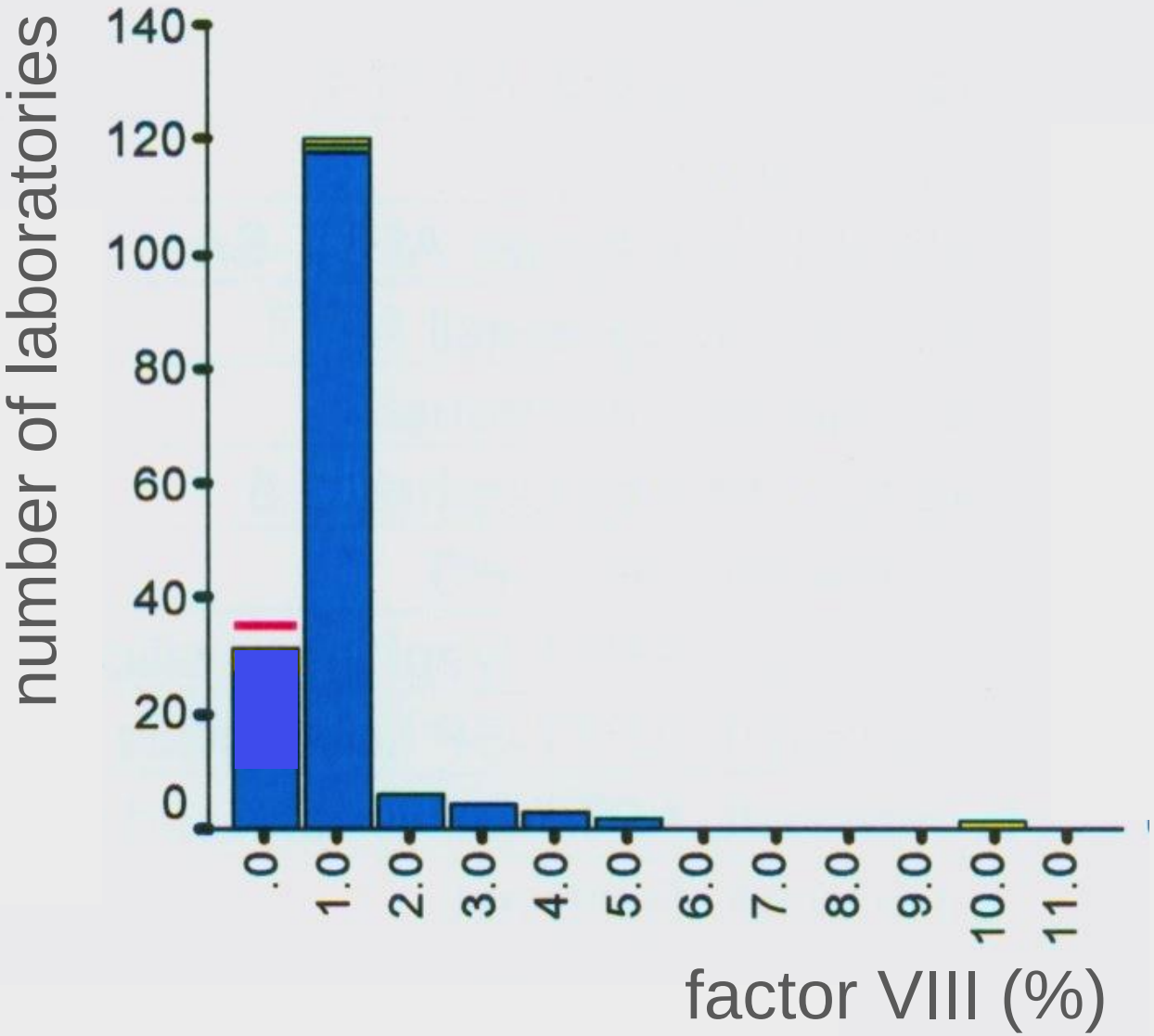
Diagnose van hemofilie A

- ernstige FVIII < 1%
- matige ernstige FVIII 1 -5%
- matige FVIII >5% and <40%

Diagnose van ernstige hemofilie A

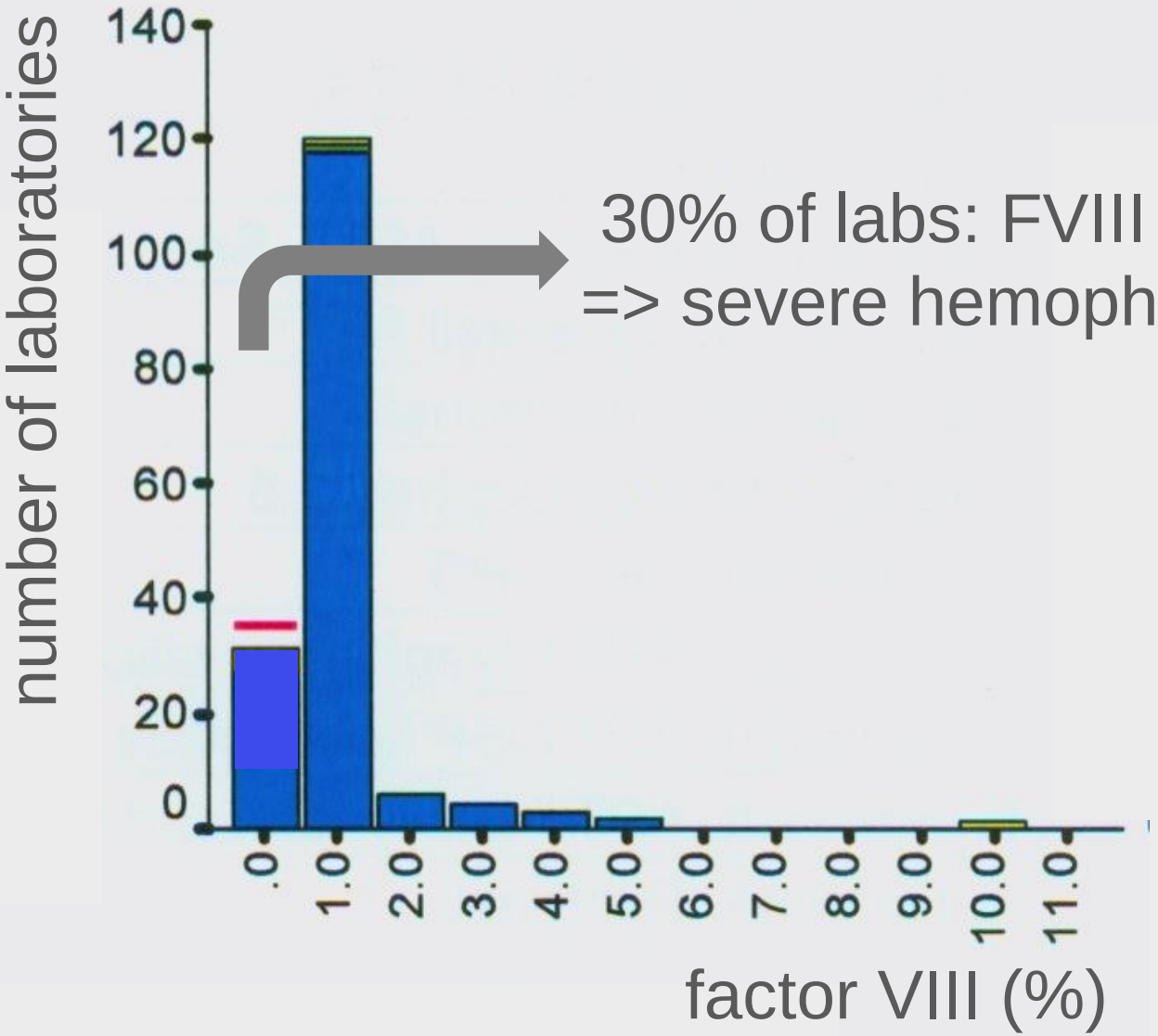
De meting van FVIII concentratie ≤1% blijft challenging

Plasma of a patient with a Factor VIII level <1%



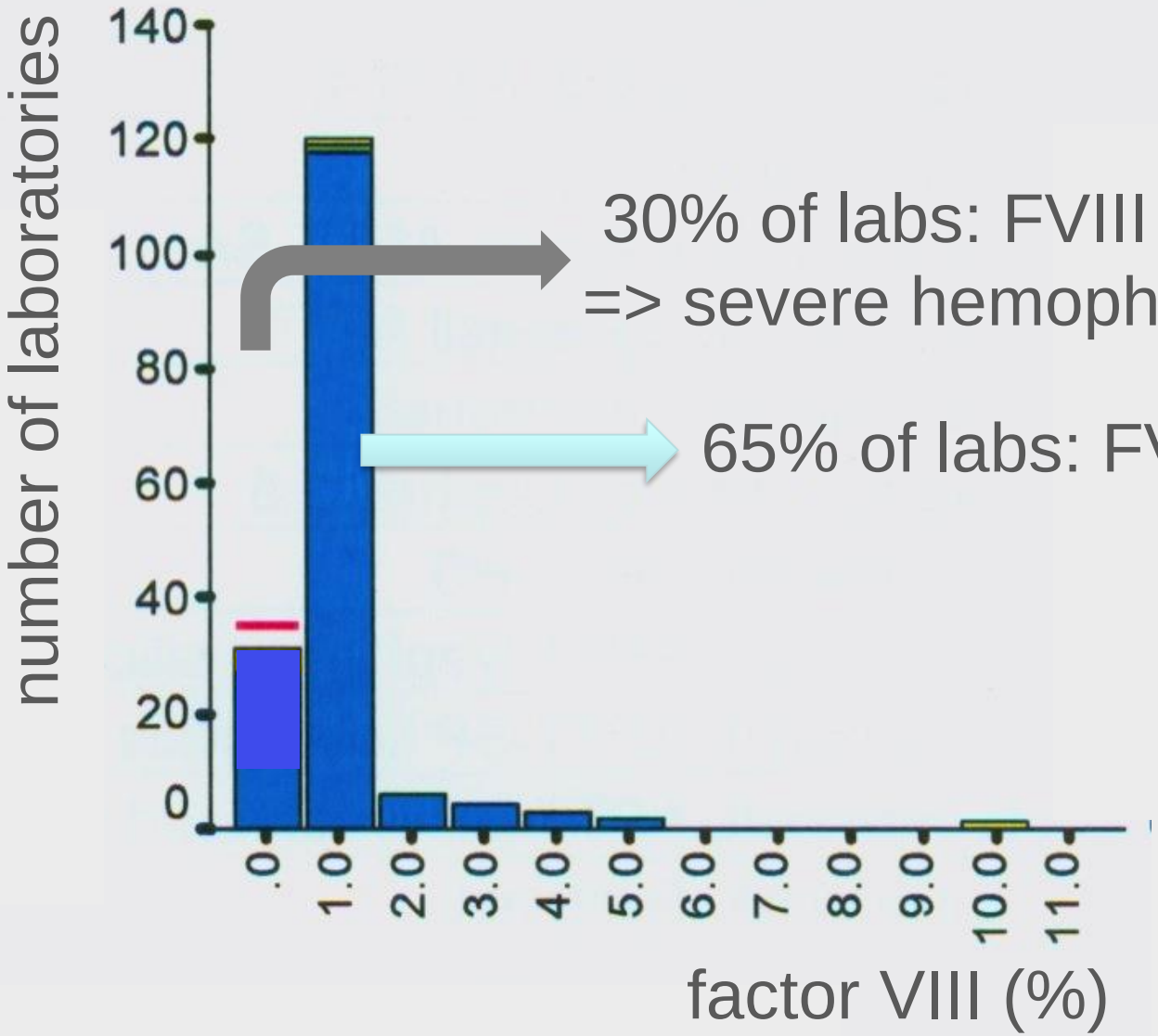
ECAT

Plasma of a patient with a Factor VIII level <1%



ECAT

Plasma of a patient with a Factor VIII level <1%

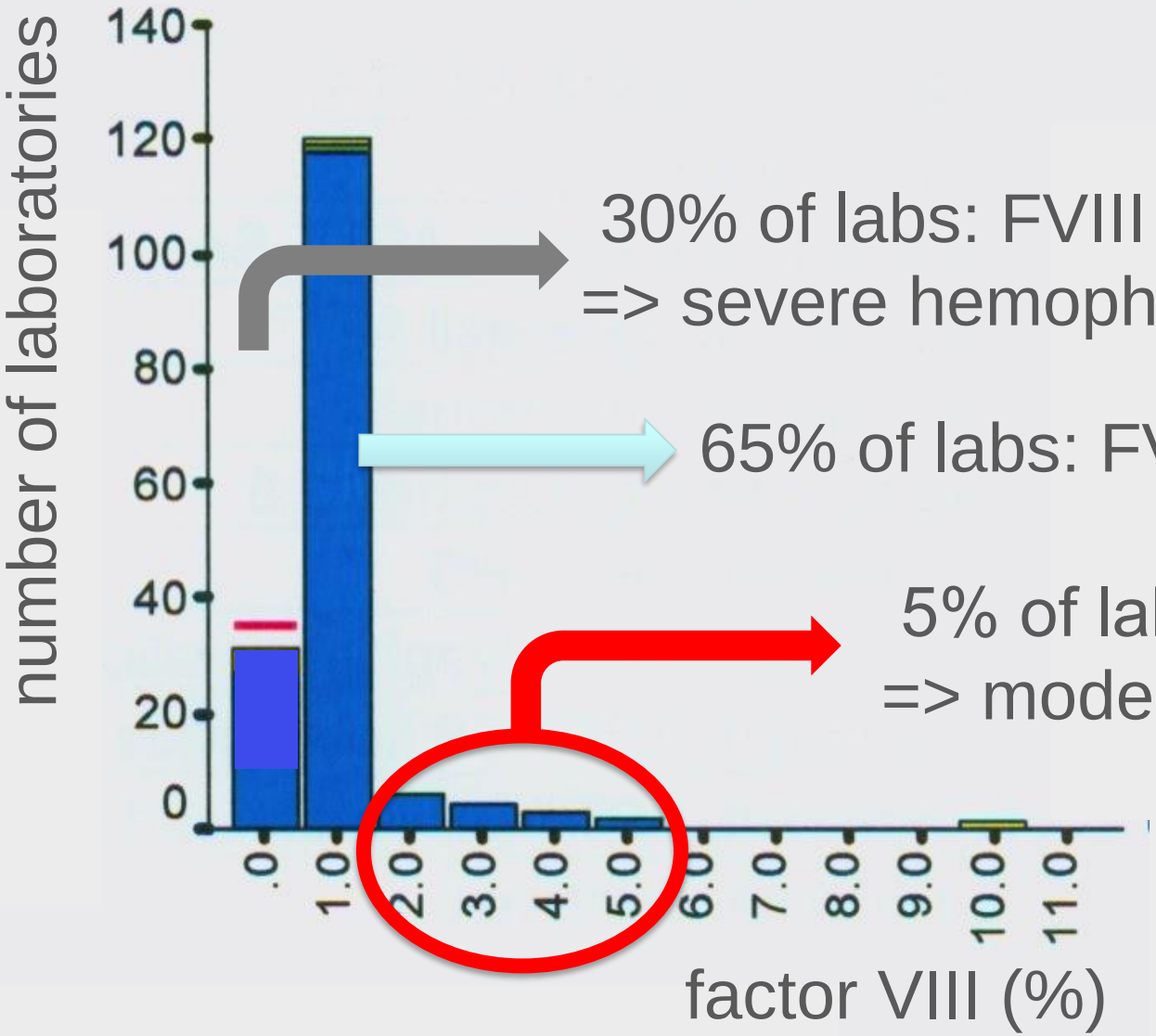


30% of labs: FVIII < 1%
=> severe hemophilia A

65% of labs: FVIII = 1%

ECAT

Plasma of a patient with a Factor VIII level <1%



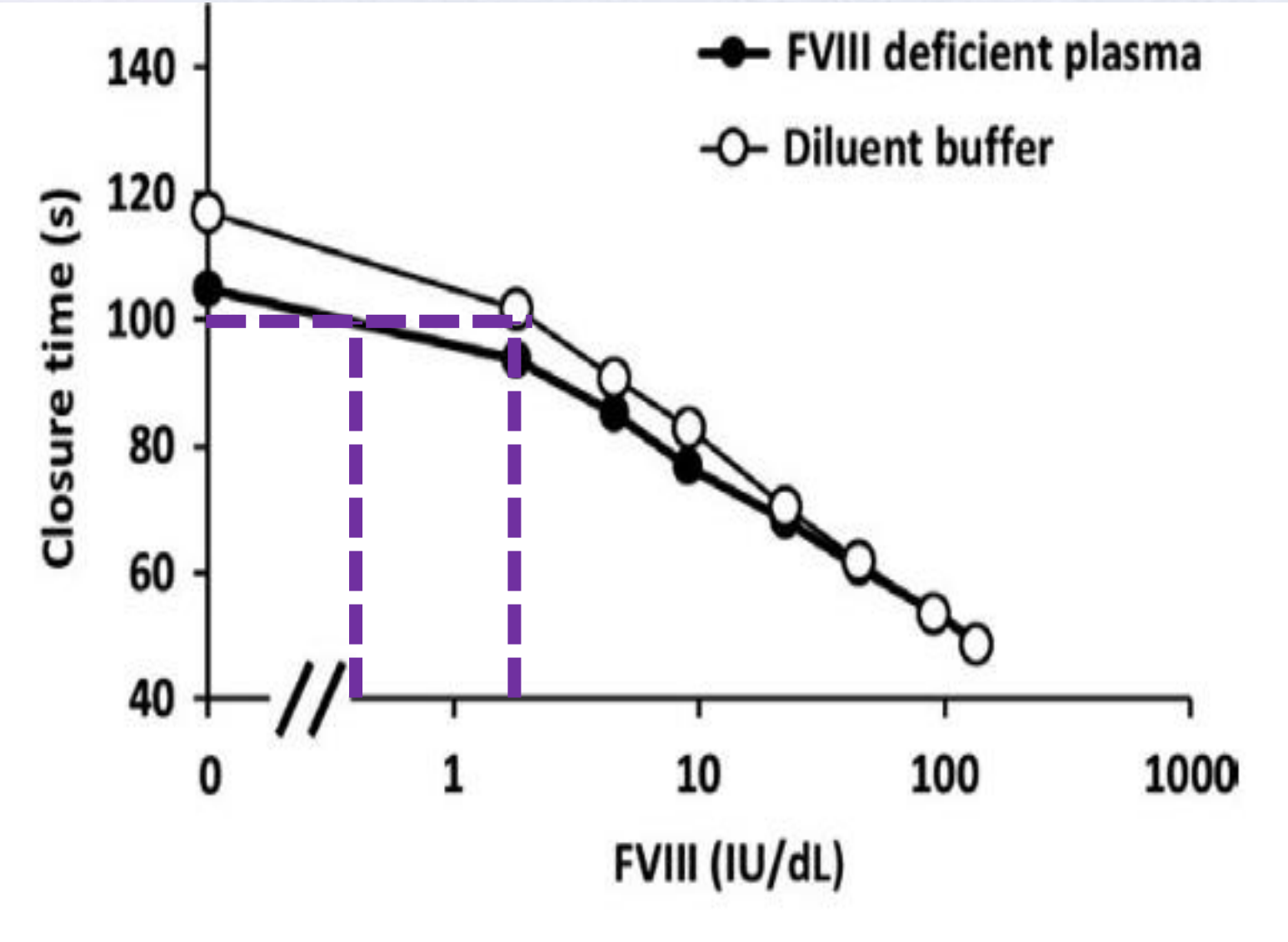
30% of labs: FVIII < 1%
=> severe hemophilia A

65% of labs: FVIII ≈1%

5% of labs: FVIII ≥ 2%
=> moderate hemophilia A

ECAT

Overestimation of FVIII with calibration curve made in diluent buffer



Van den Bosche et al. 2018 Haemophilia

De meting van FVIII concentratie ≤1% blijft challenging

- **FVIII deficient plasma met 0.5-0.9% FVIII**

Diagnose van matige hemofilie A

FVIII >5% and <40%

Diagnose van matige hemofilie A

FVIII >5% and <40%

inflammatie:
stress
fysieke activiteit

} **hogere FVIII**

Bij een FVIII > 40%: altijd een controle om een matige hemofilie A uit te sluiten

Diagnose van matige hemofilie A

FVIII >5% and <40%

kwaliteit staal!

Bij een FVIII < 40%: altijd een controle om preanalytische/analytisch probleem uit te sluiten

Stabiliteit van bloedstalen

op kamer temperatuur

! FVIII en FV: maximum 3u op kamer temperatuur

Stabiliteit van bloedstalen op kamer temperatuur

! FVIII en FV: maximum 3u op kamer temperatuur

APTT:	**48u**
PT:	**48u**
Fibrinogeen:	**> 48u**
D-dimeren:	**4u**

Stabiliteit van bloedstalen

op kamer temperatuur

! FV en FVIII: maximum 3u op kamer temperatuur

Niet op 4° C: precipitatie van het FVIII/VWF complex

Production of High-Potency Concentrates of Antihemophilic Globulin in a Closed-Bag System

plasma

Cryoprecipitate

Stabiliteit van plasma

op -20° : 2 weken

Op -80° C: > 1 jaar

Diagnose van matige hemofilie A

FVIII >5% and <40%

is 40% de optimale cut-off?

Diagnose van matige hemofilie A

FVIII >5% and <40%

Groep blood O: lagere FVIII

Diagnose van matige hemofilie A

FVIII >5% and <40%

Groep blood O: lagere FVIII

“Boven de 20 procent factor VIII/IX gehalte wordt gesproken van subhemofilie”

UMC Utrecht

Diagnose van matige hemofilie A

Cut-off: 40%?

FVIII/VWF interaction

discordant FVIII assays

combined FVIII/FV deficiency

Diagnose van matige hemofilie A

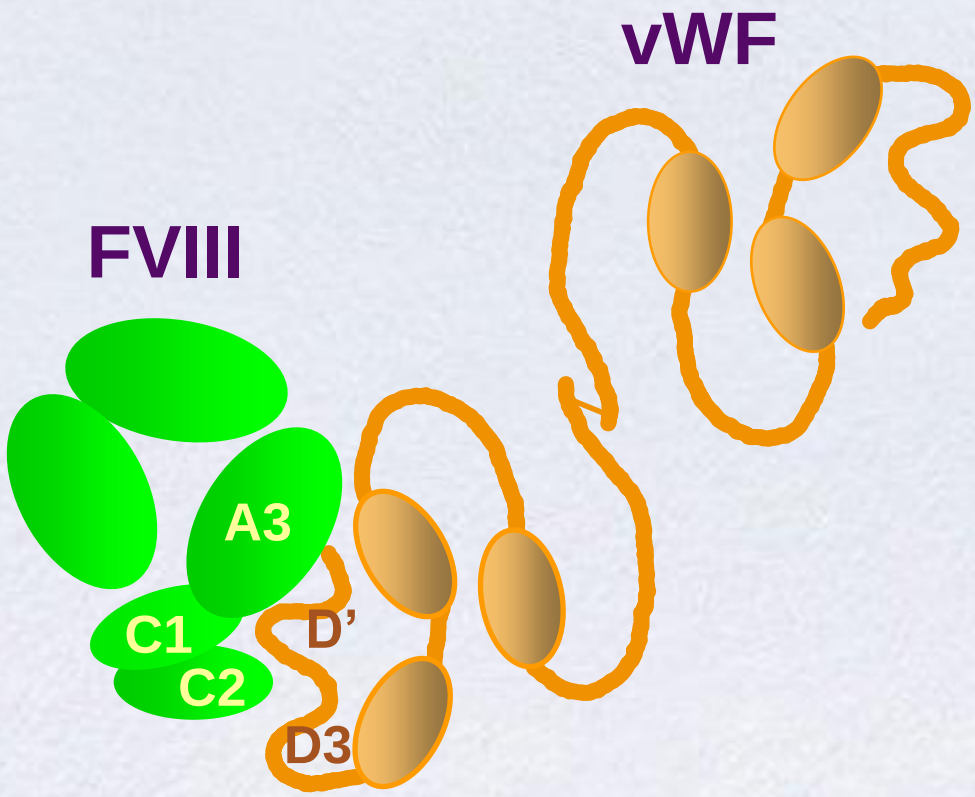
Cut-off: 40%?

FVIII/VWF interaction

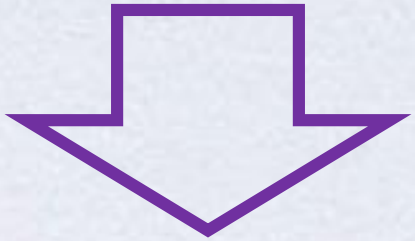
discordant FVIII assays

combined FVIII/FV deficiency

FVIII is bound to vWF in plasma

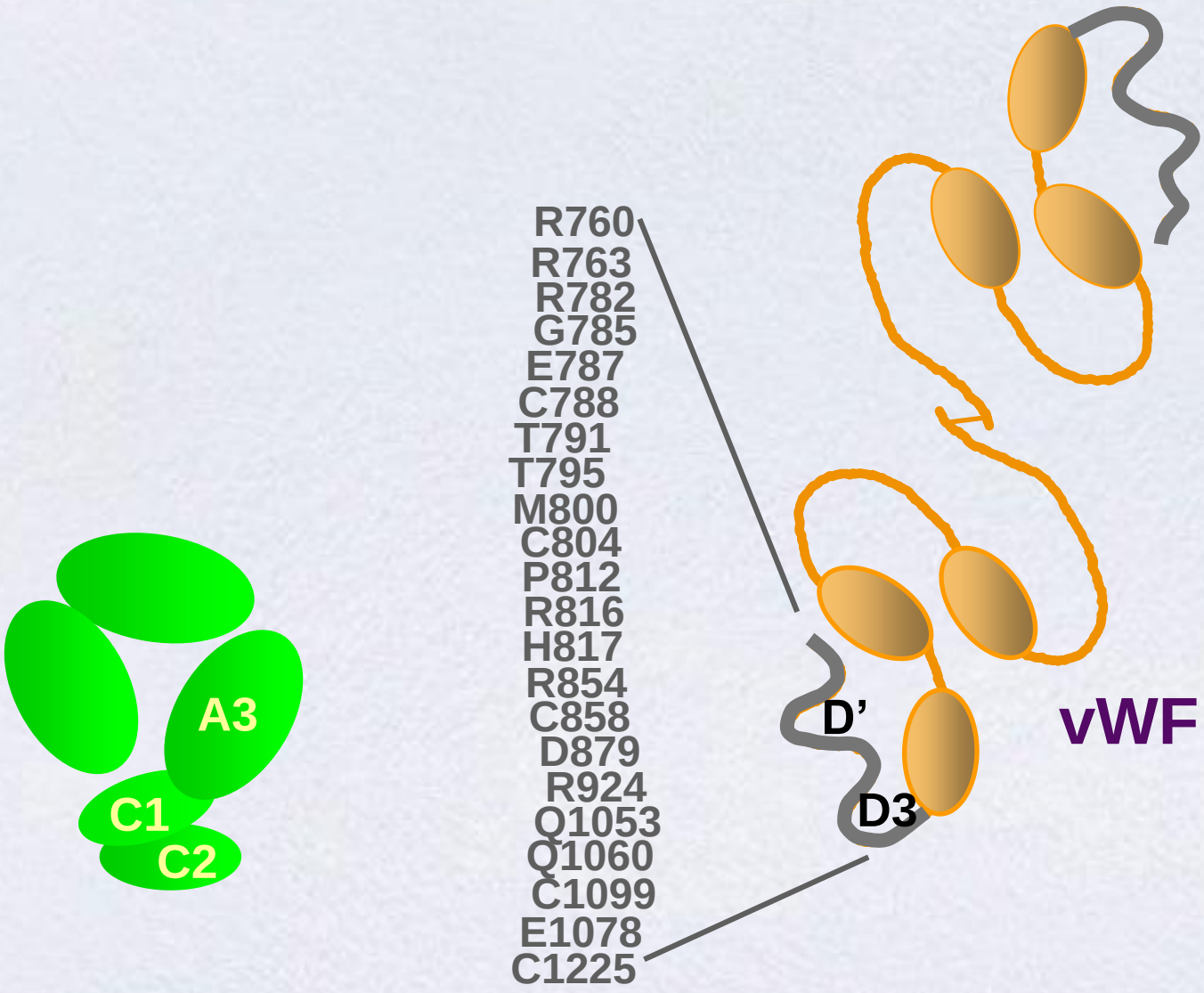


lage VWF:Ag
(von Willebrand ziekte)



lage FVIII

Mutations reducing vWF binding to FVIII are responsible for VWD type 2N (Normandy)

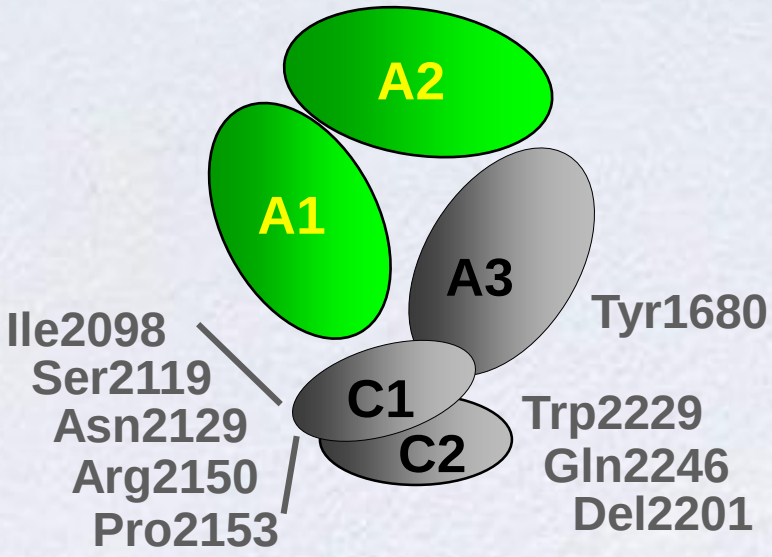


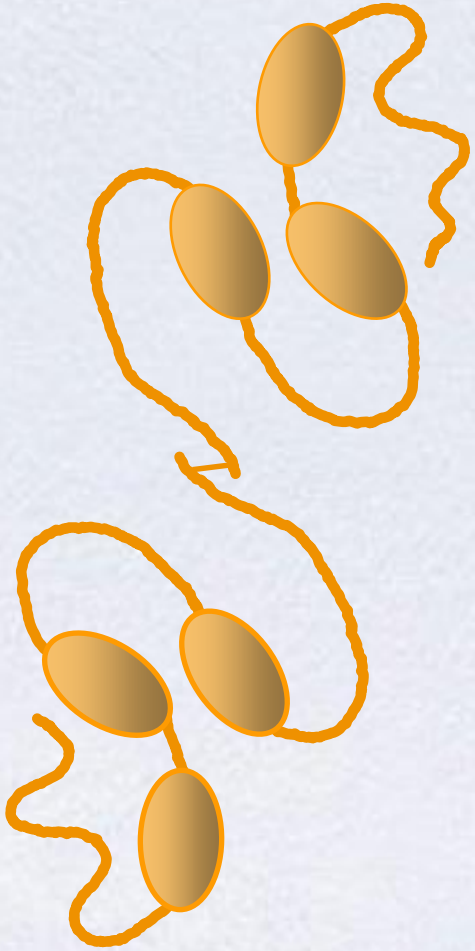
Detection of VWF Normandy

- low FVIII (similar to mild/moderate haemophilia A)
- no mutation in FVIII gene
- reduced FVIII binding to VWF in ELISA
- also in women!

- implications for: - treatment
  - genetic counseling

Mutations reducing FVIII binding to vWF result in mild/moderate hemophilia A





Blood 1996; 96: 958

Diagnose van matige hemofilie A

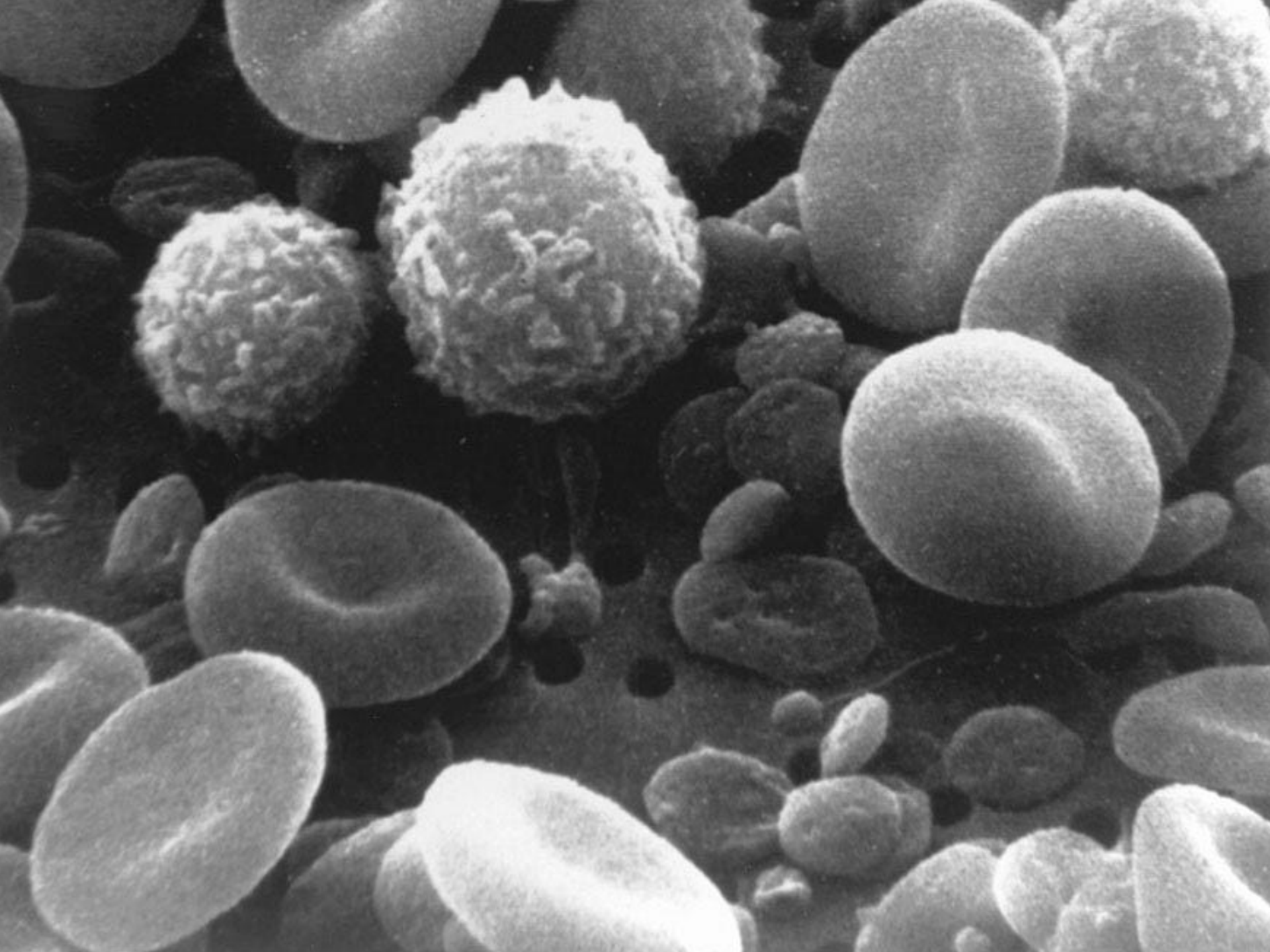
Cut-off: 40%?

FVIII/VWF interaction

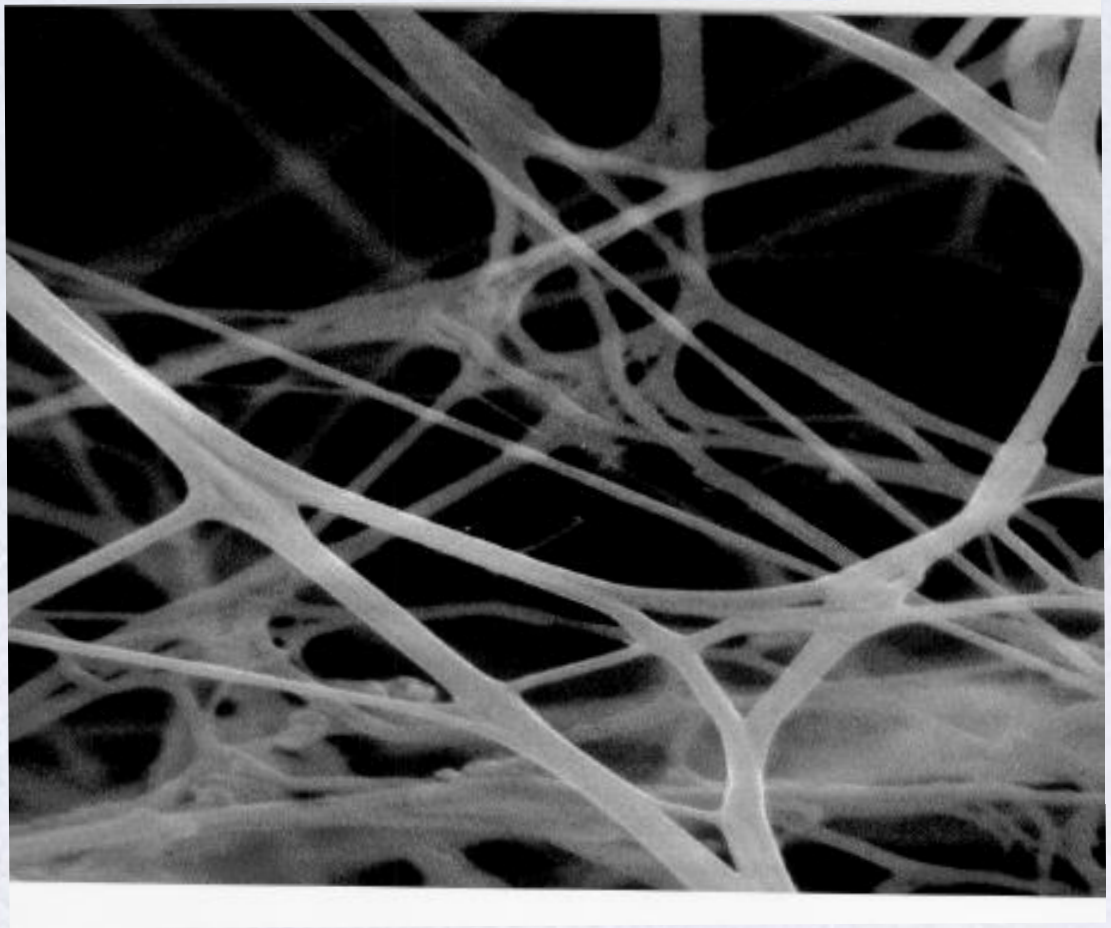
discordant FVIII assays

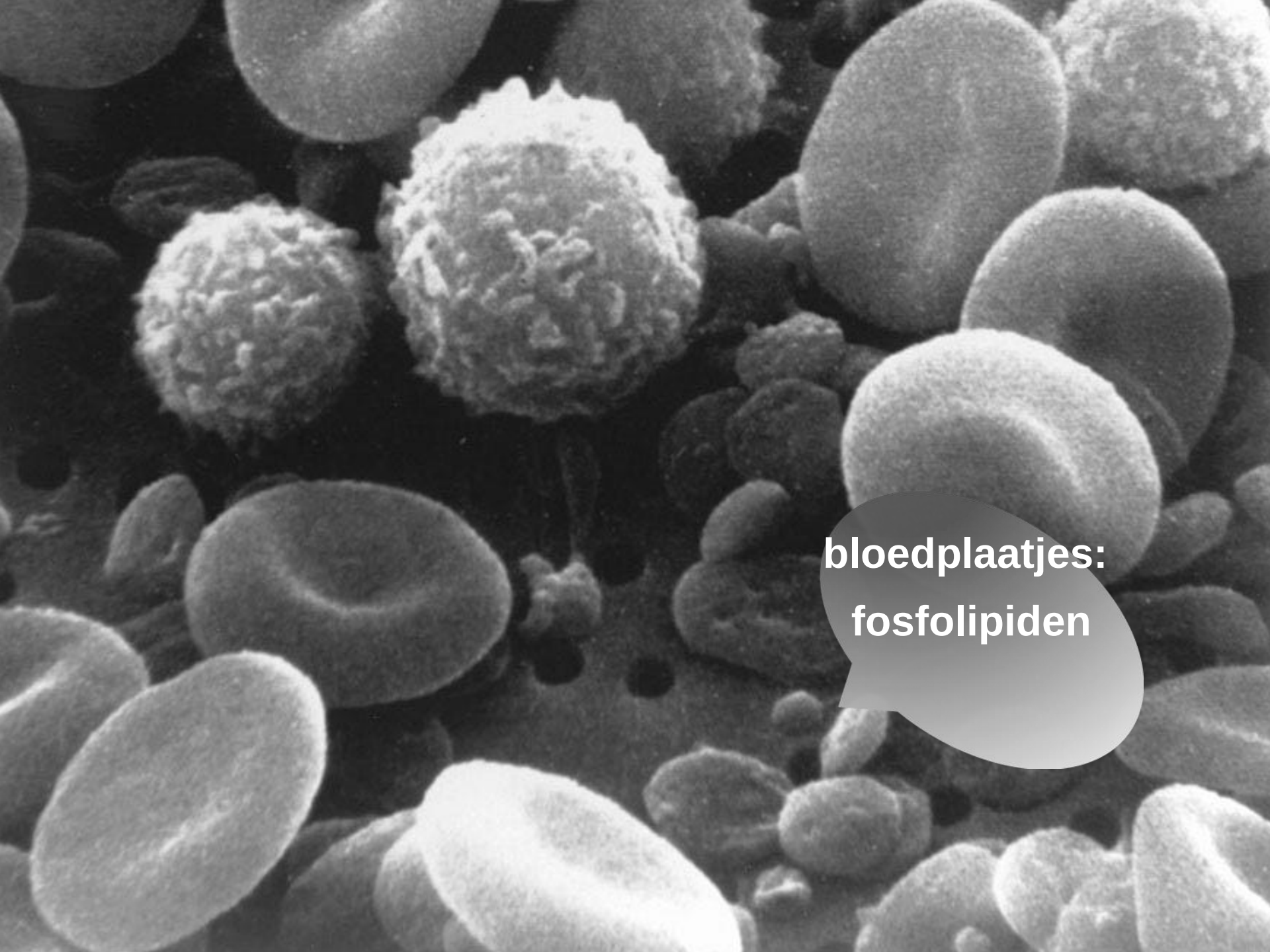
combined FVIII/FV deficiency

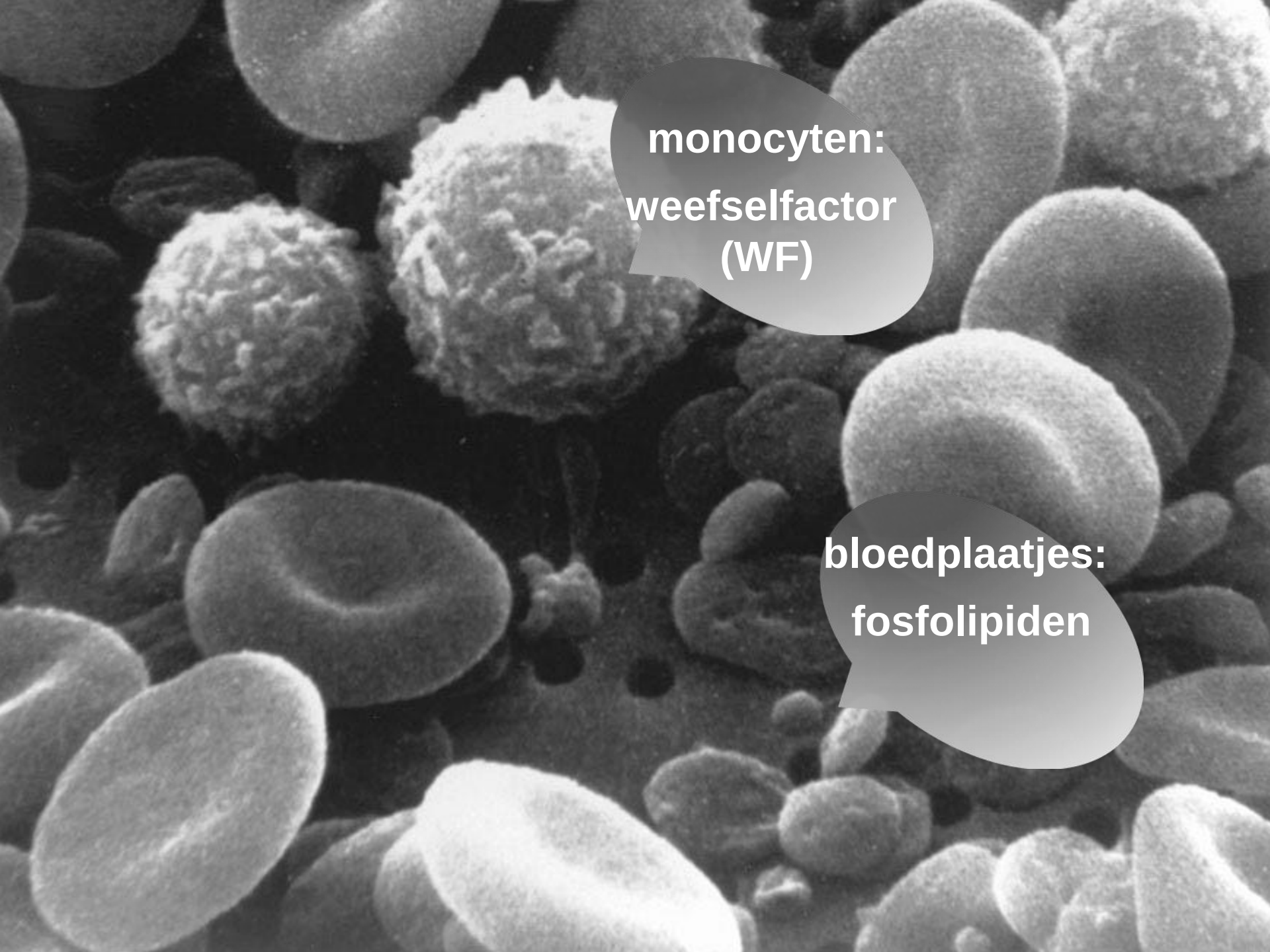
	FVIII (%)	
	Stollingstest	chromogene test
normal plasma 1/2	45,6	47,1
Arg531His	42,1	18,2
His1954Leu	78,5	28,2

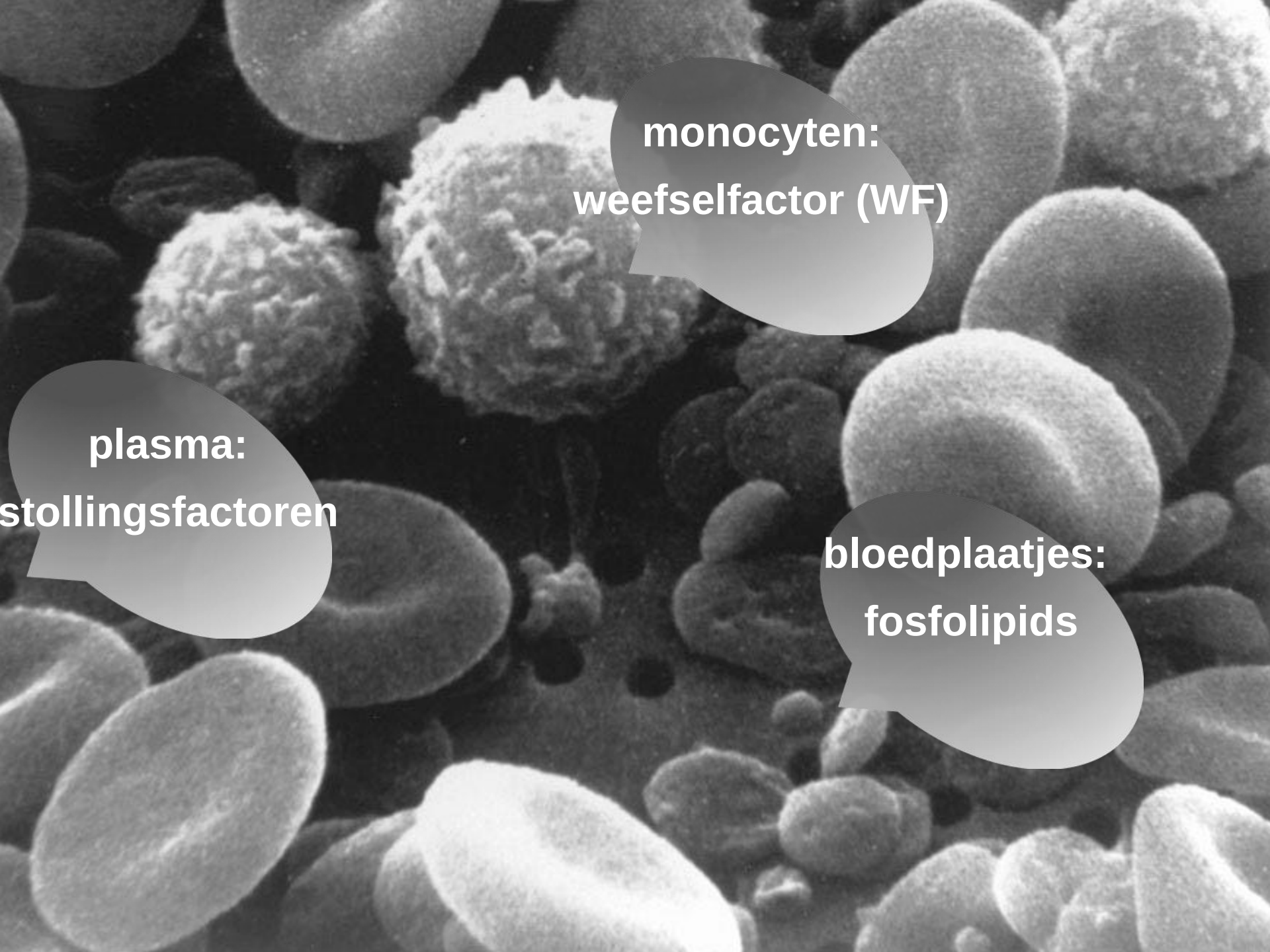


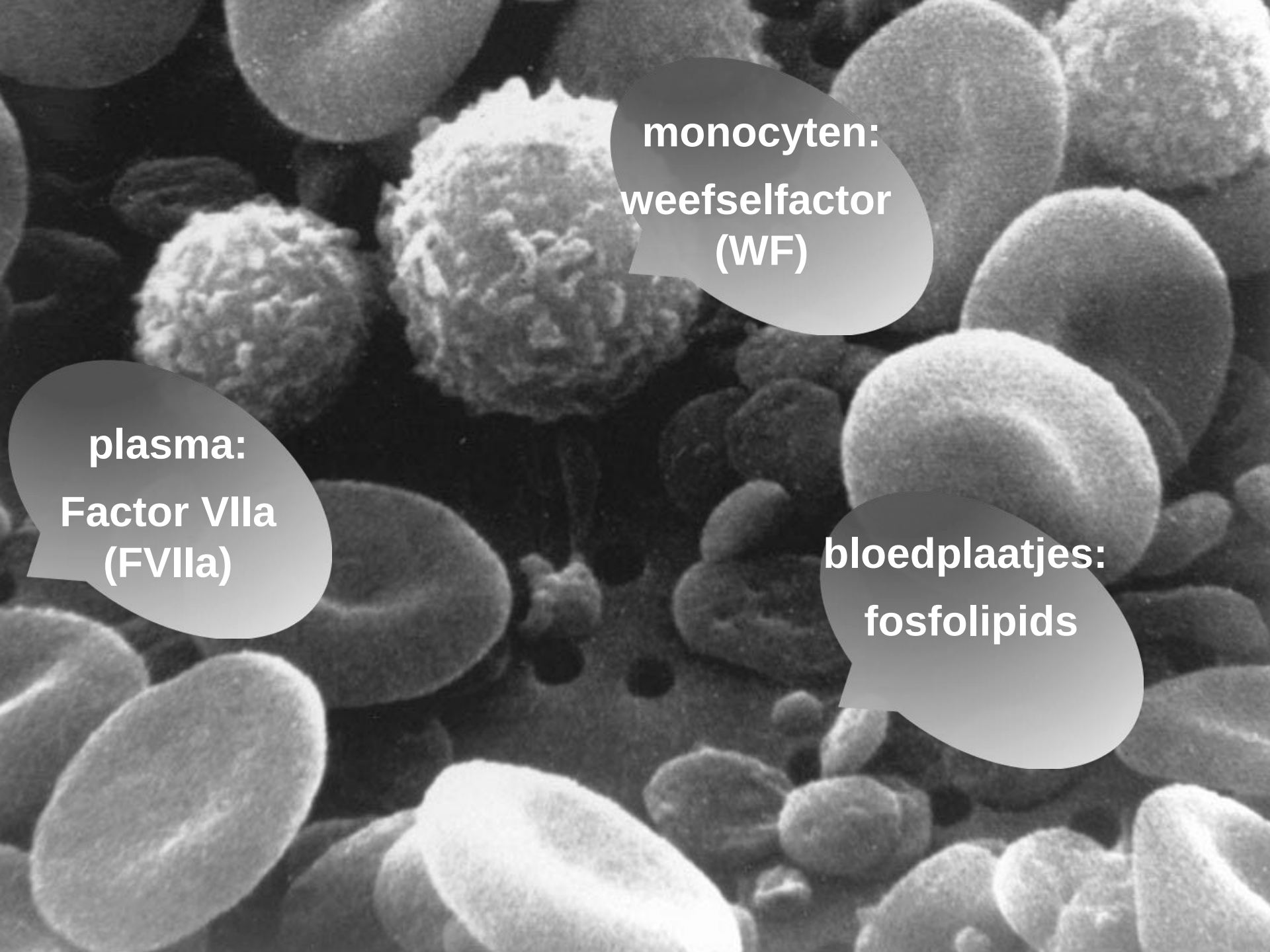
Netwerk van fibrine




bloedplaatjes:
fosfolipiden


monocyten:
weefselfactor
(WF)
bloedplaatjes:
fosfolipiden


monocyten:
weefselfactor (WF)
plasma:
stollingsfactoren
bloedplaatjes:
fosfolipids

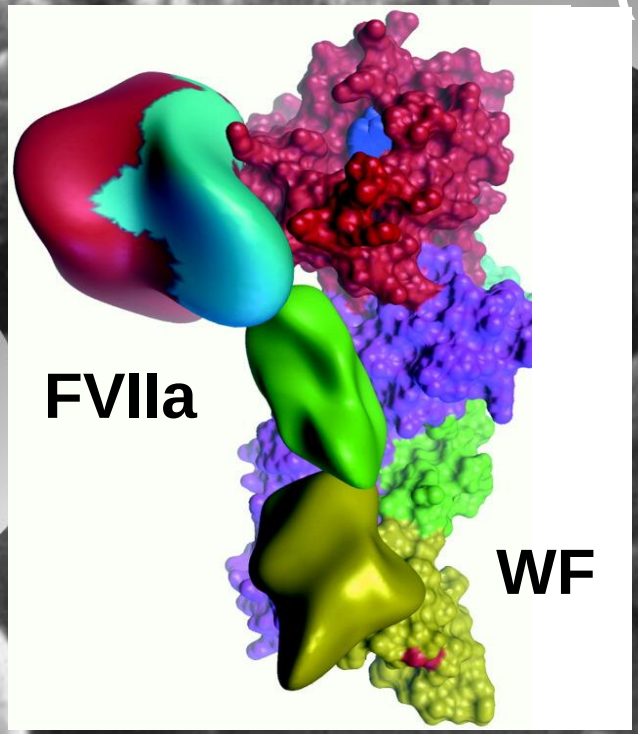

monocyten:
weefselfactor
(WF)
plasma:
Factor VIIa
(FVIIa)
bloedplaatjes:
fosfolipids

monocyten:

weefselfactor (WF)

plasma:

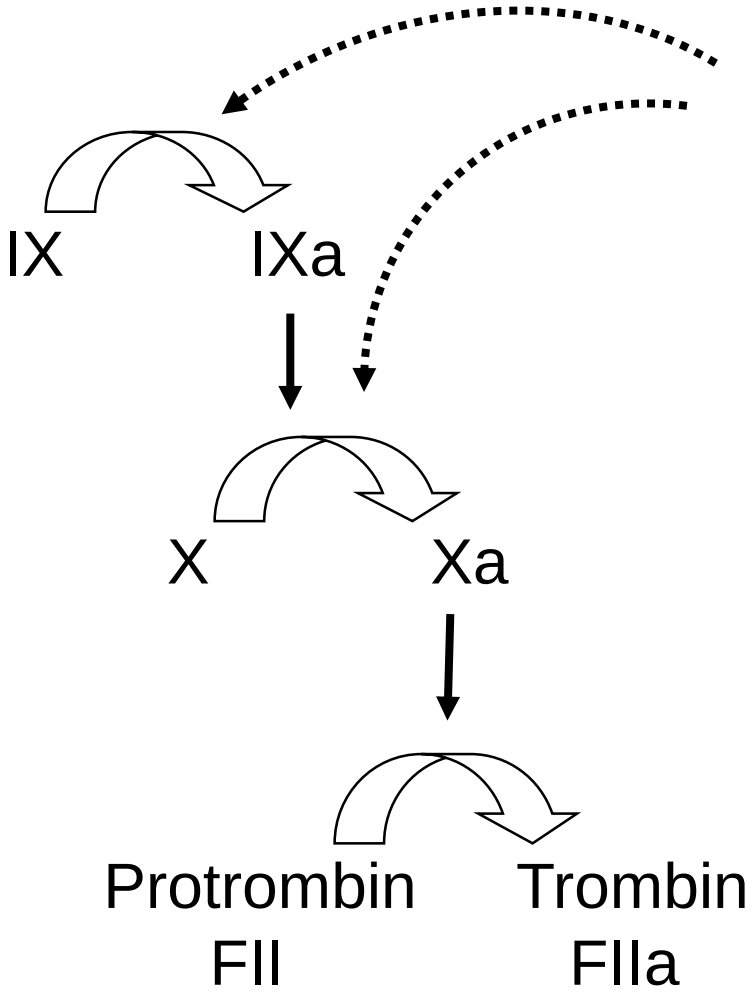
Factor VIIa (FVIIa)

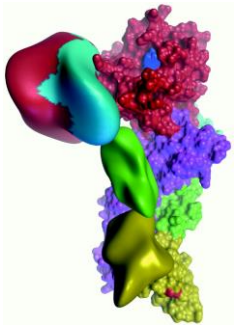


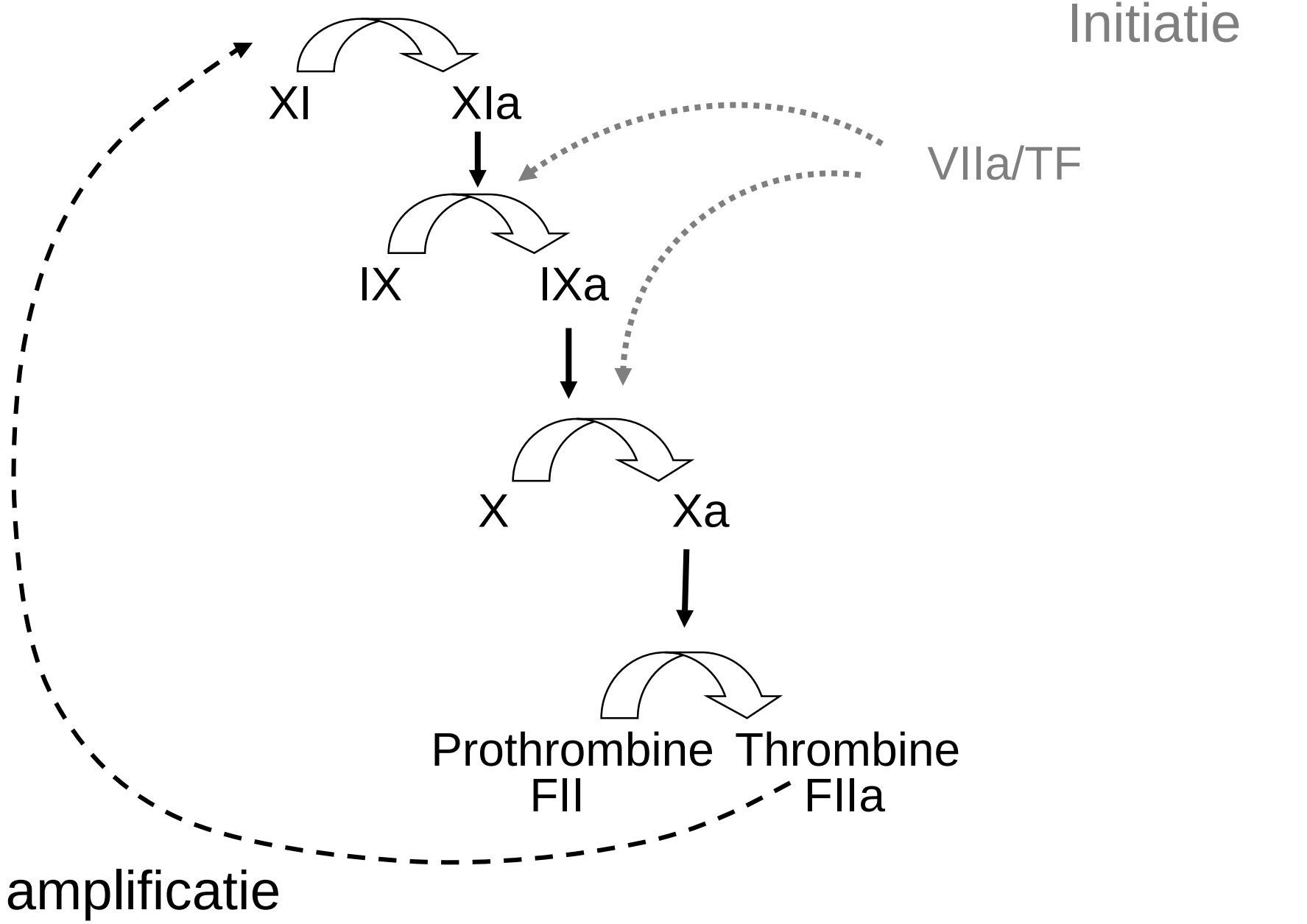
bloedplaatjes:

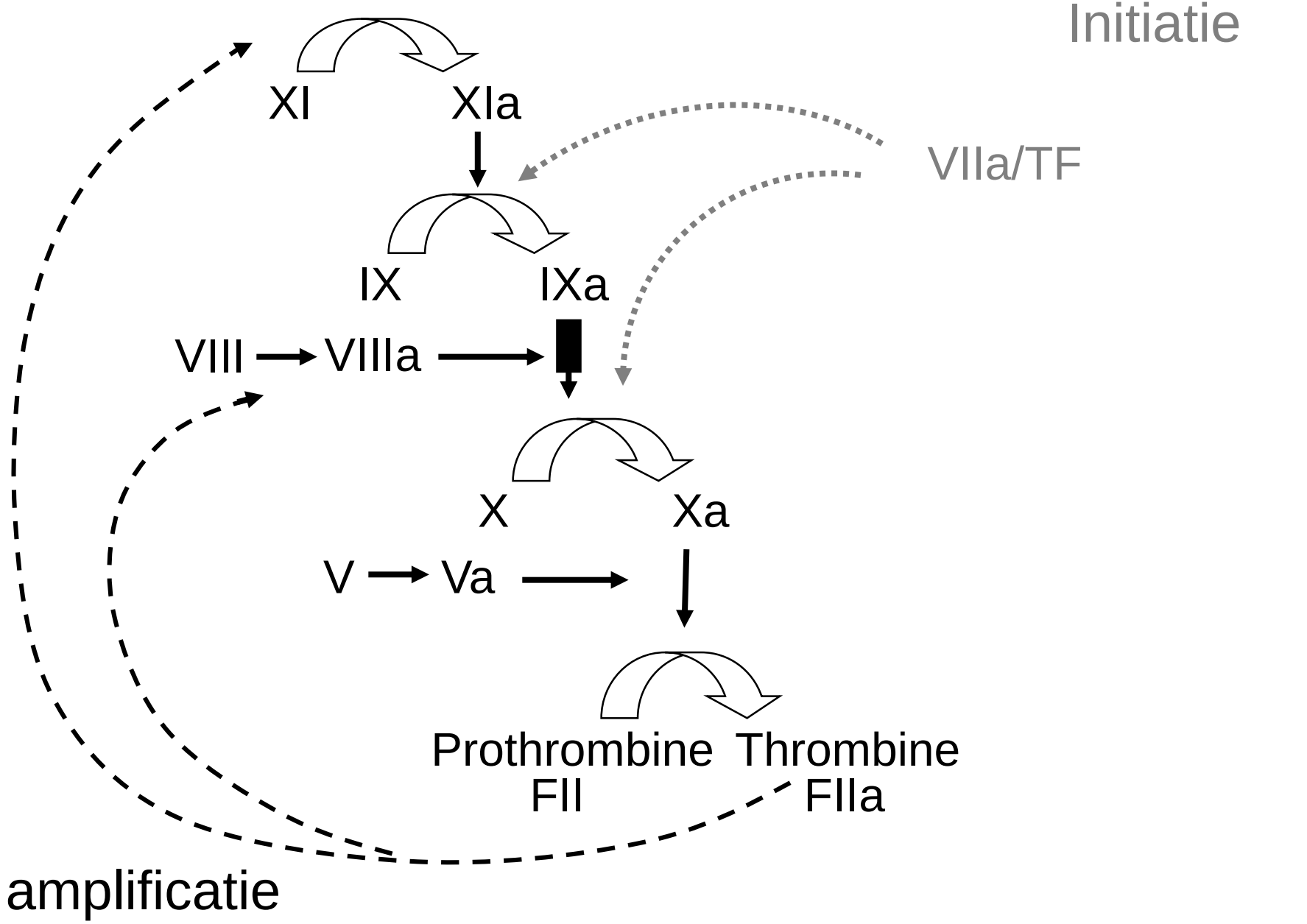
fosfolipids

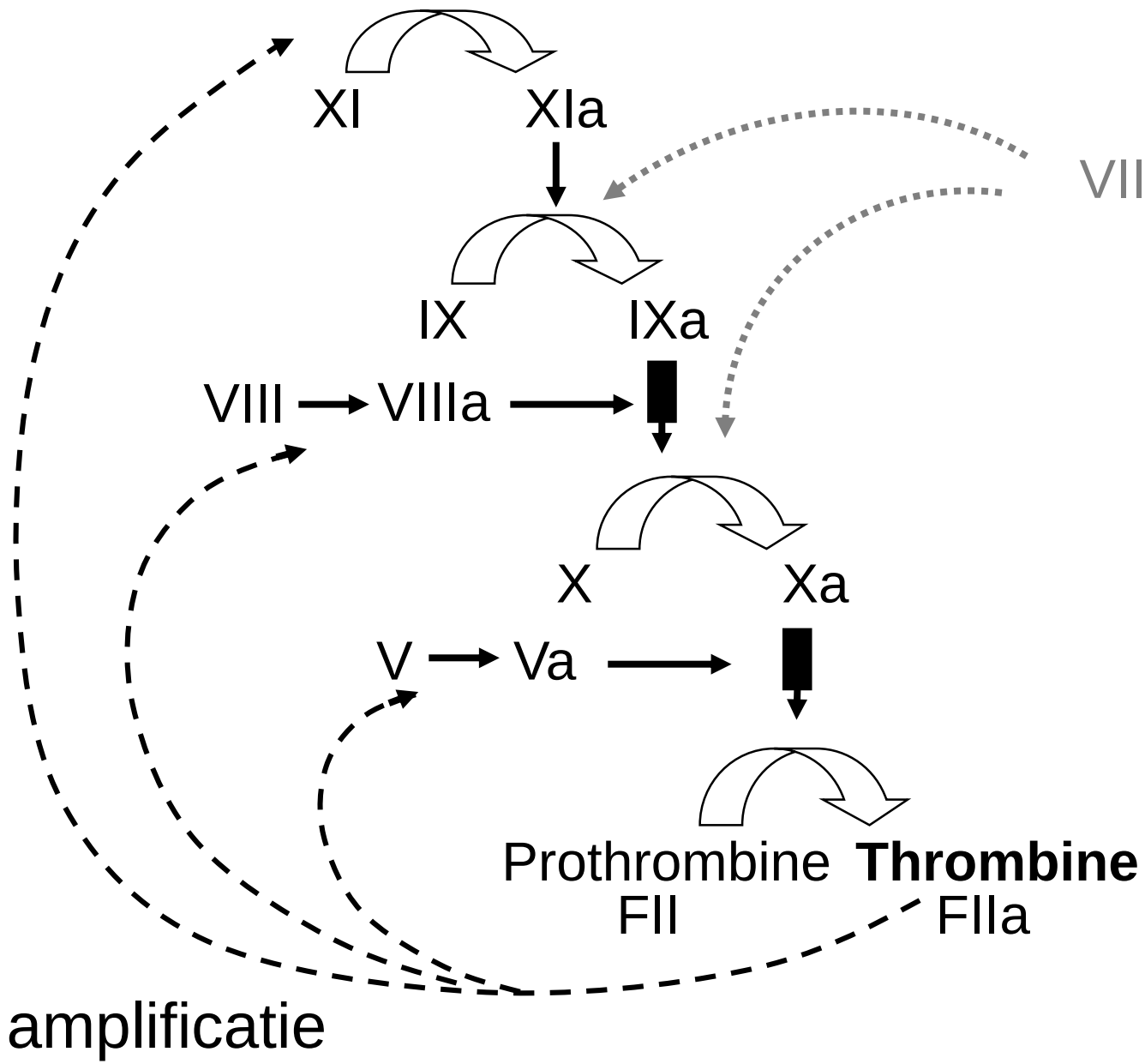
Initiatiefase

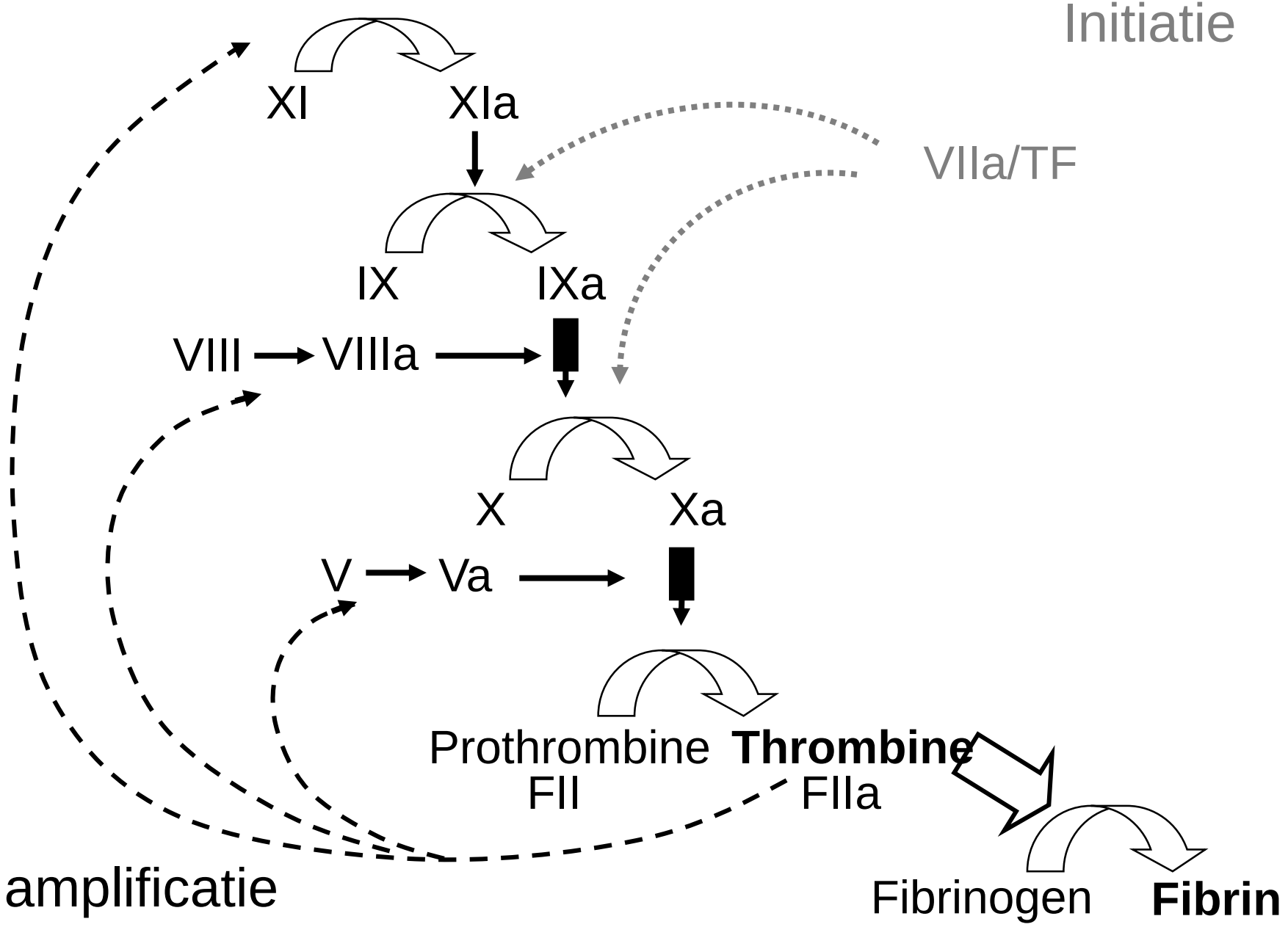


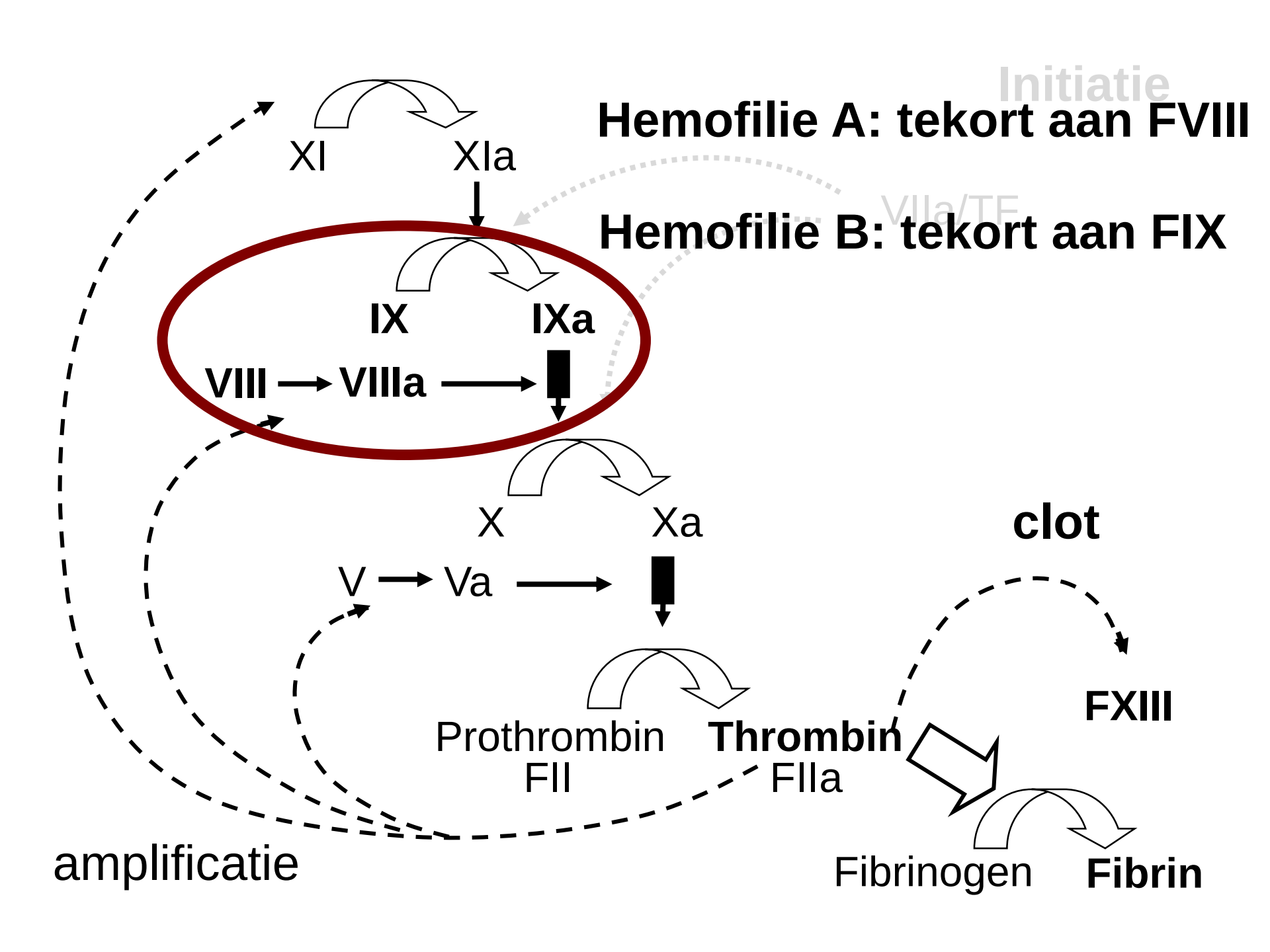



Initiatie
XI
XIa
VIIa/TF
IX
IXa
X
Xa
Prothrombine
FII
Thrombine
FIIa
amplificatie


Initiatie
XI
XIa
VIIa/TF
IX
IXa
VIII
VIIIa
X
Xa
V
Va
Prothrombine
FII
Thrombine
FIIa
amplificatie


Initiatie
XI
XIa
VIIa/TF
IX
IXa
VIII
VIIIa
X
Xa
V
Va
Prothrombine
FII
Thrombine
FIIa
amplificatie


Initiatie
XI
XIa
VIIa/TF
IX
IXa
VIII
VIIIa
X
Xa
V
Va
Prothrombine
FII
Thrombine
FIIa
amplificatie
Fibrinogen
Fibrin


Initiatie
Hemofilie A: tekort aan FVIII
Hemofilie B: tekort aan FIX
XI
XIa
VIIa/TF
IX
IXa
VIII
VIIIa
X
Xa
clot
V
Va
FXIII
Prothrombin
FII
Thrombin
FIIa
amplificatie
Fibrinogen
Fibrin

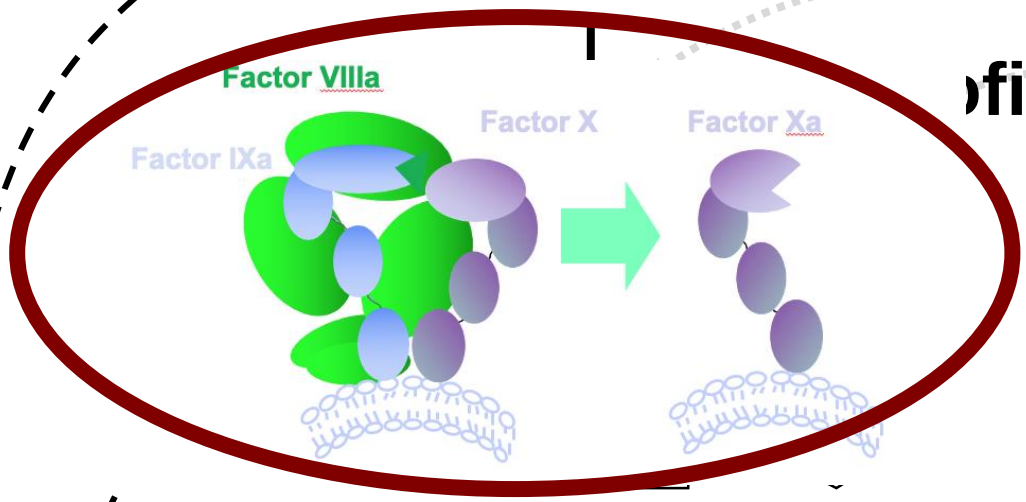
Initiatie

Hemofilie A: tekort aan FVIII

VIIa/TF

ofilie B: tekort aan FIX

XI → XIa



X → Xa

V → Va → Xa

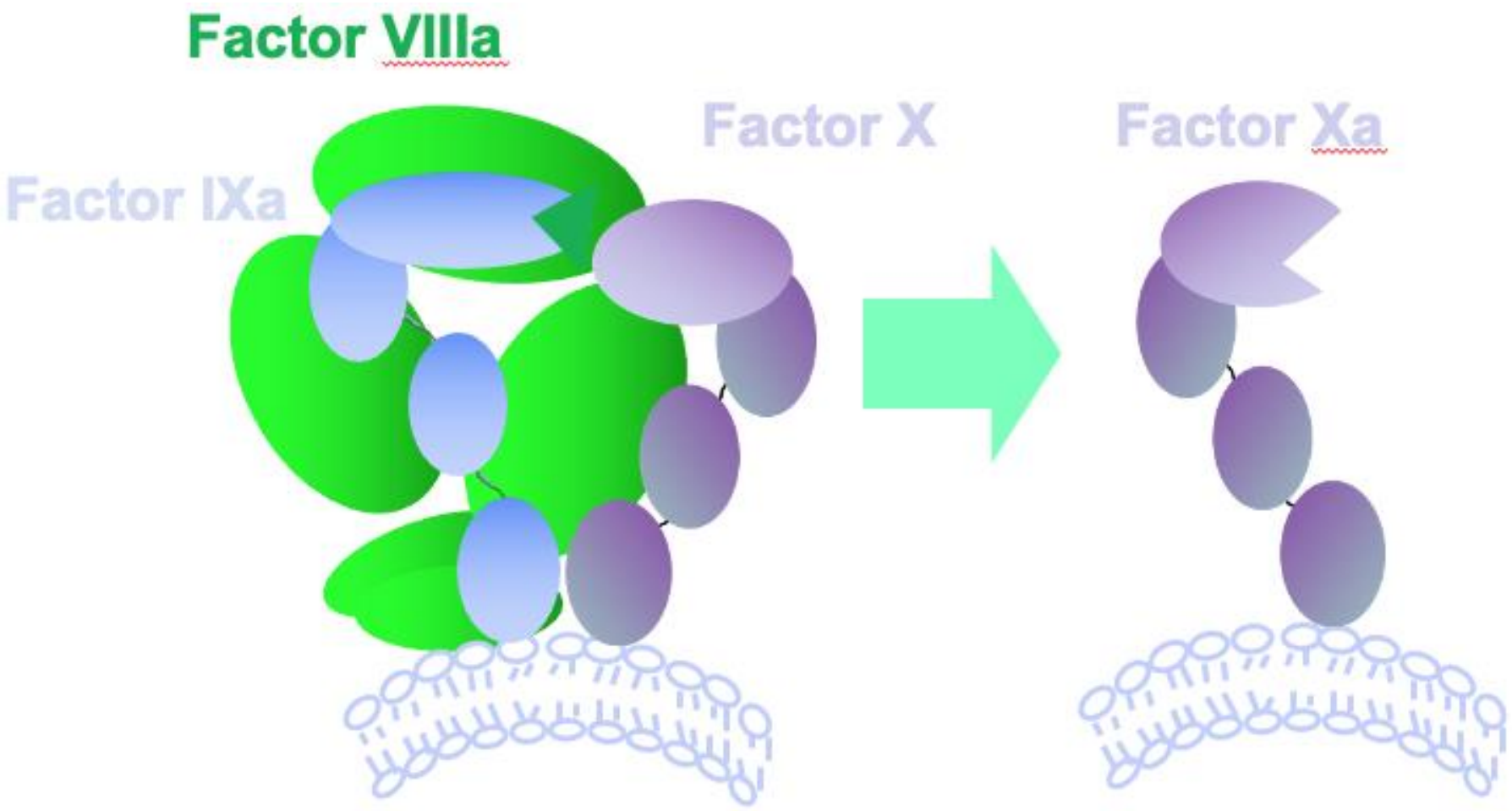
clot

Prothrombin FII → **Thrombin** FIIa

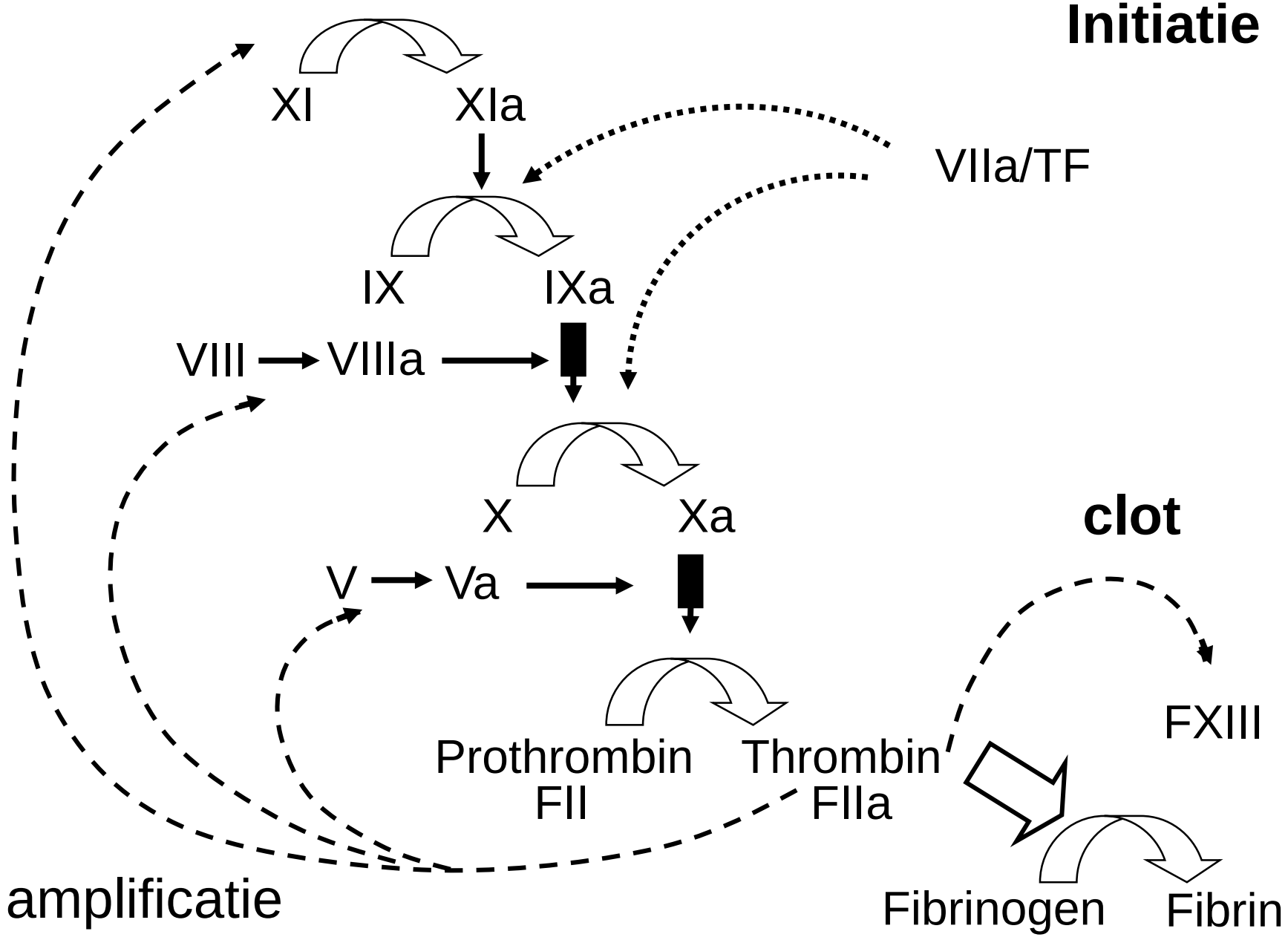
FXIII

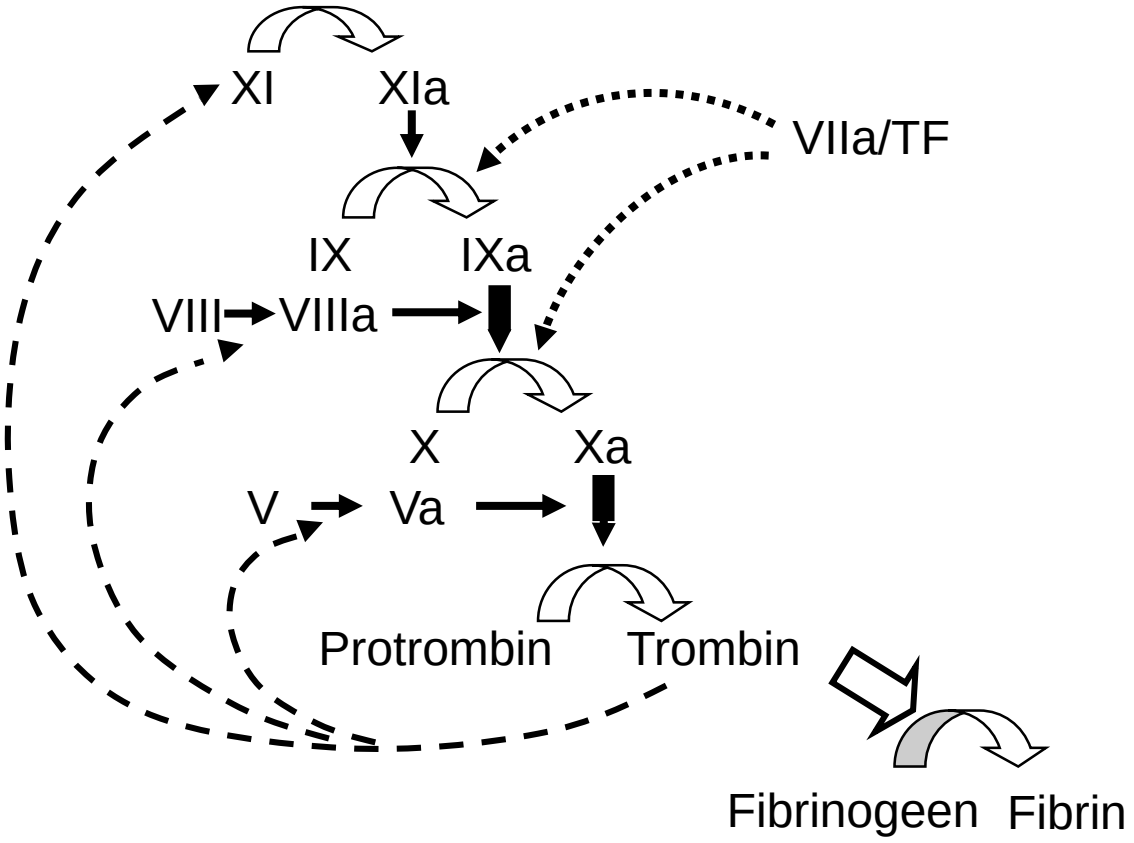
Fibrinogen → **Fibrin**

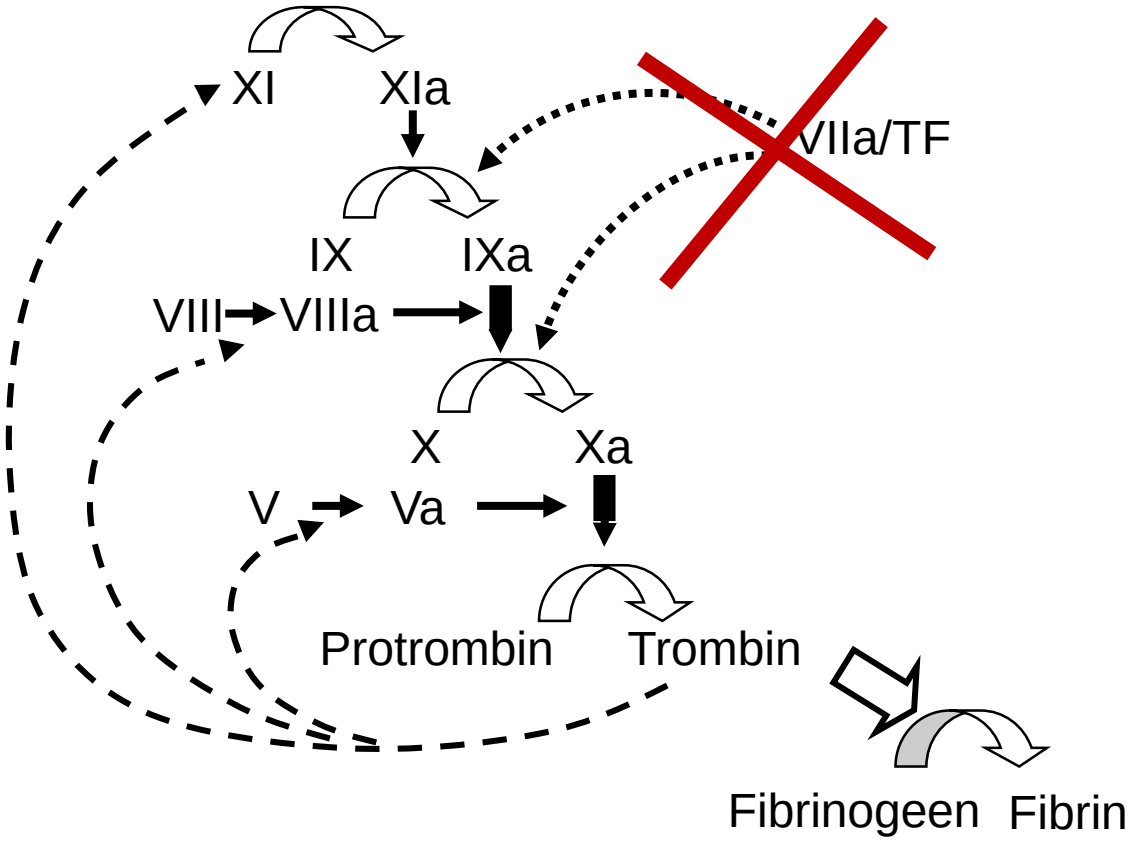
amplificatie


Factor VIIIa
Factor IXa
Factor X
Factor Xa

Opsporen van een tekort aan FVIII

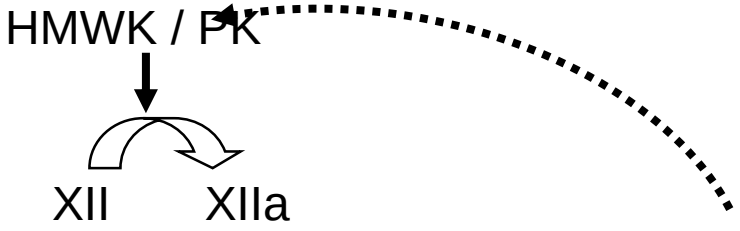

Initiatie
XI
XIa
VIIa/TF
IX
IXa
VIII
VIIIa
X
Xa
clot
V
Va
FXIII
Prothrombin
FII
Thrombin
FIIa
amplificatie
Fibrinogen
Fibrin


XI
XIa
VIIa/TF
IX
IXa
VIII
VIIIa
X
Xa
V
Va
Protrombin
Trombin
Fibrinogeen
Fibrin


XI
XIa
VIIa/TF
IX
IXa
VIII
VIIIa
X
Xa
V
Va
Protrombin
Trombin
Fibrinogeen
Fibrin

APTT (geActiveerde Partiële Thromboplastine Tijd)

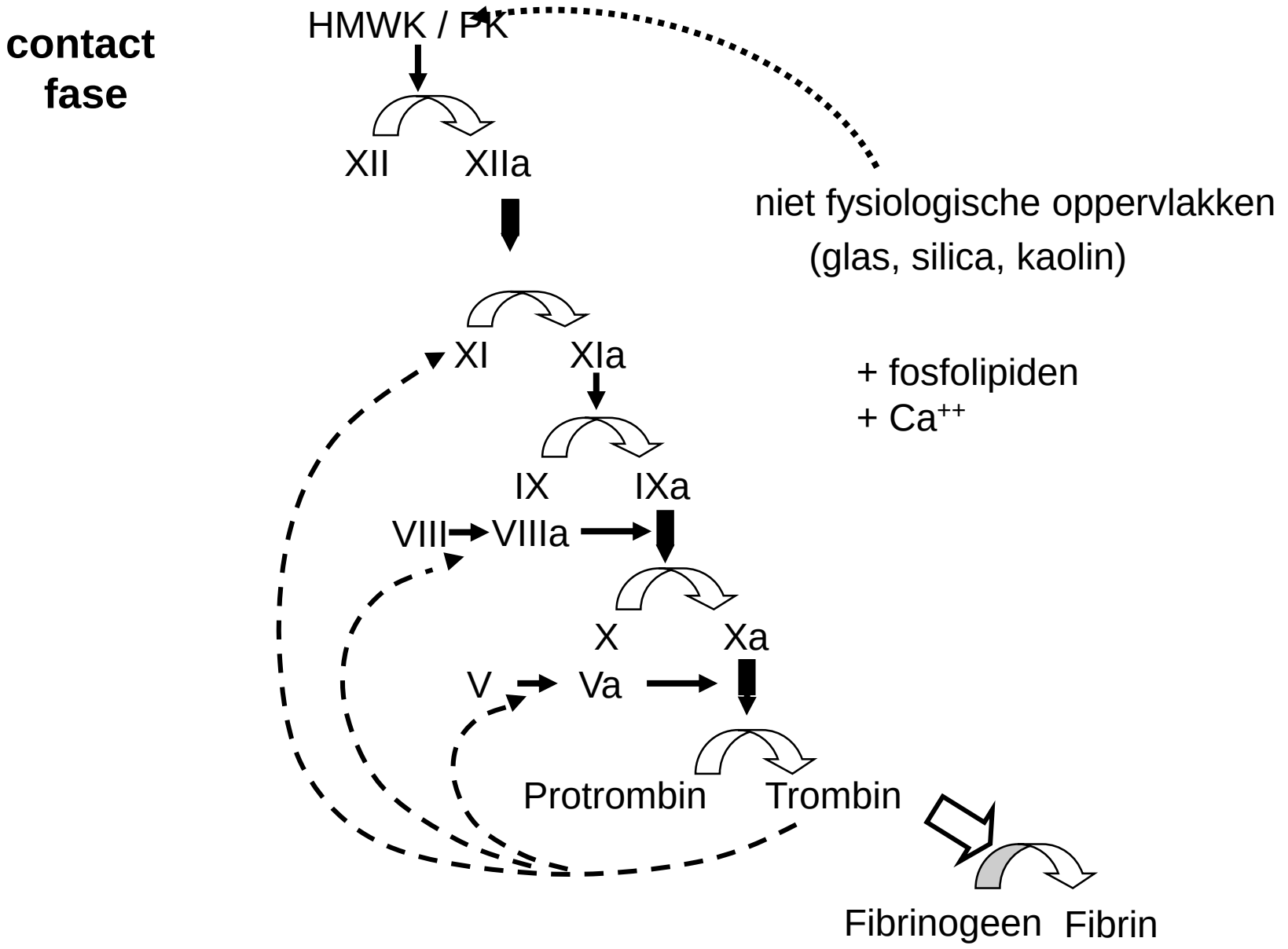
contact fase



niet fysiologische oppervlakken
(glas, silica, kaolin)

+ fosfolipiden
+ Ca^{++}

APTT (geActiveerde Partiële Thromboplastine Tijd)



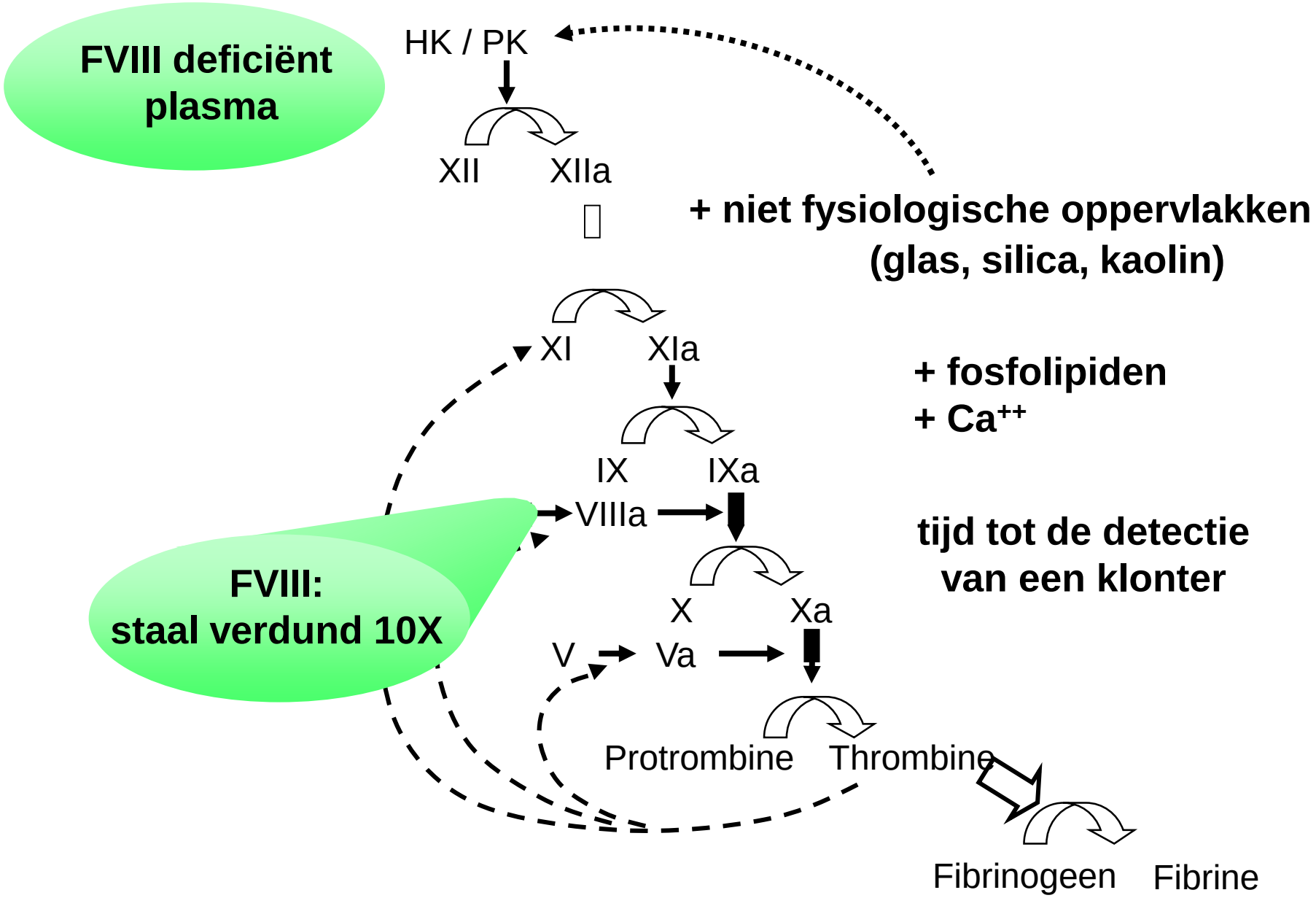
!!! een normale APTT sluit een matigehemofilie A niet uit

!!! een normale APTT sluit een matigehemofilie A niet uit

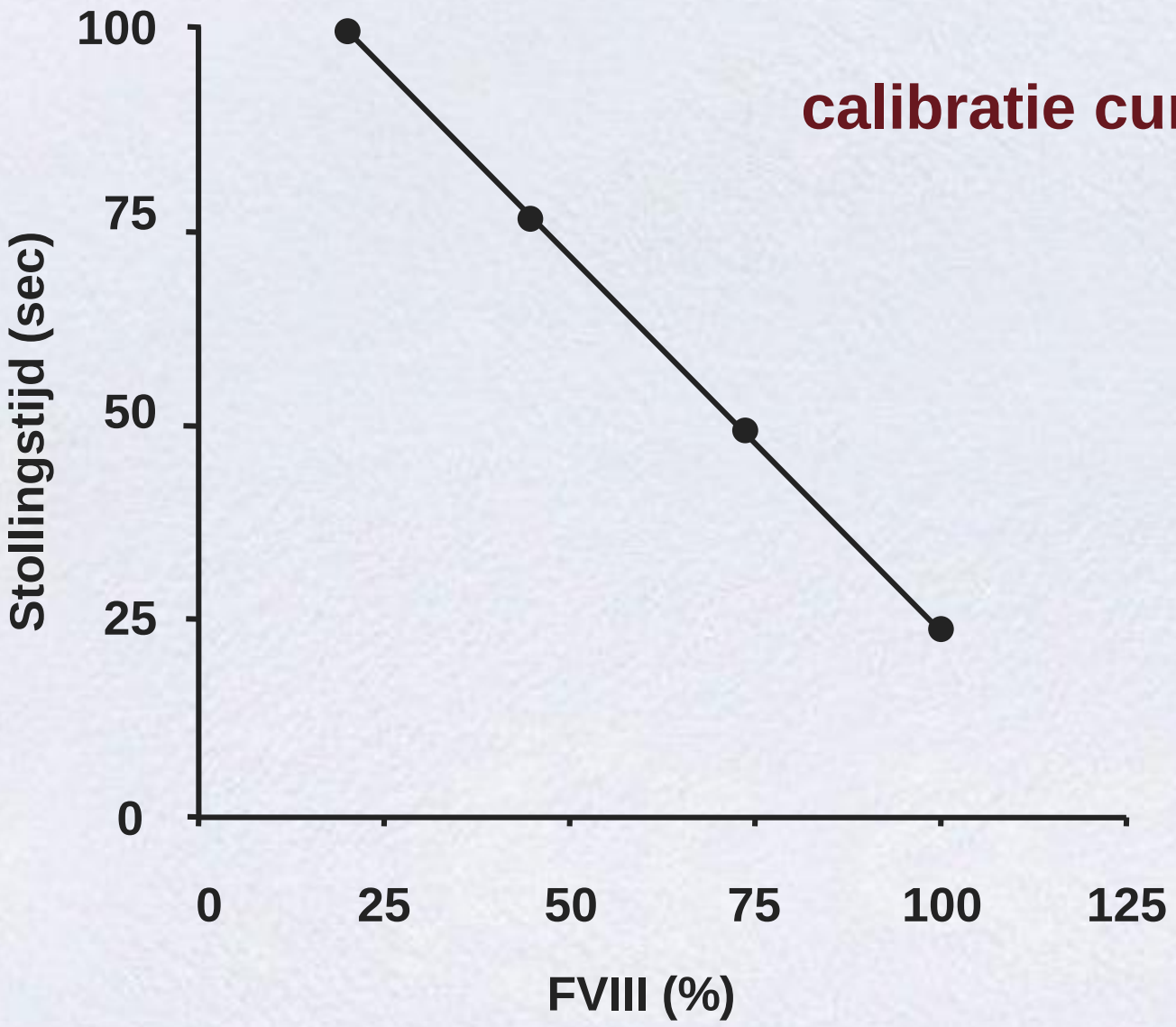
FVIII	APTT	
21,5 %	31 sec	(normaal range: 25,1-36,5 sec)
24,4 %	31,9 sec	

Meting van FVIII met een APTT gebaseerde test

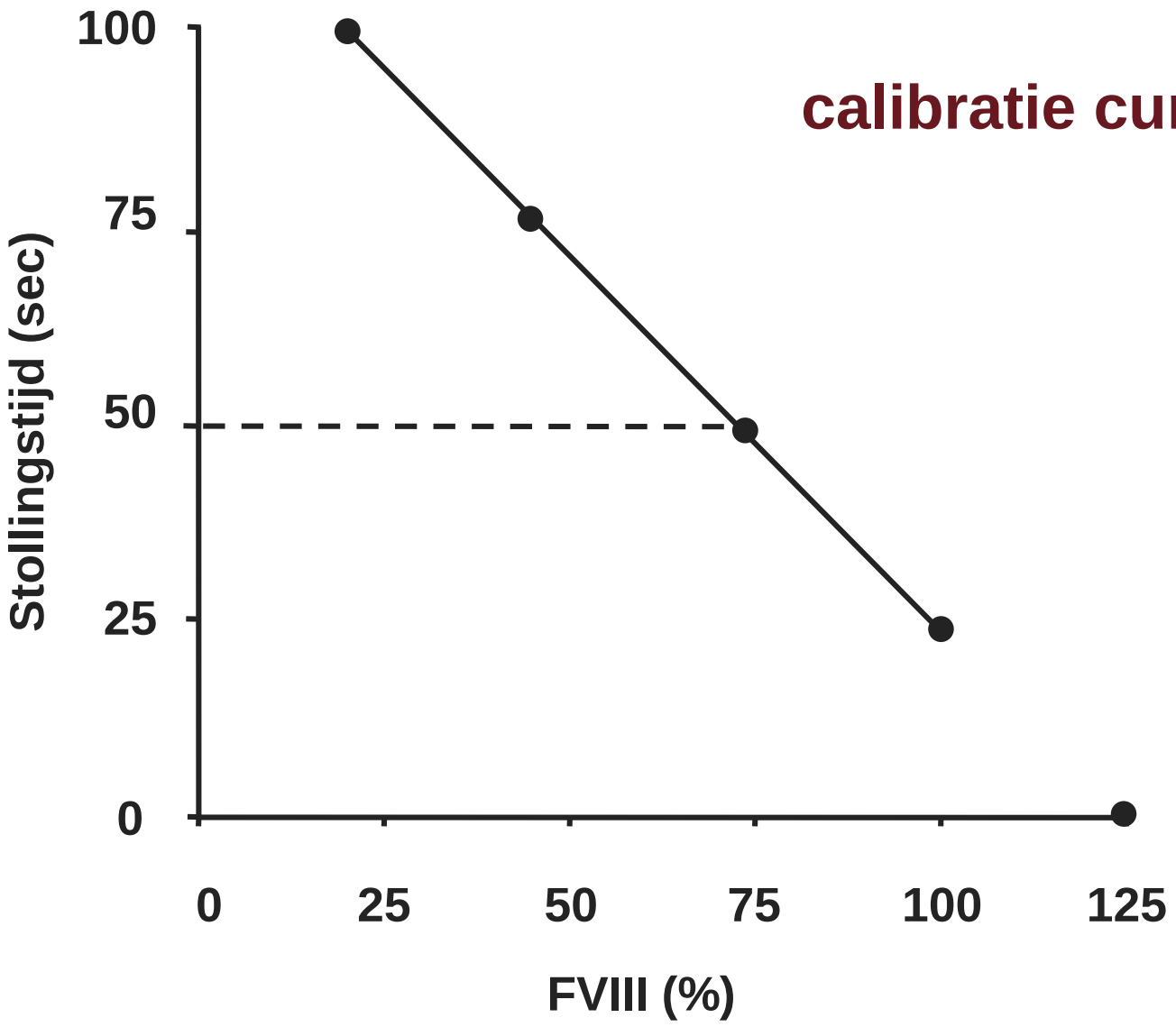
Meting van FVIII met een APTT gebaseerde test



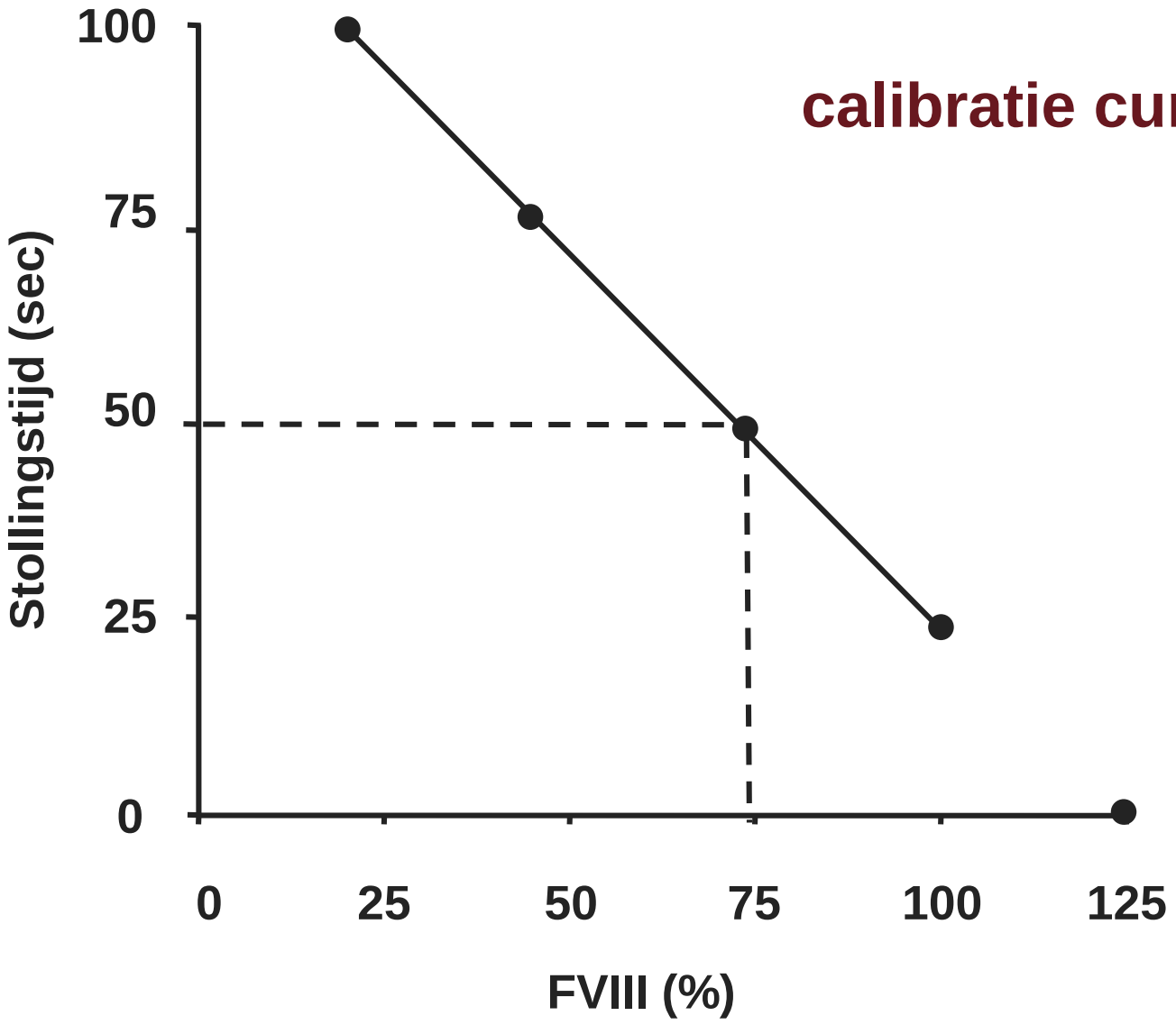
Meting van FVIII met een APTT gebaseerde test



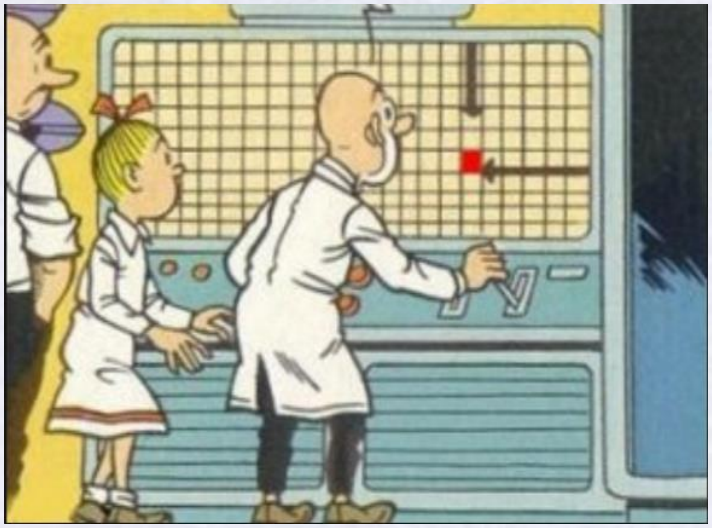
Meting van FVIII met een APTT gebaseerde test



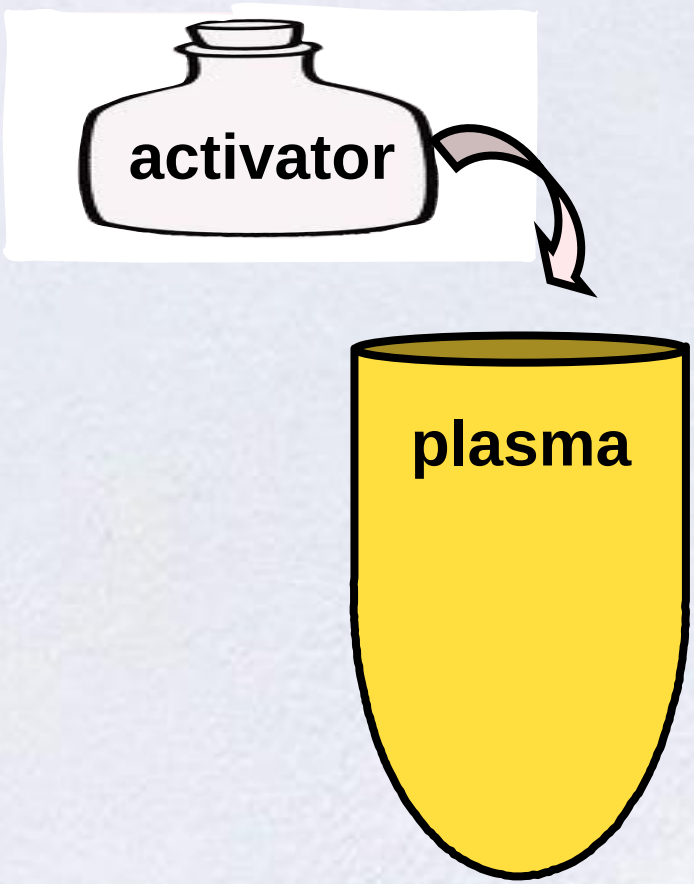
Meting van FVIII met een APTT gebaseerde test



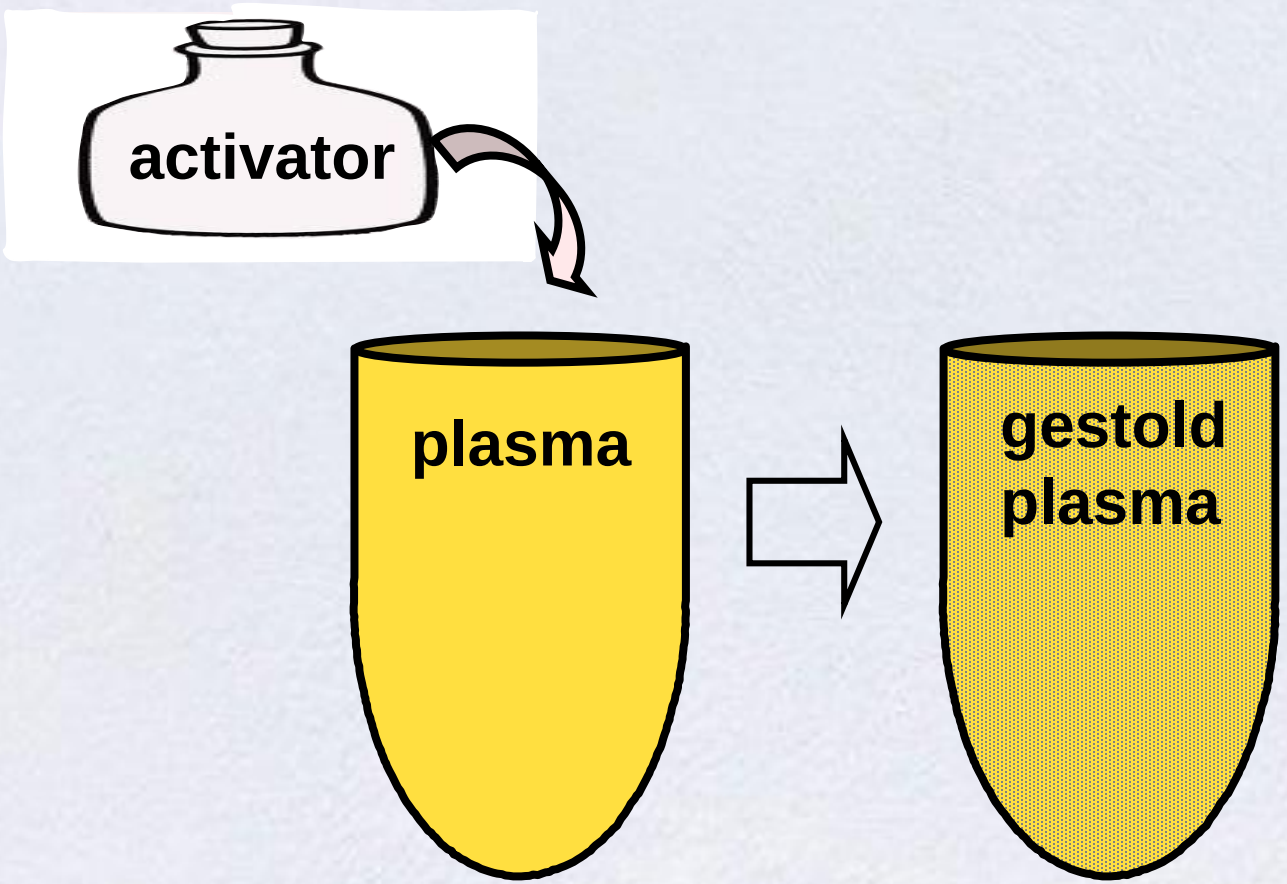
In de praktijk



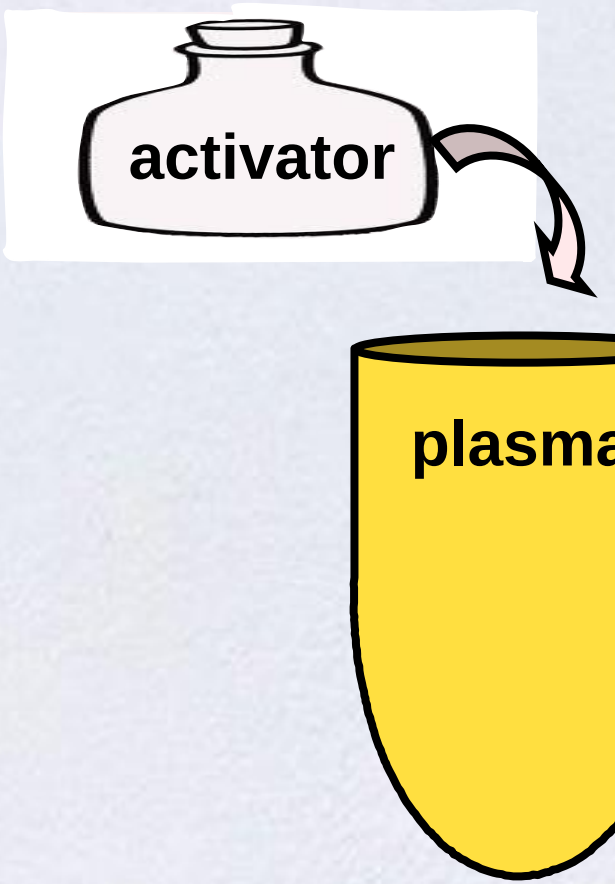
Testen voor stollingsfactoren



Testen voor stollingsfactoren

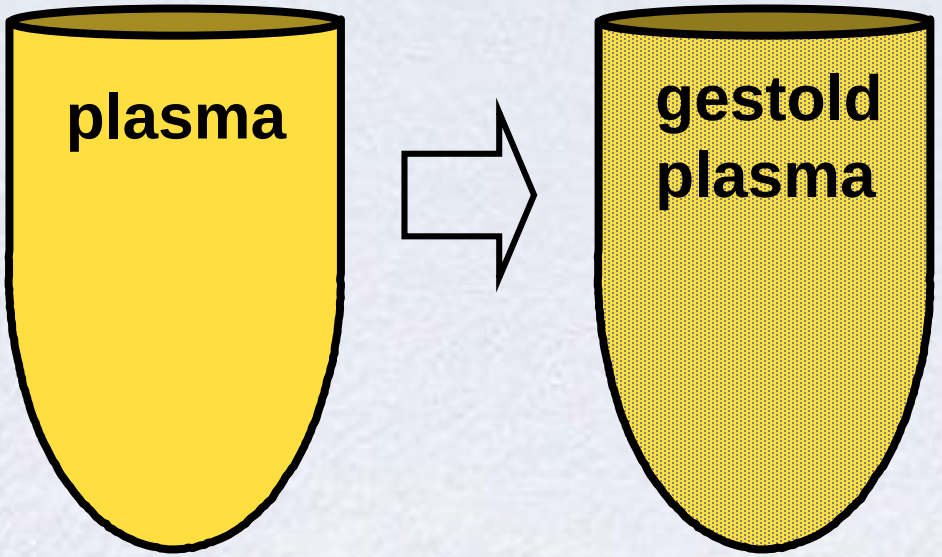


Testen voor stollingsfactoren



vermindering doorzichtigheid

Testen voor stollingsfactoren

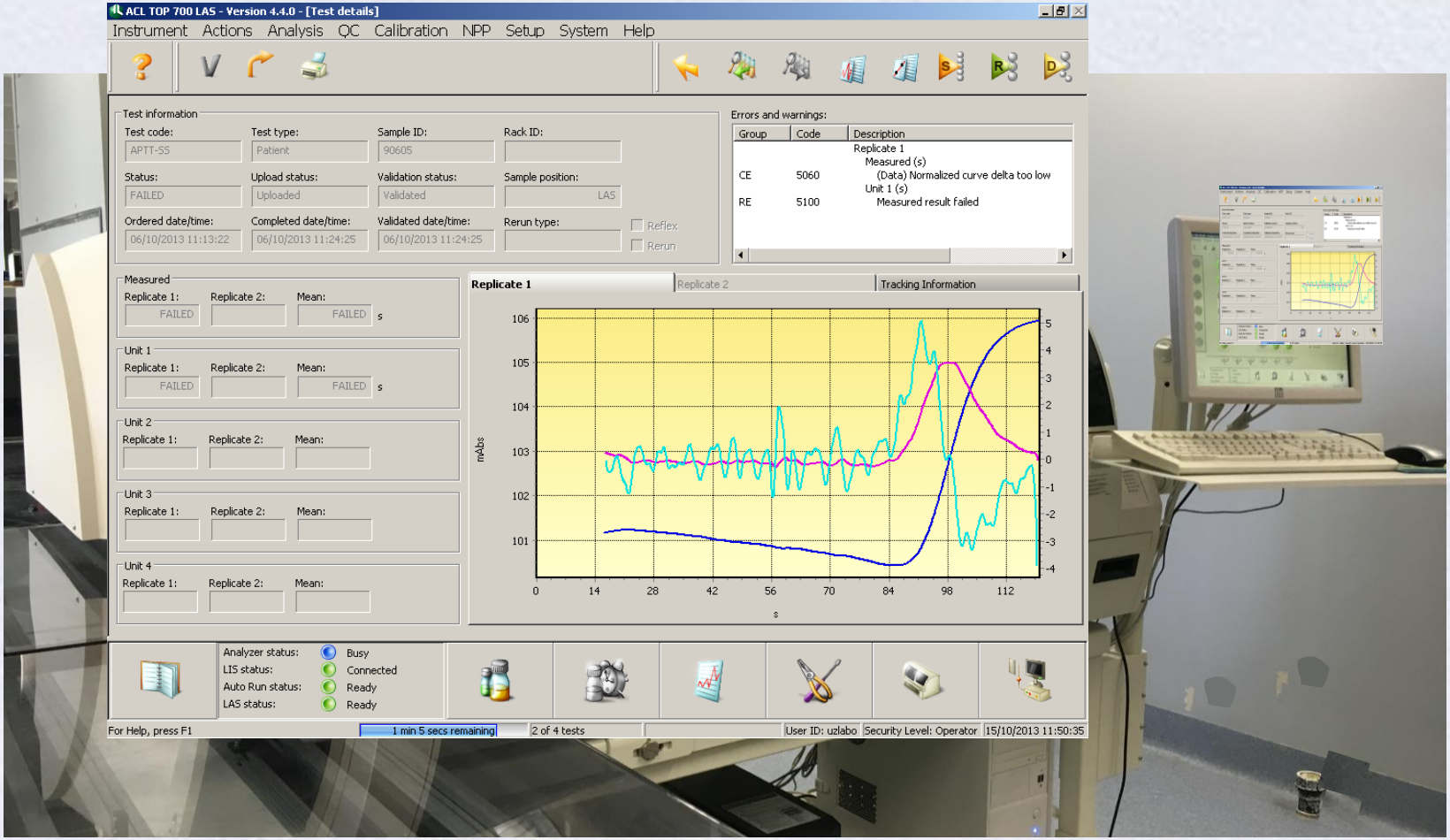


TIJD

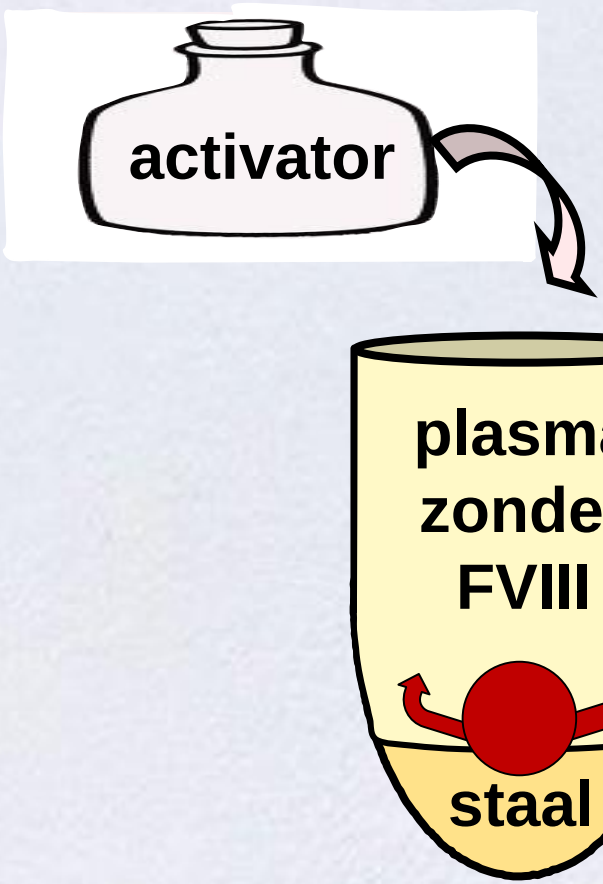
tot vermindering

doorzichtigheid


ACL TOP 700
Instrumentation Laboratory
NIET AANKOMEN


ACL TOP 700 LAS - Version 4.4.0 - [Test details]
Instrument Actions Analysis QC Calibration NPP Setup System Help
Test information
Test code: APTT-SS
Test type: Patient
Sample ID: 90605
Rack ID:
Status: FAILED
Upload status: Uploaded
Validation status: Validated
Sample position: LAS
Ordered date/time: 06/10/2013 11:13:22
Completed date/time: 06/10/2013 11:24:25
Validated date/time: 06/10/2013 11:24:25
Rerun type:
Reflex
Rerun
Errors and warnings:
Group Code Description
Replicate 1
Measured (s)
CE 5060 (Data) Normalized curve delta too low
Unit 1 (s)
RE 5100 Measured result failed
Measured
Replicate 1: FAILED
Replicate 2:
Mean: FAILED s
Unit 1
Replicate 1: FAILED
Replicate 2:
Mean: FAILED s
Unit 2
Replicate 1:
Replicate 2:
Mean:
Unit 3
Replicate 1:
Replicate 2:
Mean:
Unit 4
Replicate 1:
Replicate 2:
Mean:
Replicate 1
Replicate 2
Tracking Information
mAbs
106
105
104
103
102
101
5
4
3
2
1
0
-1
-2
-3
-4
0
14
28
42
56
70
84
98
112
s
Analyzer status: Busy
LIS status: Connected
Auto Run status: Ready
LAS status: Ready
For Help, press F1
1 min 5 secs remaining
2 of 4 tests
User ID: uzlabo
Security Level: Operator
15/10/2013 11:50:35

Meting van factor VIII:
mechanische methode



TIJD

tot vermindering

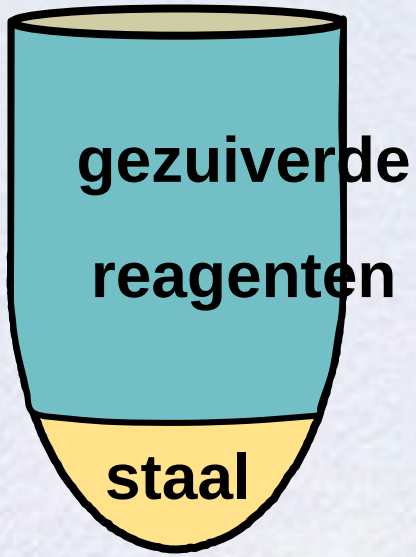
van beweging
van het bolletje

Meting van factor VIII:

chromogene testen

Meting van factor VIII:

chromogene testen



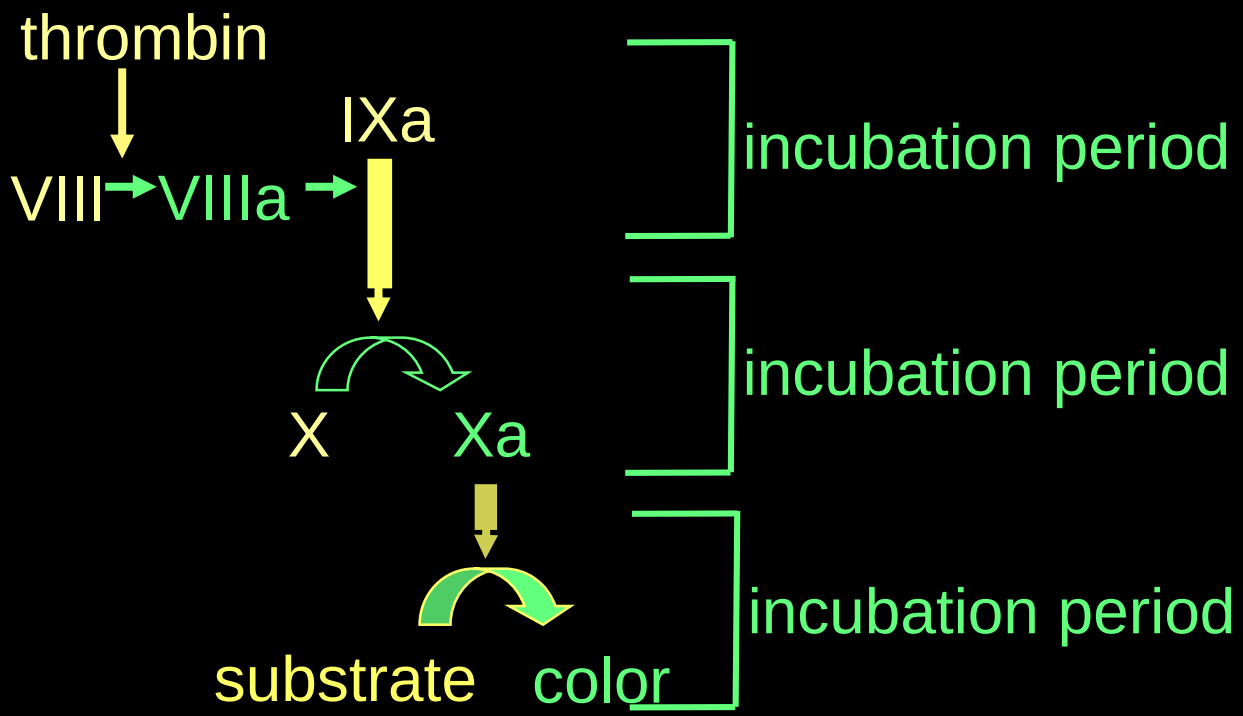
FVIII chromogenic assay

test sample (FVIII)

+ a little thrombin
+ phospholipids
+ FIXa, FX

+ Ca^{++}

+ chromogenic substrate for FXa



Diagnose van matige hemofilie A

Cut-off: 40%?

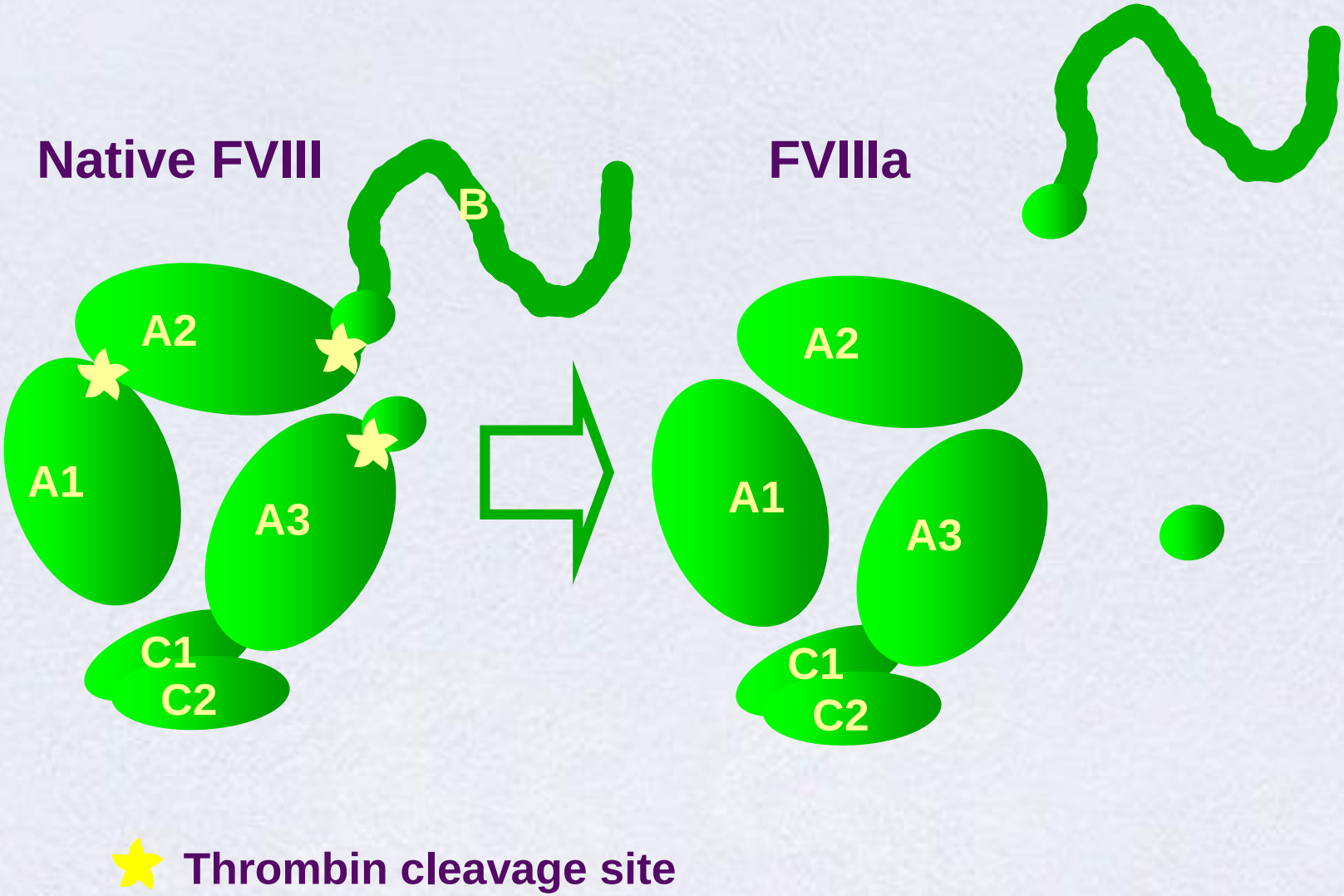
FVIII/VWF interaction

discordant FVIII assays

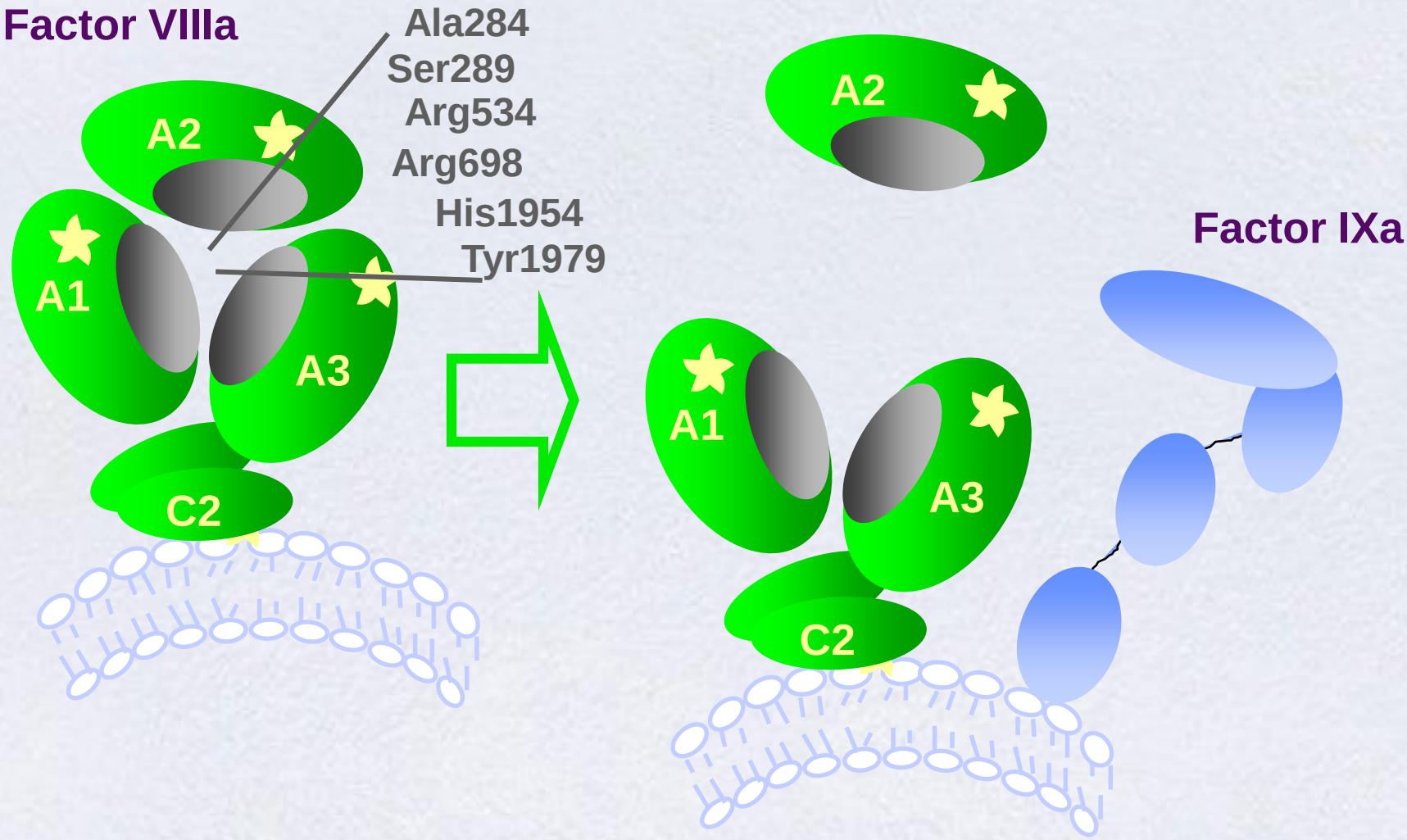
combined FVIII/FV deficiency

	FVIII (%)	
	Stollingstest	chromogene test
normal plasma 1/2	45,6	47,1
Arg531His	42,1	18,2
His1954Leu	78,5	28,2

Native and thrombin-activated FVIII



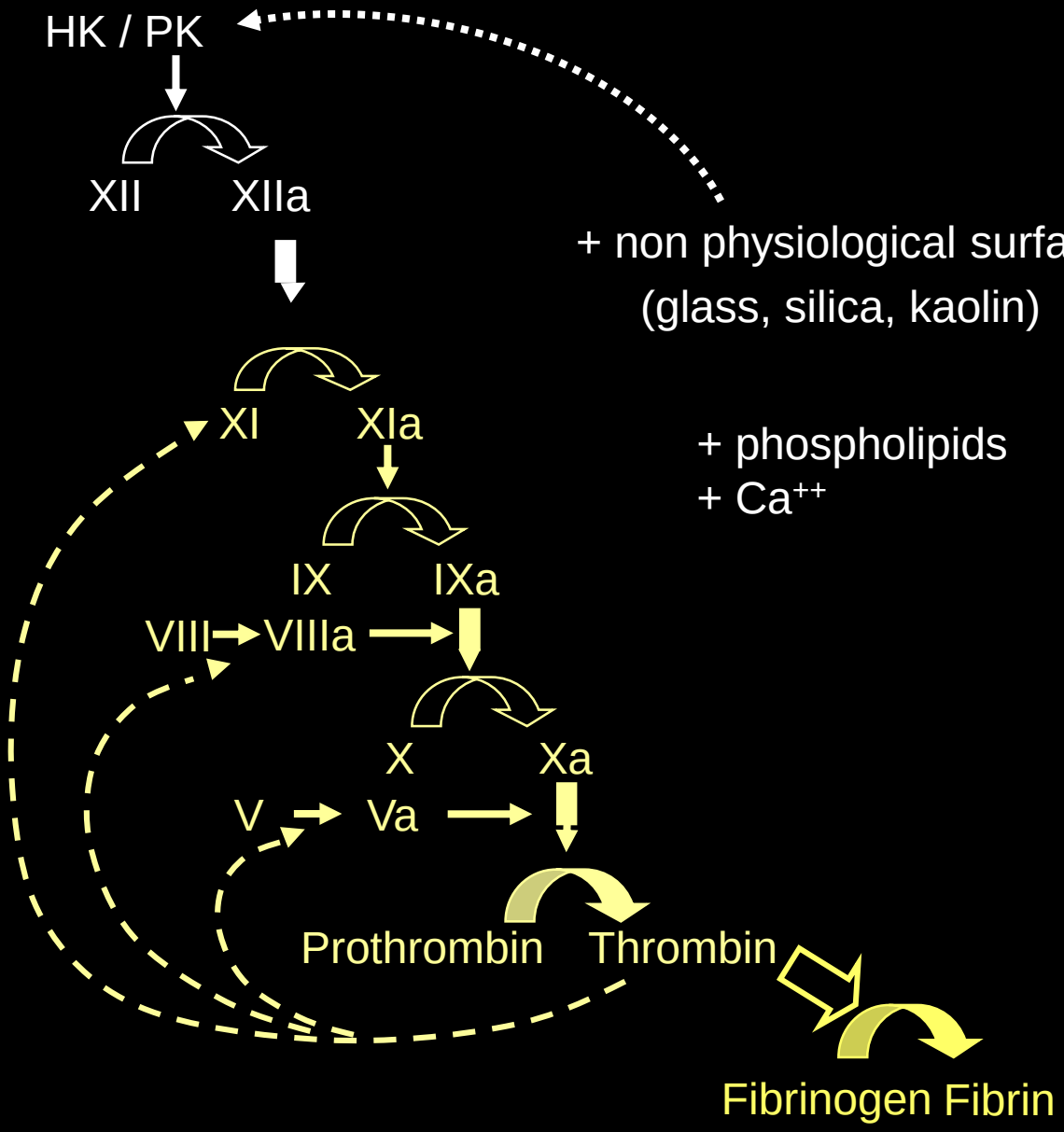
Accelerated dissociation of A2 inactivates FVIIIa



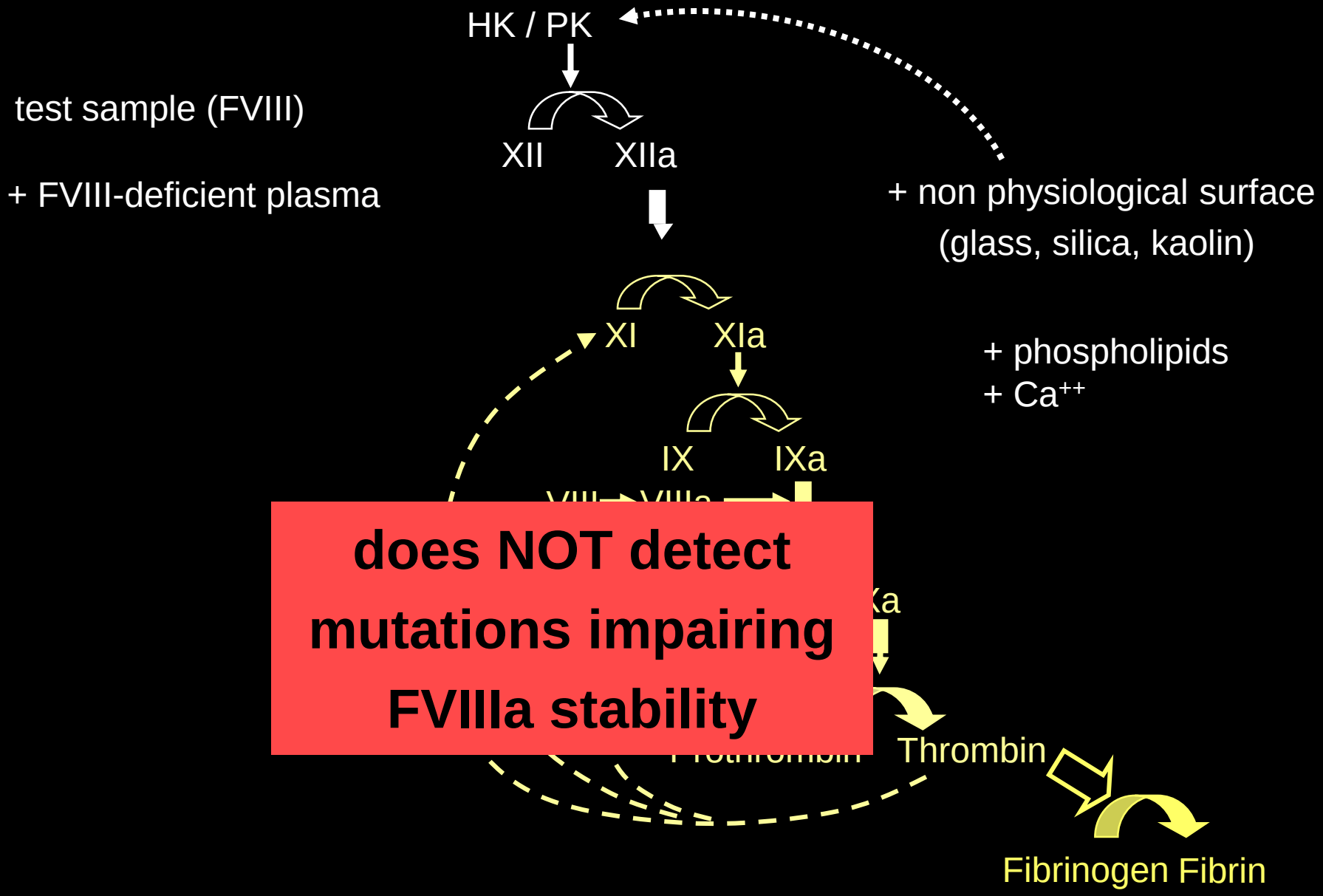
FVIII one-stage assay (APTT-based)

test sample (FVIII)

+ FVIII-deficient plasma



FVIII one-stage assay (APTT-based)



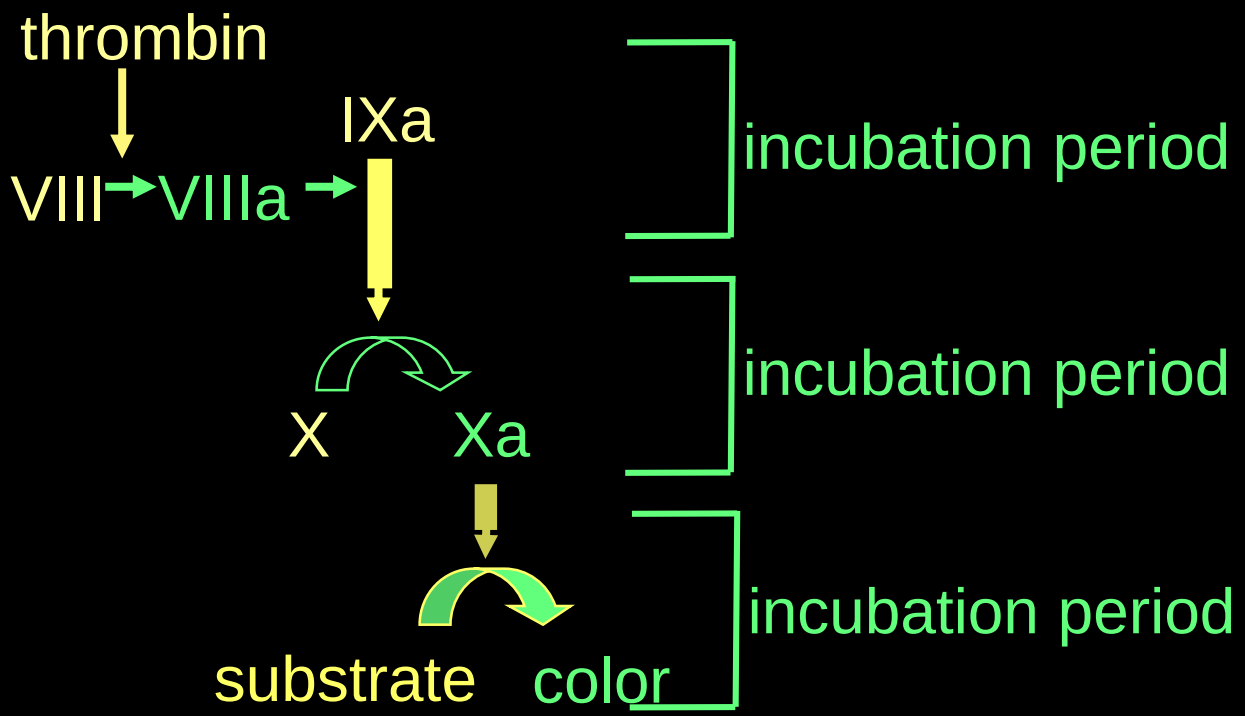
FVIII chromogenic assay

test sample (FVIII)

+ FIXa, FX,
+ a little thrombin
+ phospholipids

+ Ca^{++}

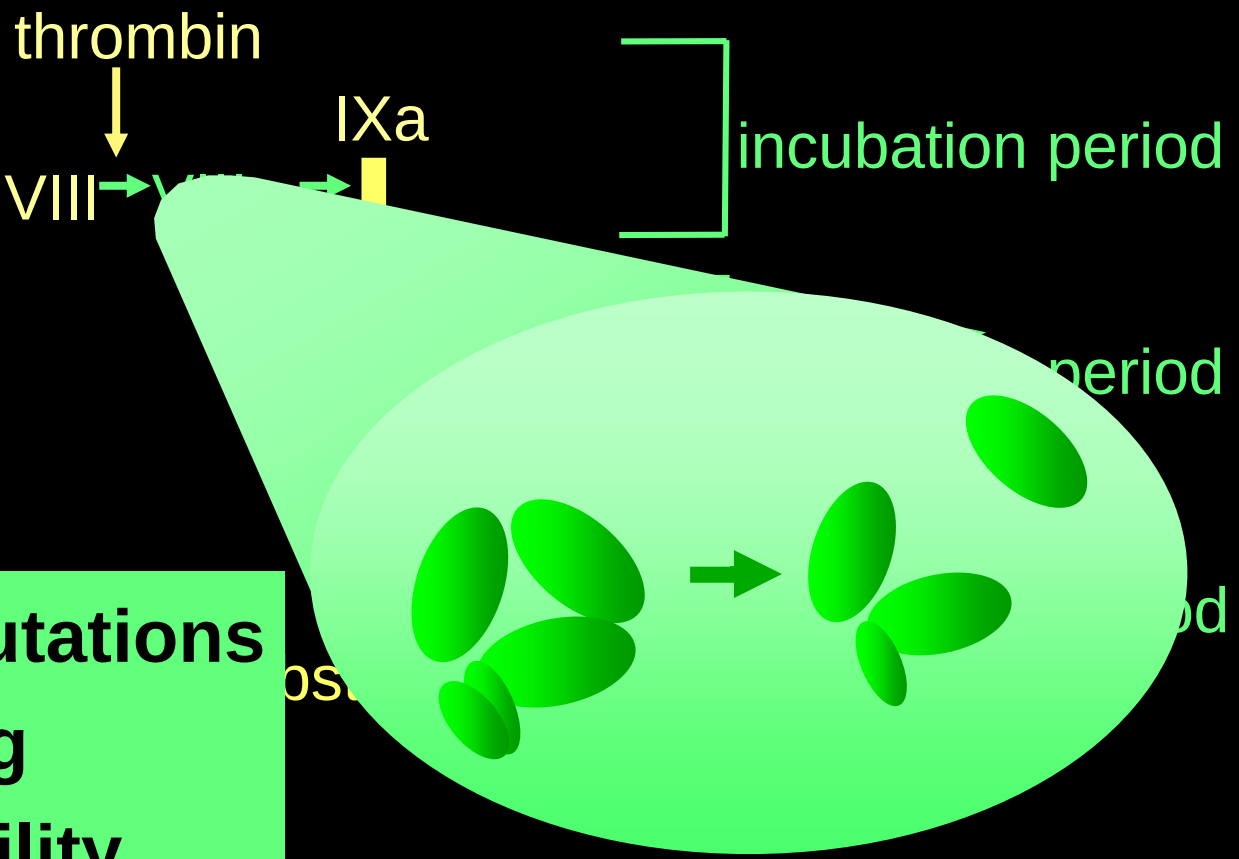
+ chromogenic substrate for FXa



FVIII chromogenic assay

test sample (FVIII)

+ FIXa, FX,
+ a little thrombin
+ phospholipids



+ Ca^{++}

sensitive to mutations impairing FVIIIa stability

Genetic analyses

gene specific analysis for FVIII or FIX (identification of inversion)

exome sequencing including 99 genes for bleeding, thrombotic and platelet disorders.

Van Laer C et. al. ThromboGenomics implementation in Belgium, Belg J Hematol 2020;12(3):99-105

Diagnose van matige hemofilie A

Cut-off: 40%?

FVIII/VWF interaction

discordant FVIII assays

combined FVIII/FV deficiency

FVIII <40 IU dL^{-1}

confirmed on a repeat sample (preanalytical problems).

AND
- known family history of hemophilia A
or
- pathogenic FVIII mutation
or
- exclude von Willebrand disease, including VWD type 2N

ISTH guidelines (JTH 2018;16: 2530)

>40 IU dL^{-1}

confirmed on a repeat sample (at the time of resolution of inflammation).

AND

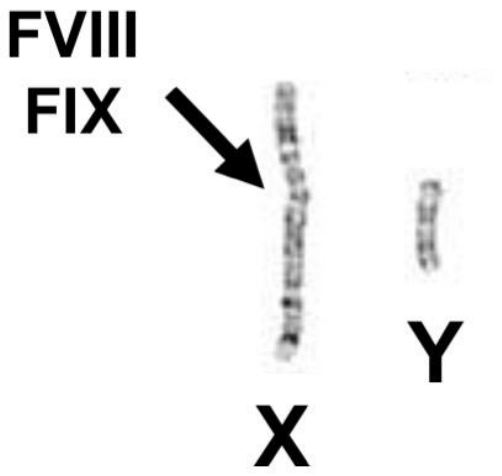
a pathologic DNA change in the F8 gene

ISTH guidelines (JTH 2018;16: 2530)

Mannen en vrouwen met hemofilie

Hemofilie A en B zijn X-gebonden ziekten

FVIII en FIX op de X chromosoom



mannen: 1 X chromosoom

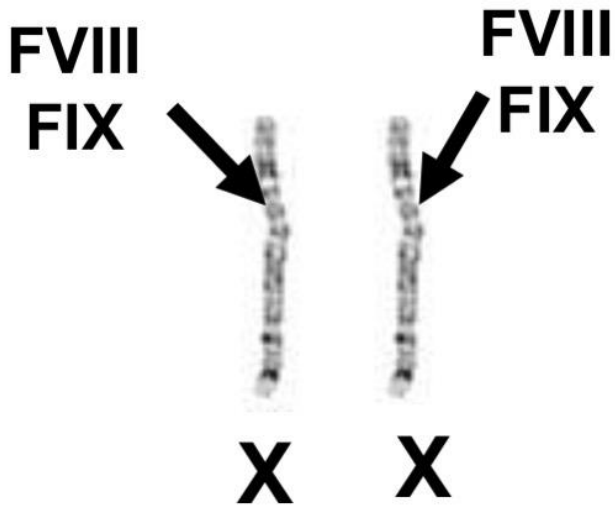
Hemofilie A: 1/10.000

Hemofilie B: 1/50.000

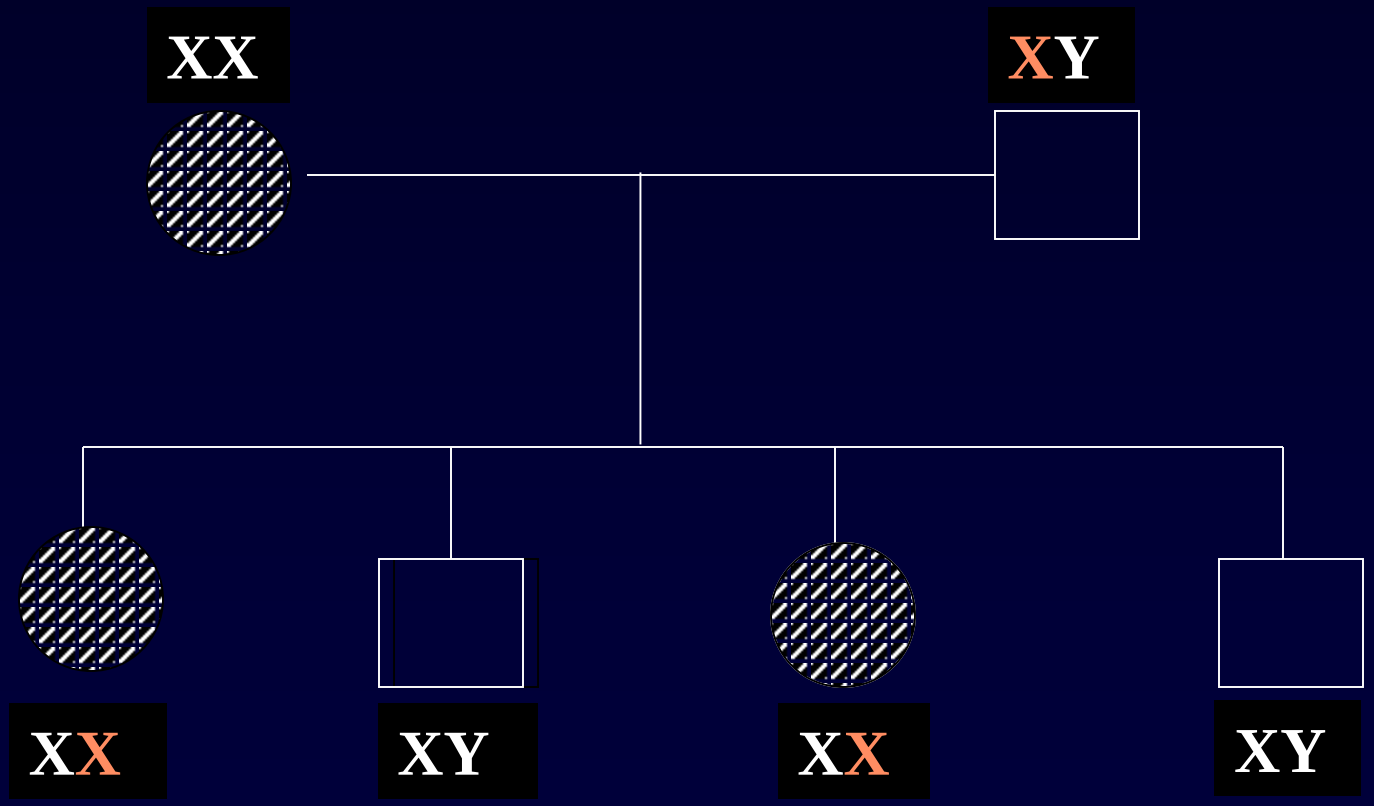
vrouwen: 2X chromosomen

meestal draagsters;

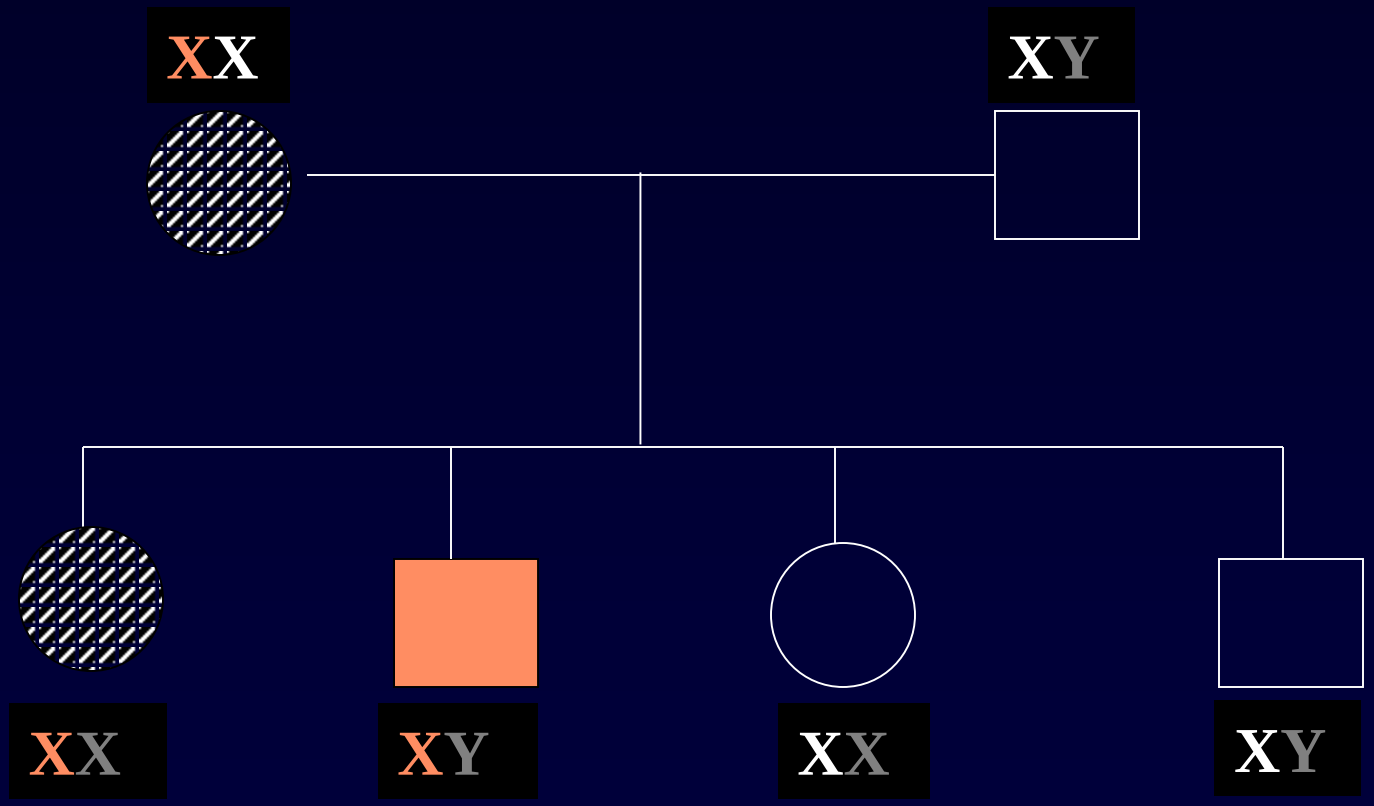
heel zeldzaam symptomen



X-linked inheritance



X-linked inheritance



Haemophilia A is an X linked disorder

- Virtually exclusively in boys: 1 in 10000
- Transmission by women
  - “carriers”
  - in most cases, no bleeding problem
- No family history in one case out of three

LIFESTYLE

Amal and George Clooney welcome twins

George Clooney, the 56-year-old Oscar-winning actor,

Female carriers with FVIII:C levels < 40 IU dL-1 should be considered as having hemophilia and be managed as such.

ISTH guidelines (JTH 2018;16: 2530)

Monitoring van de behandeling

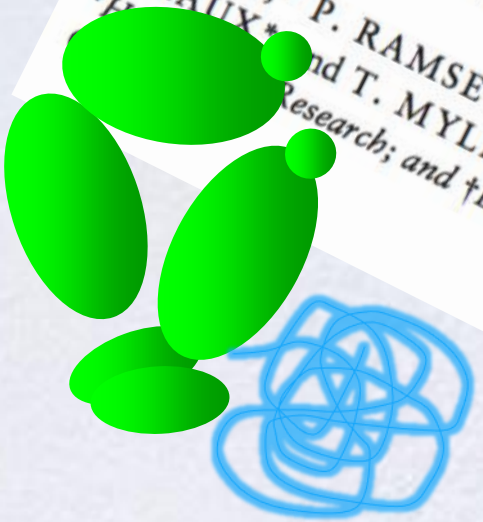
Haemophilia (2014), 20, 593–600

ORIGINAL ARTICLE *Laboratory science*

Evaluation of the activated partial thromboplastin time assay for clinical monitoring of PEGylated recombinant factor VIII (BAY 94-9027) for haemophilia A

J-M. GU,* P. RAMSEY,* V. EVANS,* L. TANG,† H. APELER,† L. LEONG,* J. E. MURPHY,† V. LAUX* [illegible] and T. MYLES*
*[illegible] Research; and †Biological Research, US Innovation Center, Bayer HealthCare Pharmaceuticals, San Francisco,

DOI: 10.111



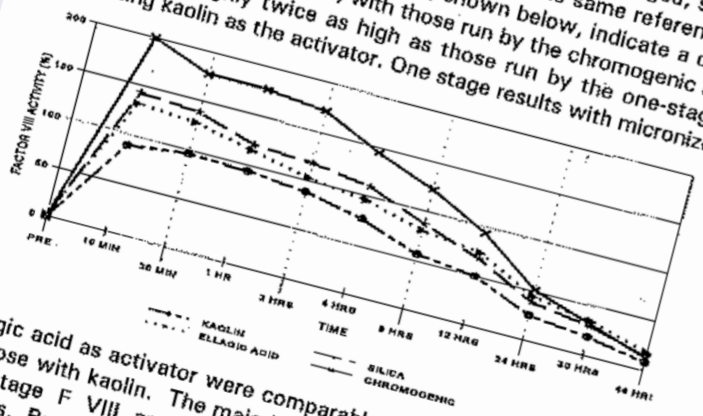
10 maal onderschatting met bepaalde stollingstesten

Will the real FVIII please stand up?

Lusher J 1995 Blood 86 supll 1 ab

PLASMA FVIII LEVELS MEASURED AFTER INFUSION OF RECOMBINANT F VIII (rFVIII) VARY SIGNIFICANTLY WITH DIFFERENT ASSAY METHODS - (WILL THE REAL FVIII LEVEL PLEASE STAND UP!). J. M. Lusher, C. Hillman-Wiseman, P. Simpson *, D. Hurst, Children's Hospital of Michigan, Detroit, Mi. and Bayer Corp., Berkeley, Ca.

As part of a bioequivalence study comparing two lots of rFVIII (Kogenate®), 8 persons with hemophilia A were infused with rFVIII from each lot, and multiple plasma samples were then obtained from each subject for determination of FVIII levels over 48 hours. In view of reported discrepancies between FVIII levels measured by different assay methods, we assayed patient samples by 4 different methods: chromogenic (Coatest, F VIII, Kabi), and one stage APTT method using 3 different types of activators- micronized silica (Organon Teknika), ellagic acid (Dade), and kaolin (Diagnostica Stago). All samples were immediately centrifuged, snap frozen and stored at -70°C until assayed in duplicate. The same reference plasma standard was used throughout. Results, shown below, indicate a consistent difference in FVIII assay values, with those run by the chromogenic substrate method being roughly twice as high as those run by the one-stage APTT method using kaolin as the activator. One stage results with micronized silica

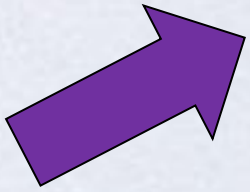


or ellagic acid as activator were comparable, and were consistently higher than those with kaolin. The majority of clinical laboratories in the U.S. use a one-stage F VIII assay, employing micronized silica or ellagic acid activators. Because significantly different results can be obtained depending on F VIII assay method used, multicenter studies must carefully standardize F VIII assay techniques and reagents in order to combine data for analysis.

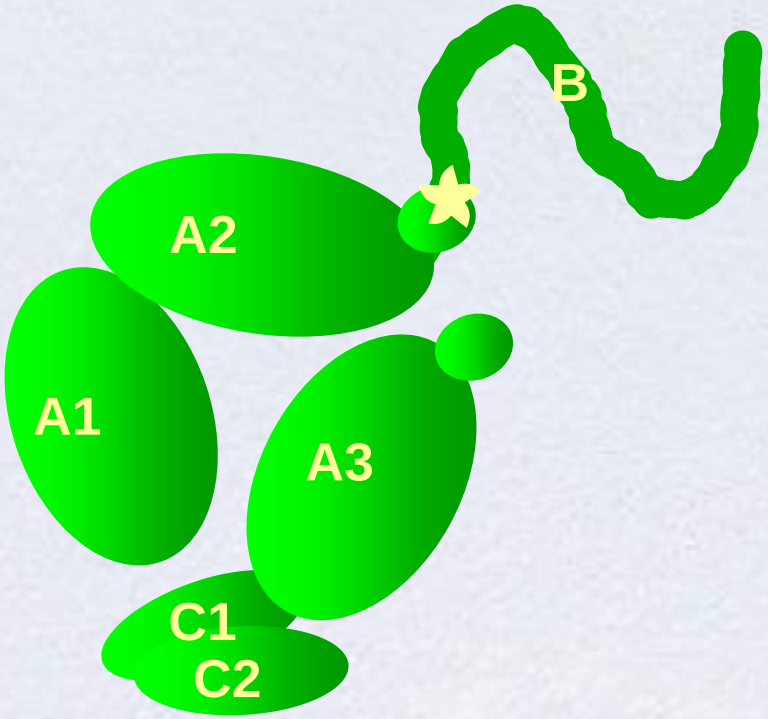
Will the real FVIII please stand up?

Lusher J 1995 Blood 86 supll 1 abstract 755

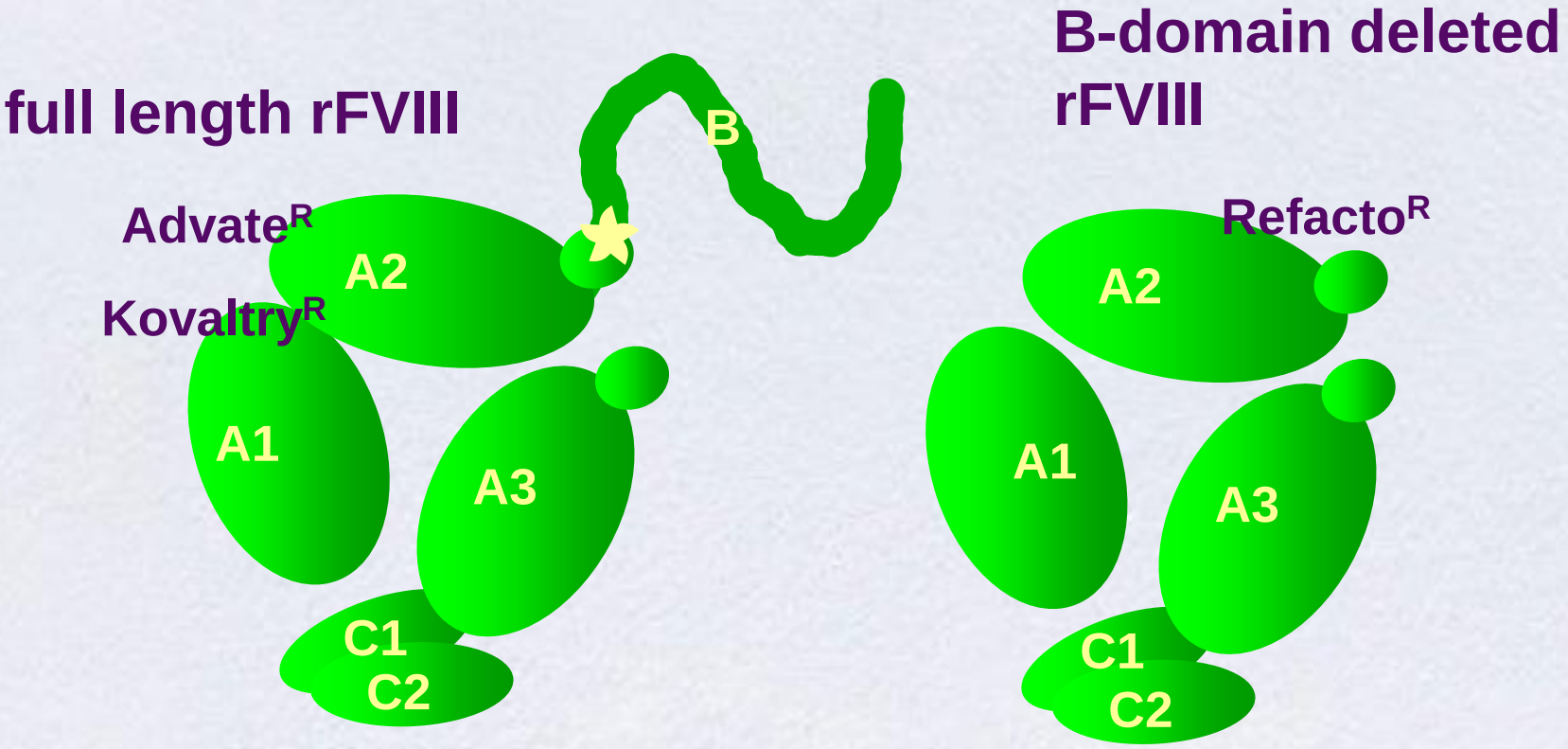
FVIII concentraten



plasma derived FVIII

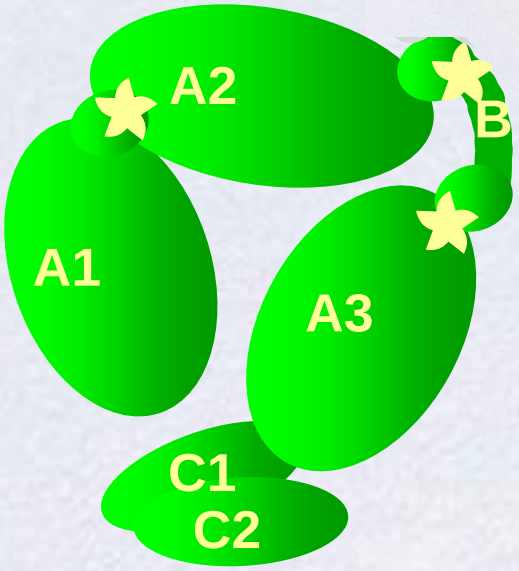


recombinant FVIII (rFVIII)



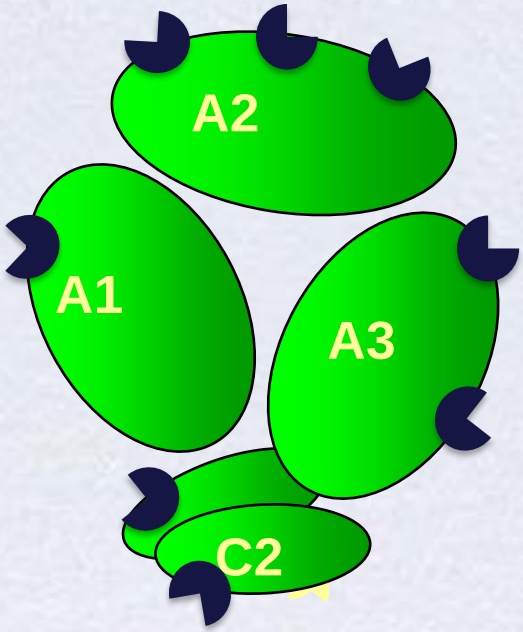
Single chain-rFVIII

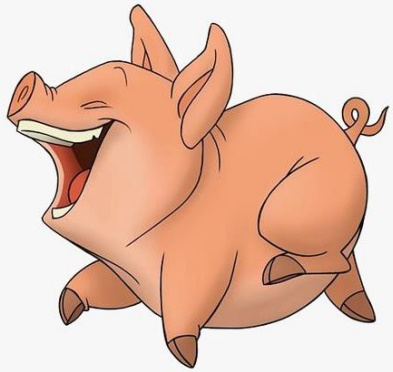
Afstyla^{R}



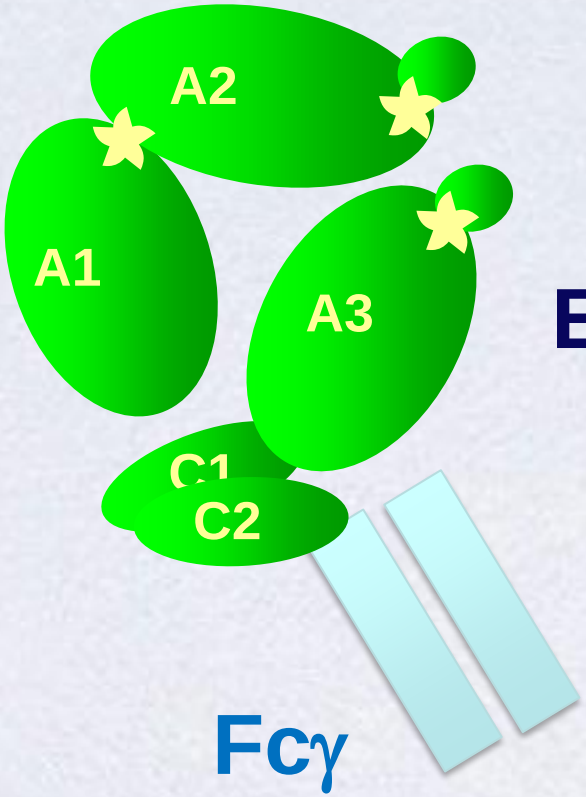
Obizur^{R}, recombinant FVIII met varkens aminozuur-sequentie

Obizur^{R}

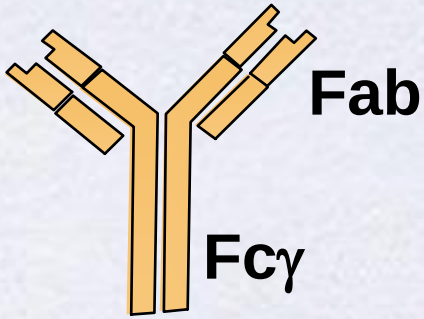


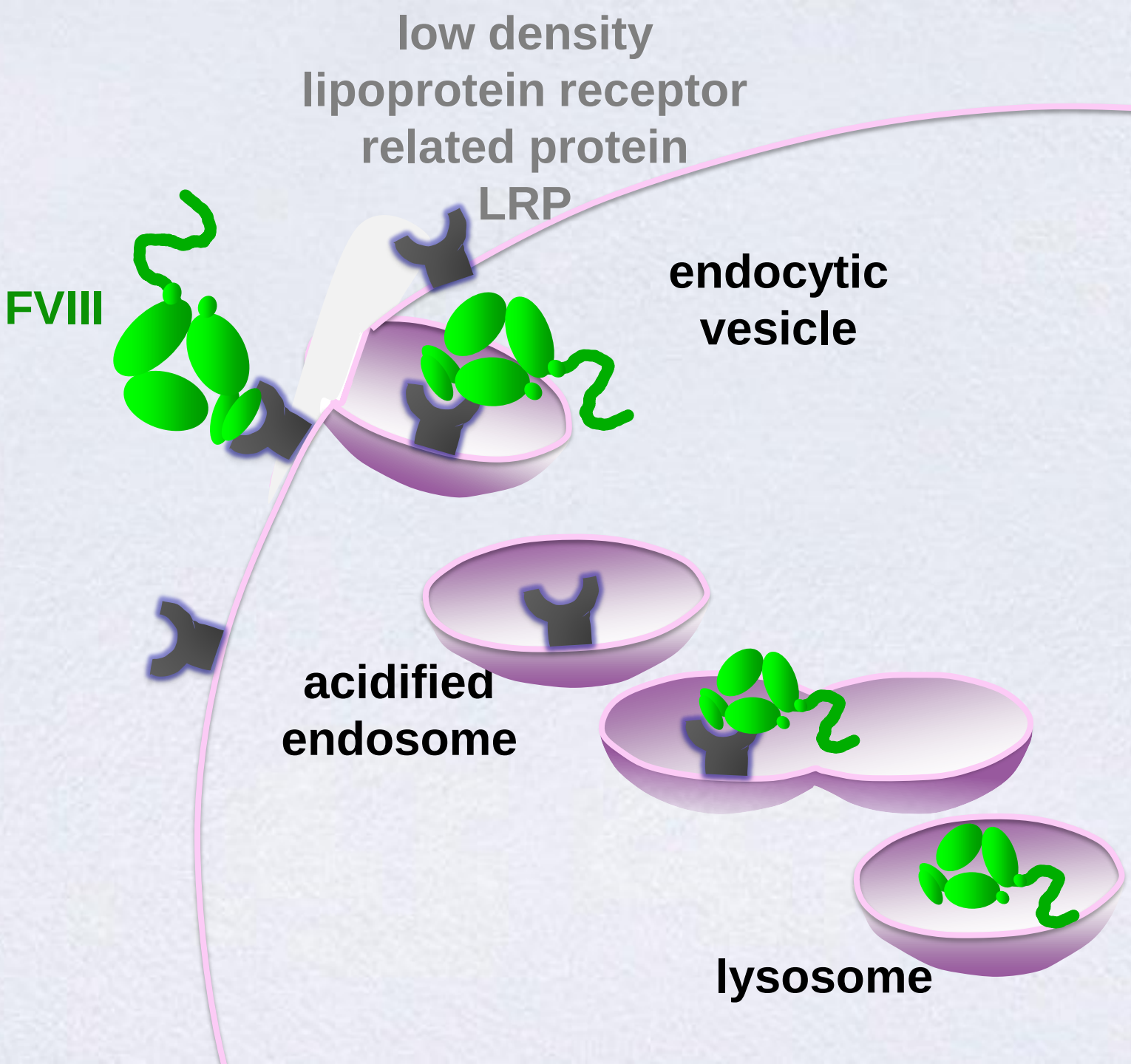


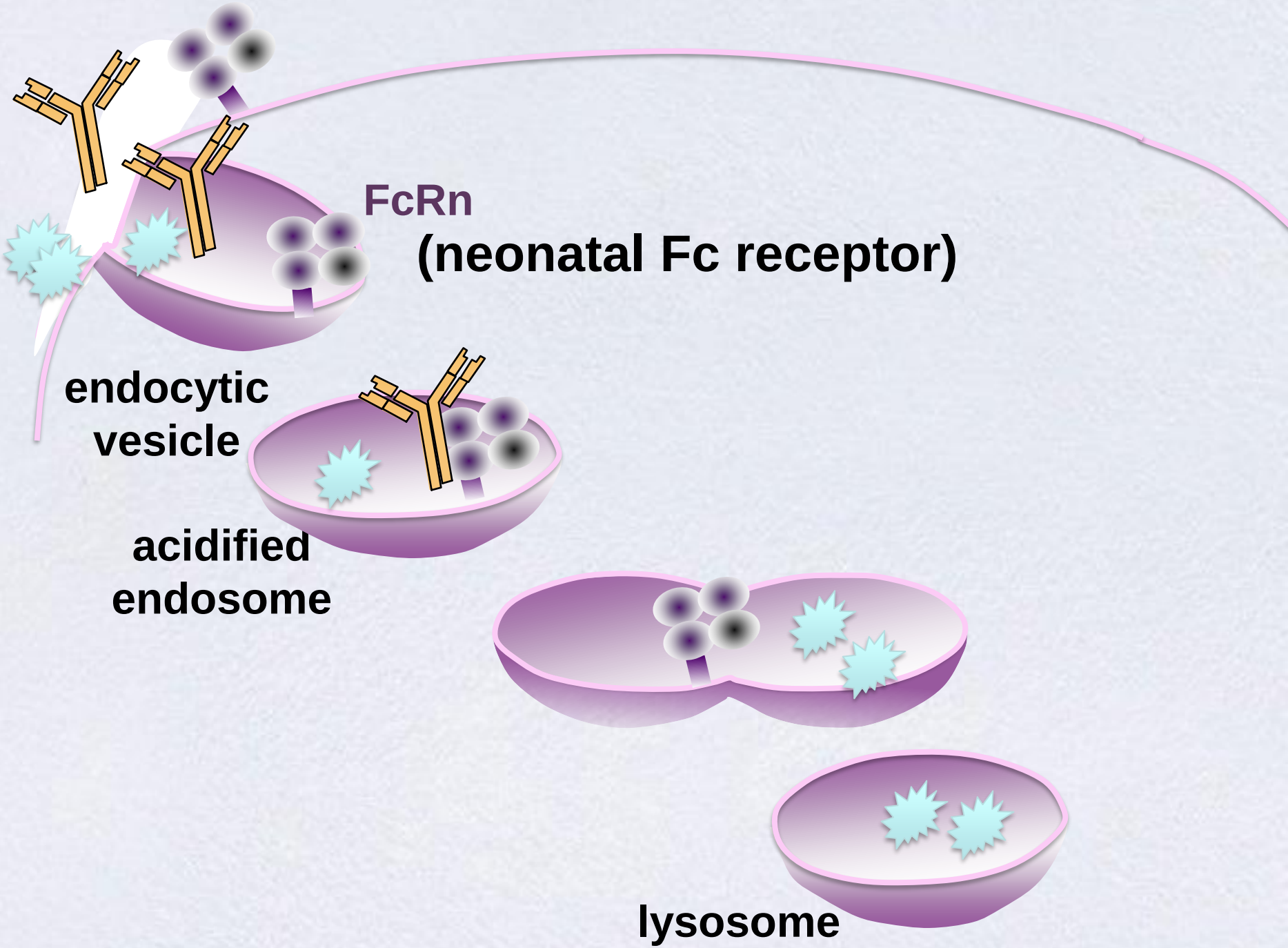
Fcγ-rFVIII

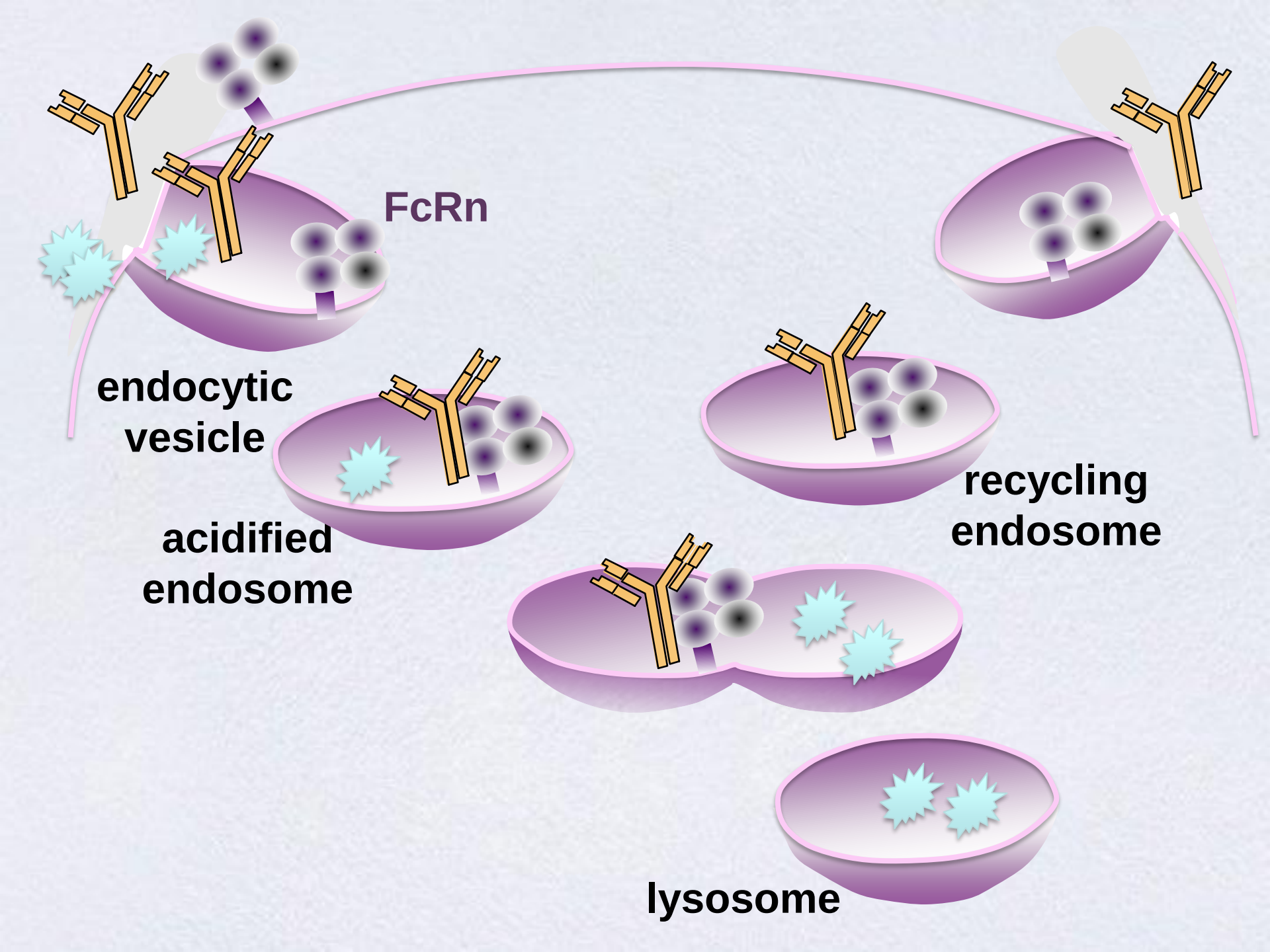


Elocta^{R}




low density
lipoprotein receptor
related protein
LRP
FVIII
endocytic
vesicle
acidified
endosome
lysosome

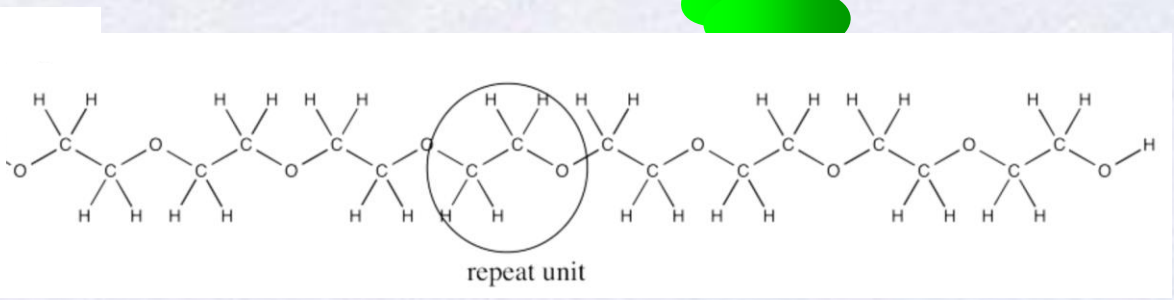

FcRn
(neonatal Fc receptor)
endocytic vesicle
acidified endosome
lysosome


FcRn
endocytic vesicle
acidified endosome
recycling endosome
lysosome

Peggylated rFVIIIs

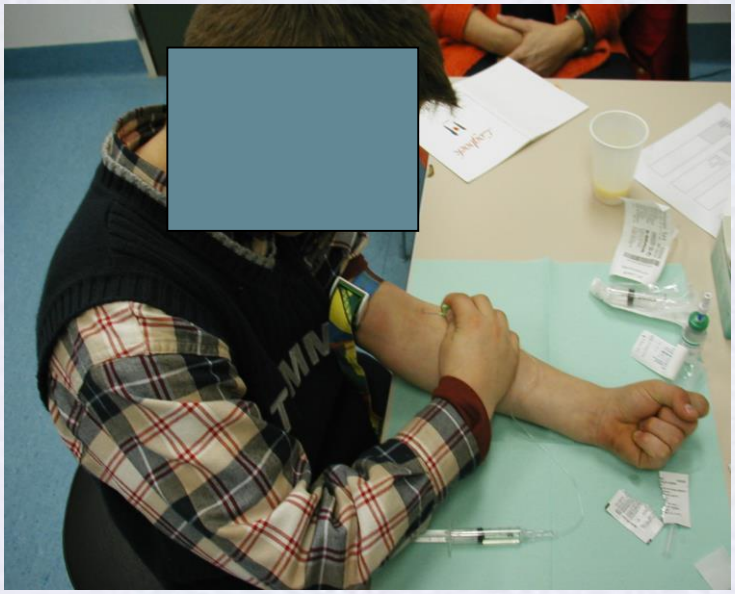
Bay 94-9027

Adynovate^{R}



prophylaxis with FVIII

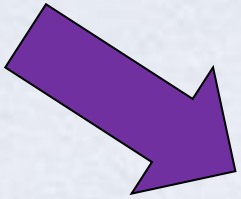
Lu	Ma	Me	Je	Ve	Sa	Di
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				



prophylaxis with extended half-life rFVIII

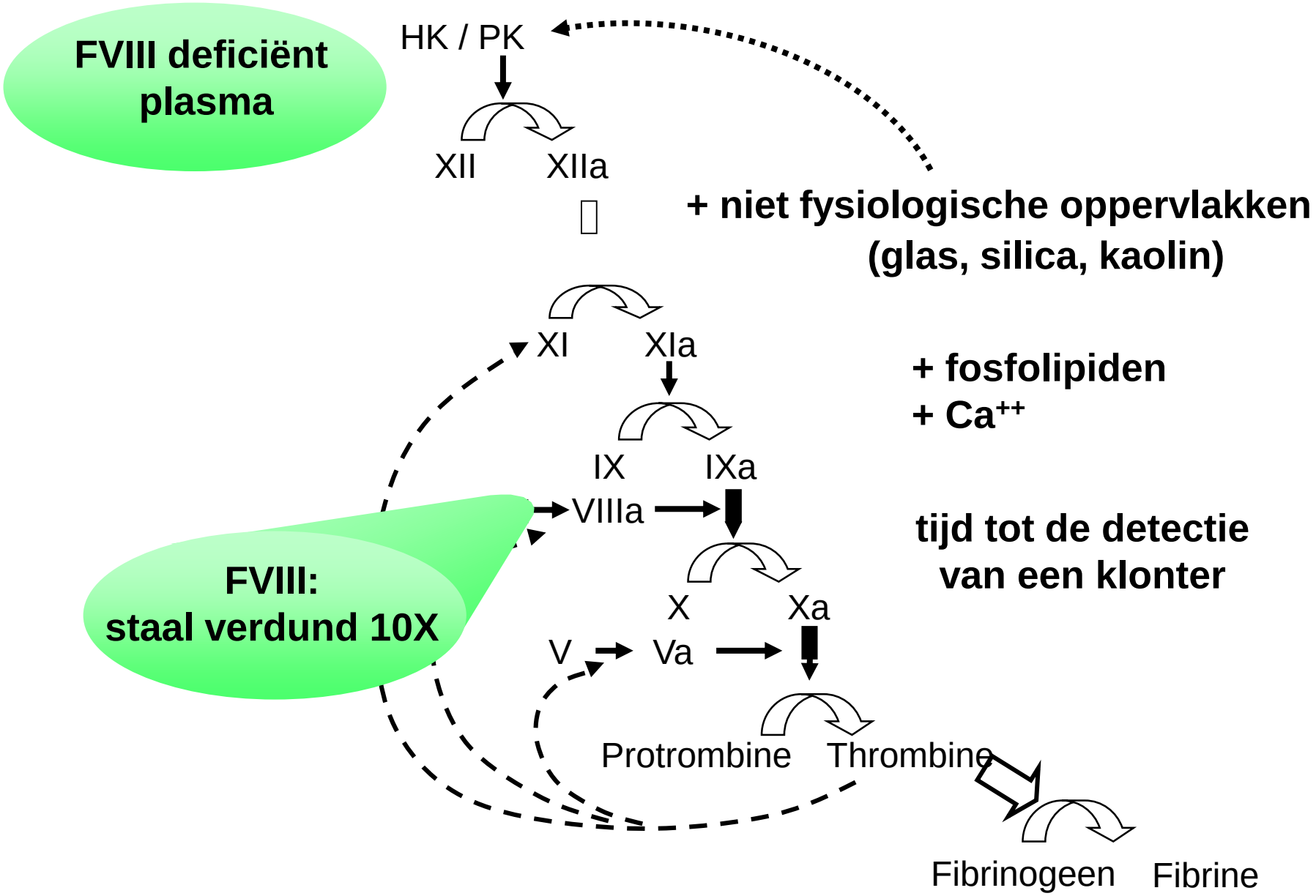
Lu	Ma	Me	Je	Ve	Sa	Di
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

By courtesy of K. Peerlinck

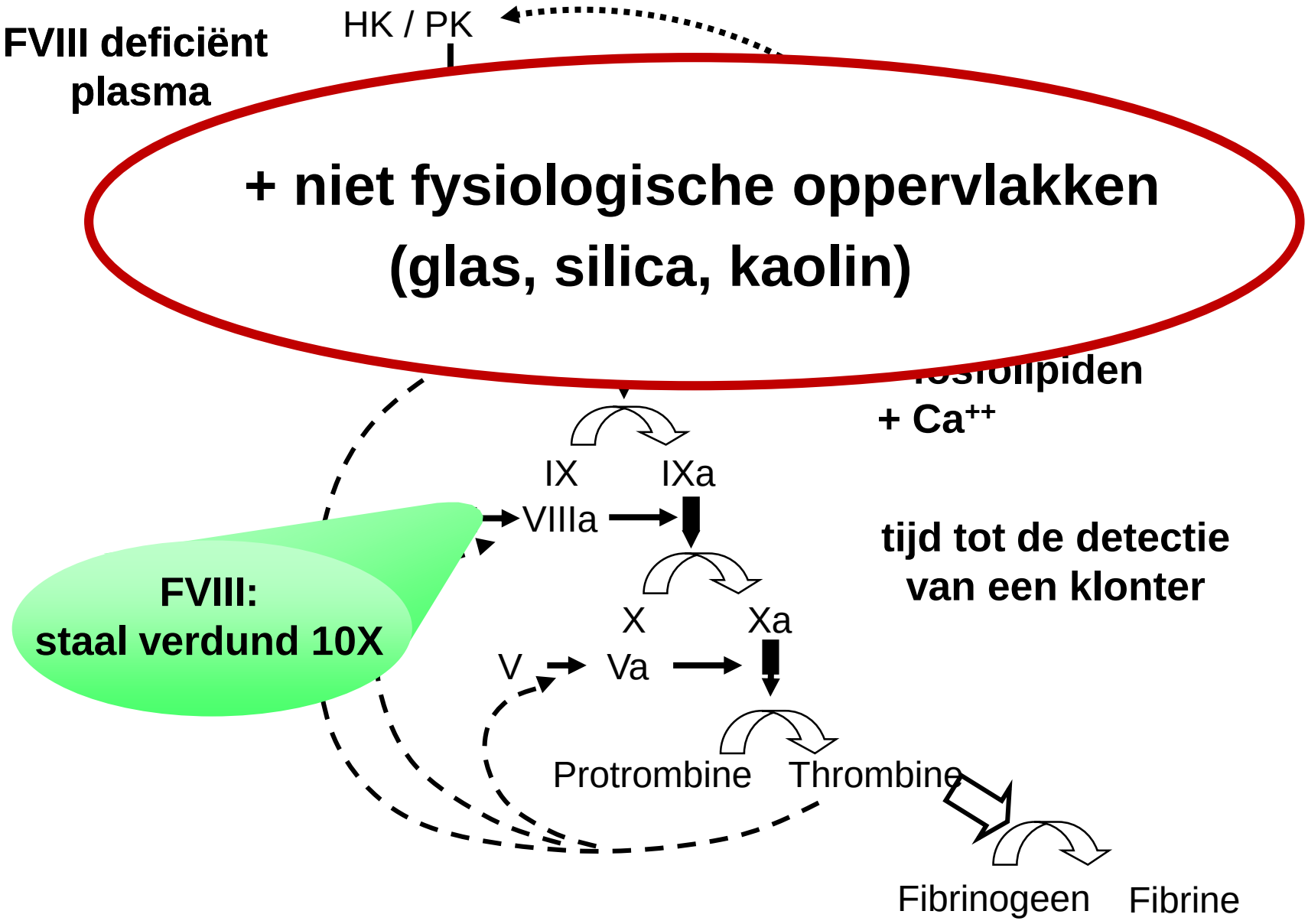


FVIII testen

Meting van FVIII met een APTT gebaseerde test



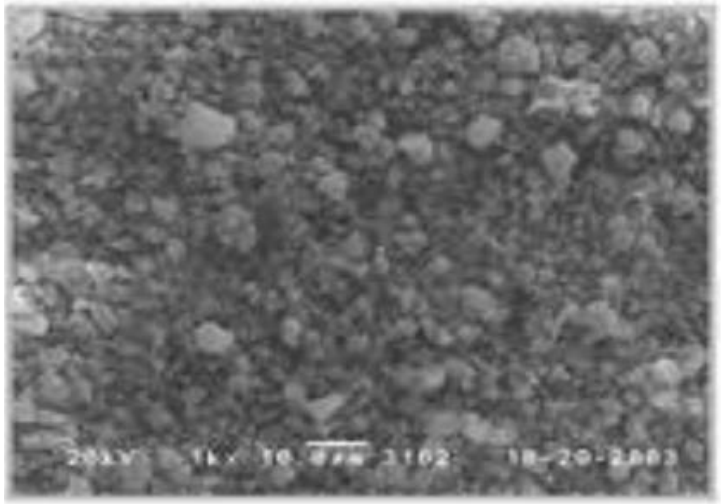
Meting van FVIII met een APTT gebaseerde test



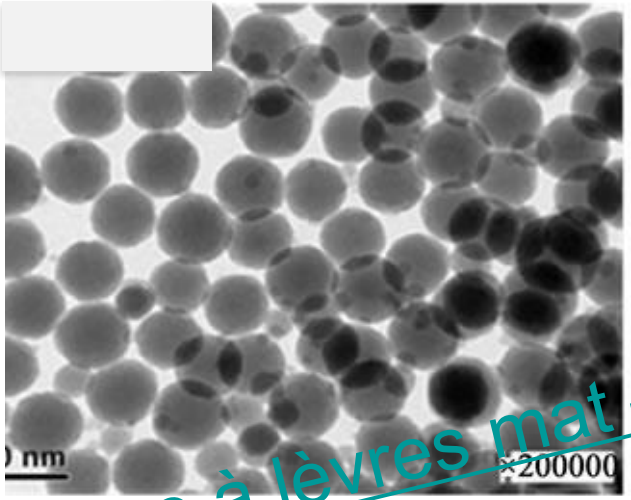
Silica: SiO_2

Silica: SiO_2

micronisated silica



colloidal silica



Patent EP0566442B1 - Rouge à lèvres mat –

3 janv. 1996 - Composition de **rouge à lèvres mat** caractérisée par... un mica enrobé … de sphères de **silice**.

Kaolin

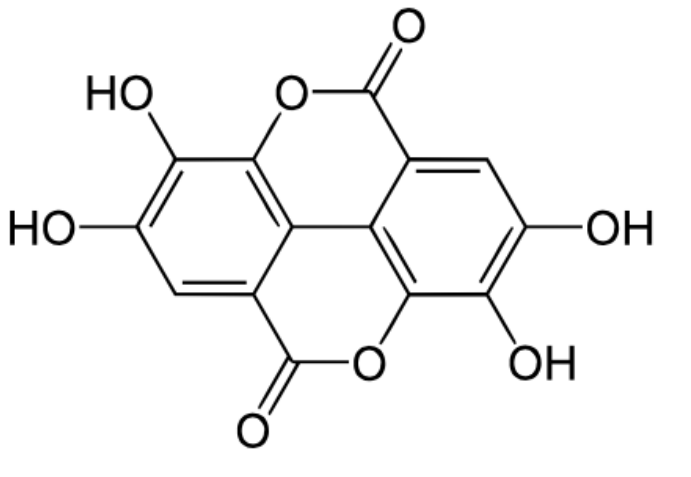
silicate d’aluminium hydraté

$Al_2Si_2O_5(OH)_4$

Ellaginezuur (oplosbaar) + Ca^{++}, Zn^{++}, Co^{++} or Fe^{++}

onoplosbaar

negatief geladen



Bock P et al. Activation of intrinsic blood coagulation by ellagic acid: insoluble ellagic acid-metal ion complexes are the activating species. Biochemistry, 1981, 20:7258

Ellagic acid

Le whisky est riche en antioxydants, dont un
en particulier que l'on connaît sous le petit
Non content de réduire

Extrait de poudre de grenade avec des polyphénols d'acide ellagique pour antioxydant

Obtenir le Dernier Prix

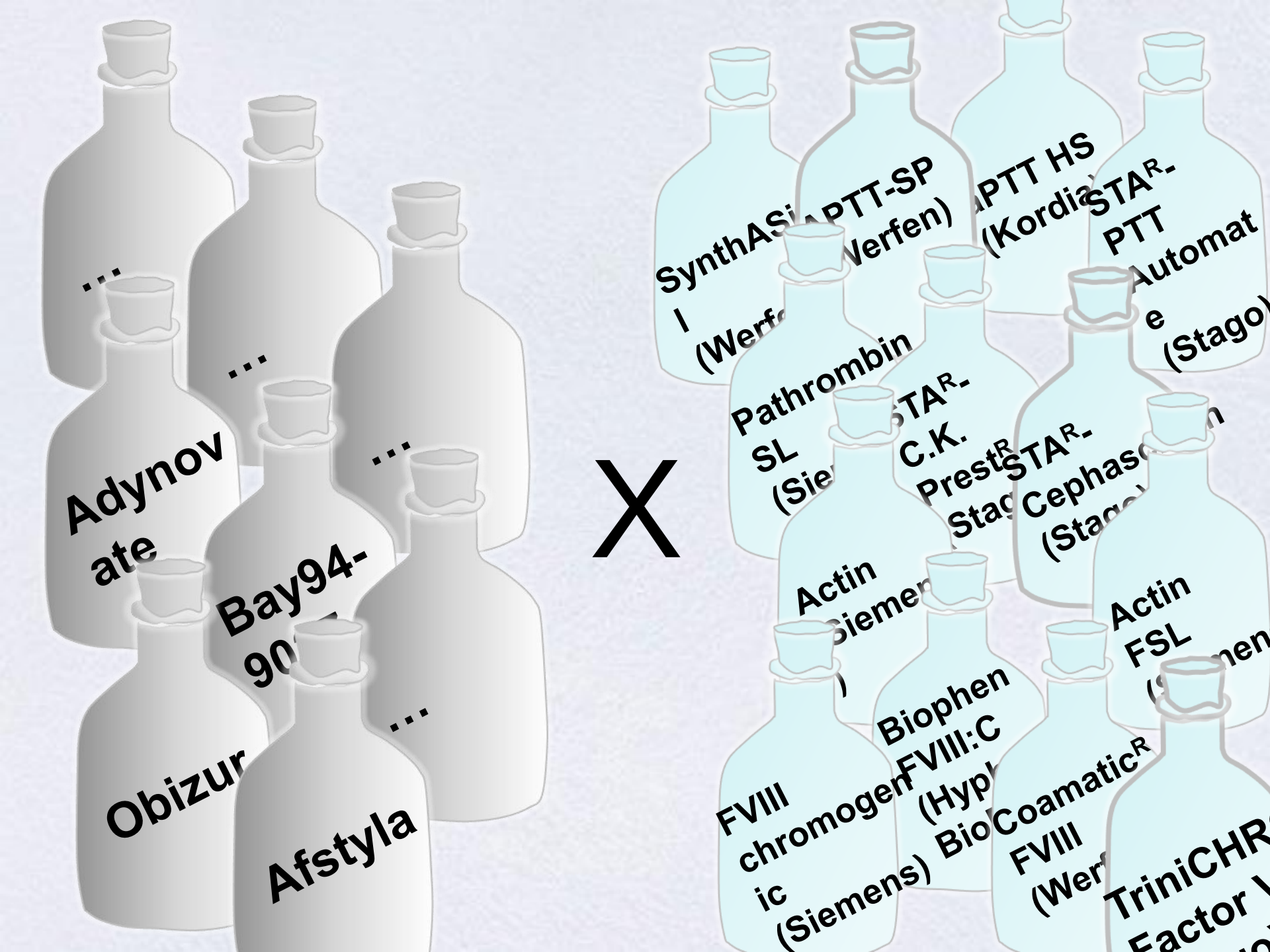
Prix Unitaire:	
Commande Minimum:	25 kg
Conditions de Paiement:	LC, T/T, Western Union, PayPal
Marque Déposée:	Meiya

Pomegranate Powder Extract

L'acide ellagique des fruits rouges : un super soin coup d'éclat | E-Santé

www.e-sante.fr › Beauté › Beauté de la peau

21 nov. 2016 - Puissant **antioxydant**, anti-taches, anti-rides, l'**acide ellagique** est un actif prisé en cosmétique. On le retrouve au cœur de nombreux fruits, et il ...


...
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Adynov
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Bay94-
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PTT-SP
Verfen)
PTT HS
(Kordia)
STA R-
PTT
Automat
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Pathrombin
SL
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C.K.
Prest R
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(Stag
Actin
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Actin
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FVIII
chromogen
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Biophen
FVIII:C
(Hyp
Bio
Coamatic R
FVIII
(Wer
TriniCHR
Factor

“acceptable” assays?

	N8 GP	Bay 94–9027	rFVIIIFc
Relevant references	29, 30, 31, 34	35	25, 28, 29
Chromogenic FVIII assay	Yes[a]	Yes[a]	Yes[a]
One-stage reagents			
STA-PTT A	No	No	Yes[b]
STA-C.K. Prest	Yes	?	Yes[b]
Actin FS	Yes	?	Yes[b]
Actin FSL	Yes	?	Yes[a]
Pathromtin SL	?	?	Yes[a]
SynthASil	?	?	Yes[a]
SynthAFax	No	Yes	?
DG Synth	Yes	?	?
Cephascreen	Yes	Yes	Yes[a]
APTT Sp	No	No	?

Kitchen 2017 Semin Thromb Hemost 43: 331

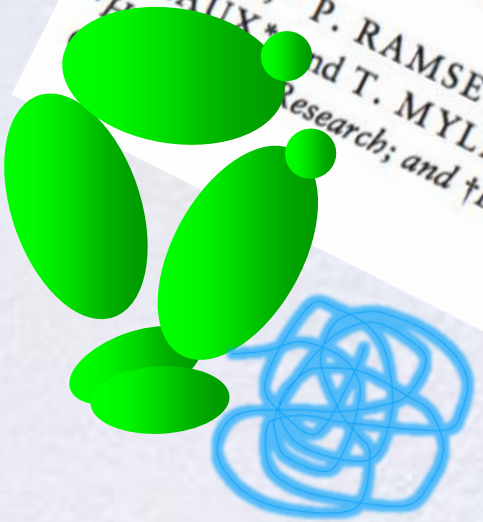
Haemophilia (2014), 20, 593–600

ORIGINAL ARTICLE *Laboratory science*

Evaluation of the activated partial thromboplastin time assay for clinical monitoring of PEGylated recombinant factor VIII (BAY 94-9027) for haemophilia A

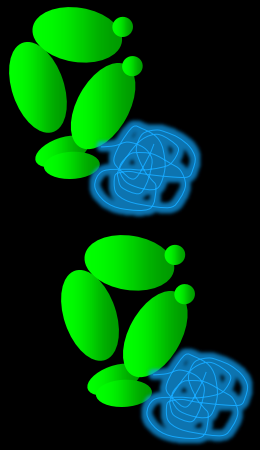
J-M. GU,* P. RAMSEY,* V. EVANS,* L. TANG,† H. APELER,† L. LEONG,* J. E. MURPHY,† V. LAUX* [illegible] and T. MYLES*
*[illegible] Research; and †Biological Research, US Innovation Center, Bayer HealthCare Pharmaceuticals, San Francisco,

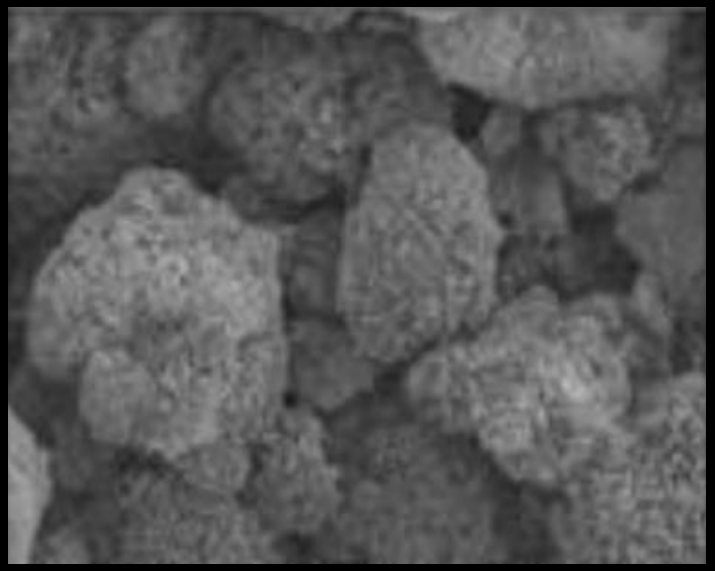
DOI: 10.111



10-fold underestimation with some one-stage clotting assays

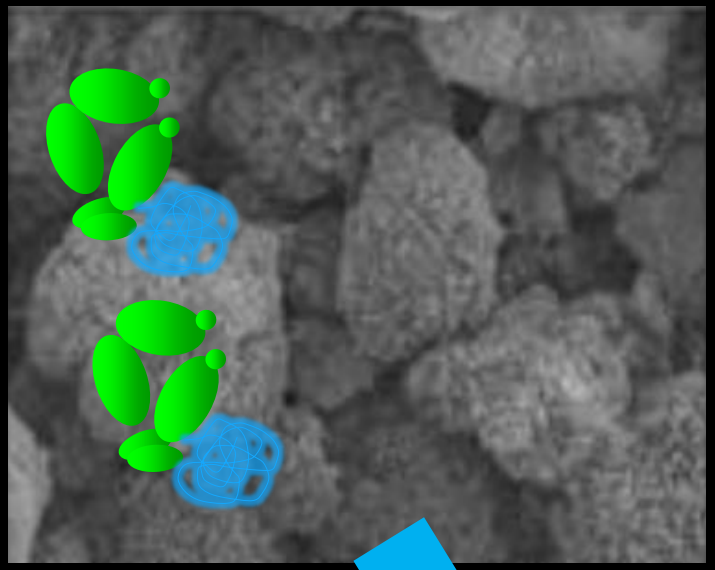
FVIII one-stage assay (APTT-based)





+ silica

FVIII one-stage assay (APTT-based)



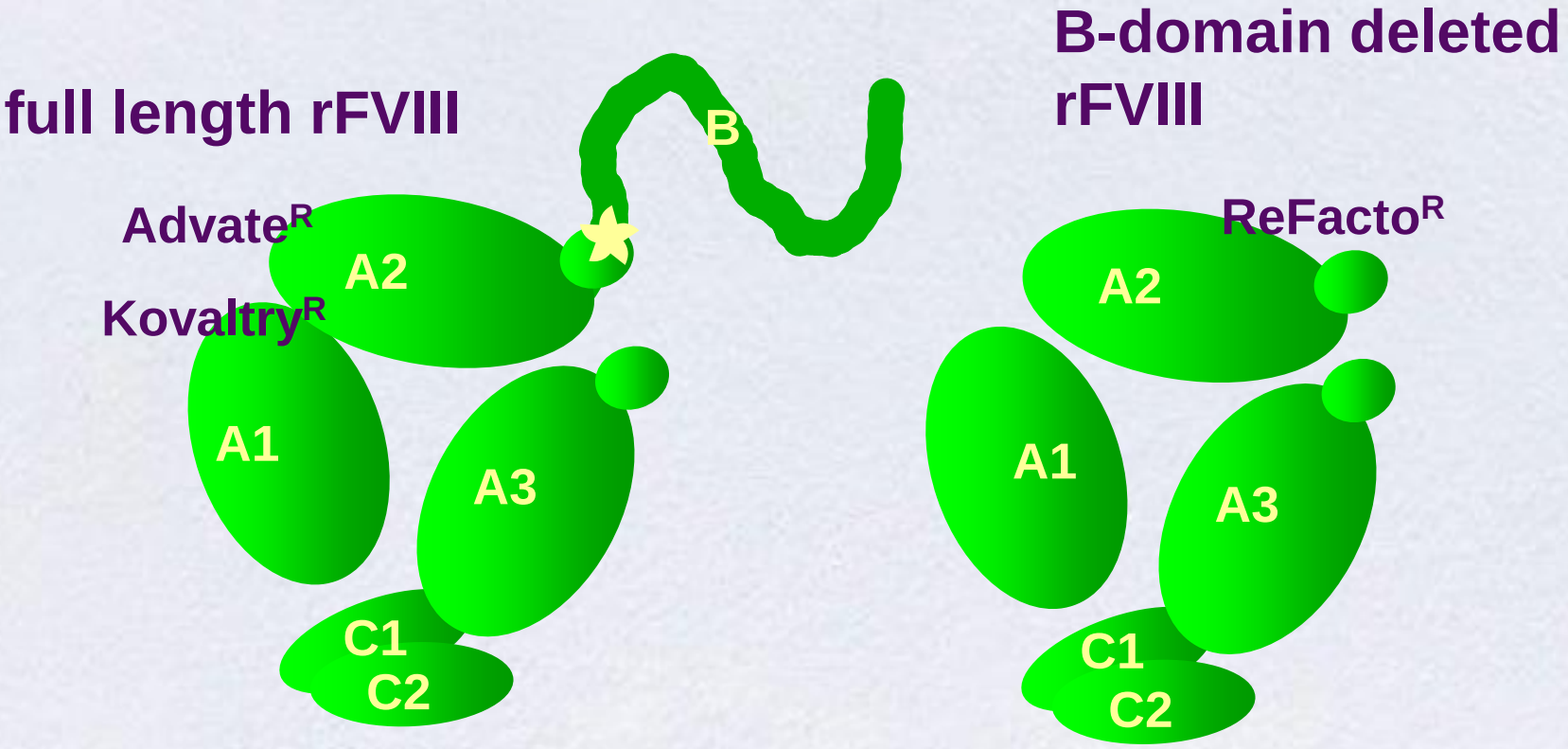
+ silica

geen FVIII

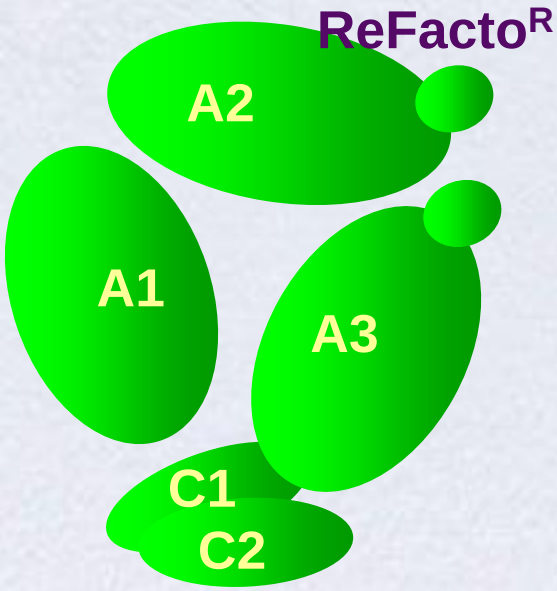
onderschatting

ReFacto

recombinant FVIII (rFVIII)



B-domain deleted rFVIII

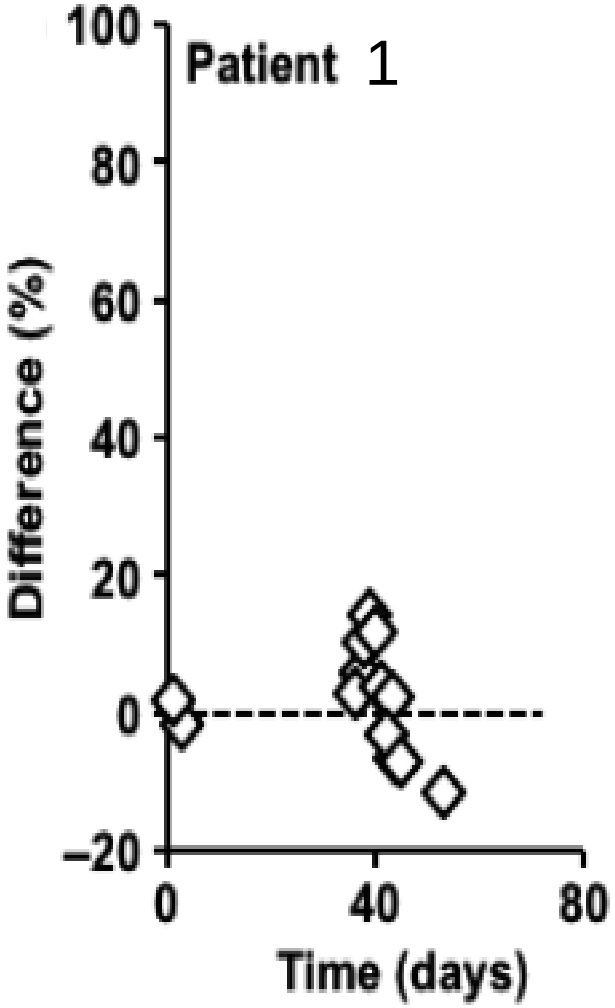


FVIII chromogene test = 2 x FVIII coagulation test

Product specific calibrator
for FVIII chromogene and coagulation tests

calibration with a product-specific calibrator for the measurenent of ReFacto AF

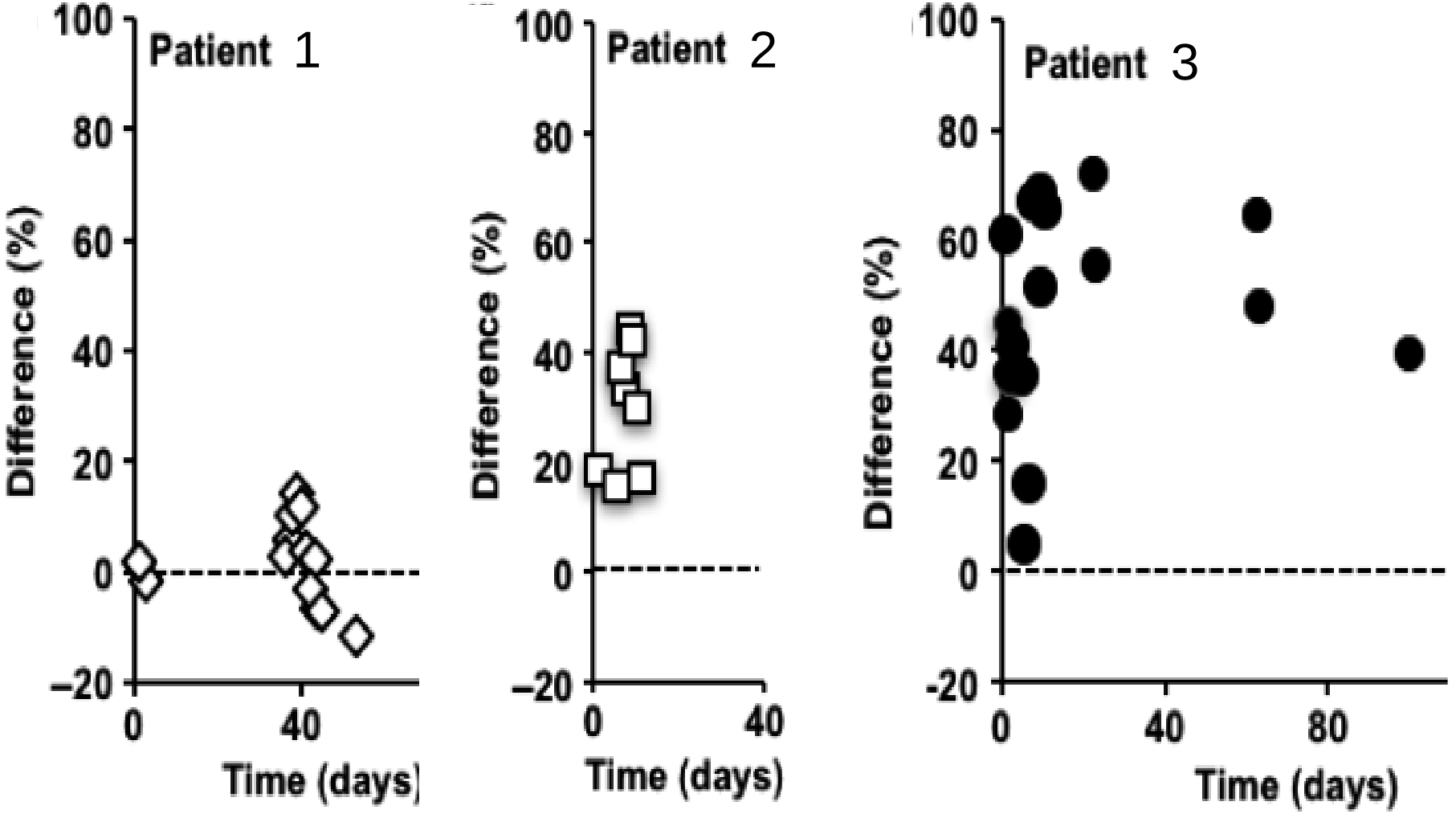
chromogenic versus coagulation FVIII assay



Haemophilia. 2018;24:675-682.

calibration with a product-specific calibrator for the measurenent of ReFacto AF

chromogenic versus coagulation FVIII assay



Gene therapy for hemophilia A

N Engl J Med 2022 386 1013

5 patients in UZLeuven (Prof. K. Peerlinck)

FVIII gene therapy

- adenovirus associated vector
- B-domain deleted FVIII
- hepatocytes

ReFacto (B-domain deleted rFIII)

Gene therapy for FVIII (B-domain deleted rFIII)

ReFacto (B-domain deleted rFIII)

chromogenic > coagulation test

Gene therapy for FVIII (B-domain deleted rFIII)

chromogenic < coagulation assay !!!

Gene therapy for hemophilia A

N Engl J Med 2022 386 1013

5 patients in UZLeuven (K. Peerlinck)

For one patient:

2% FVIII with the coagulation assay
not detectable in the central lab (chromogenic assay)

Gene therapy for hemophilia A

Discordance chromogenic versus coagulation assay:

post-translational modifications?

Gene therapy for hemophilia A

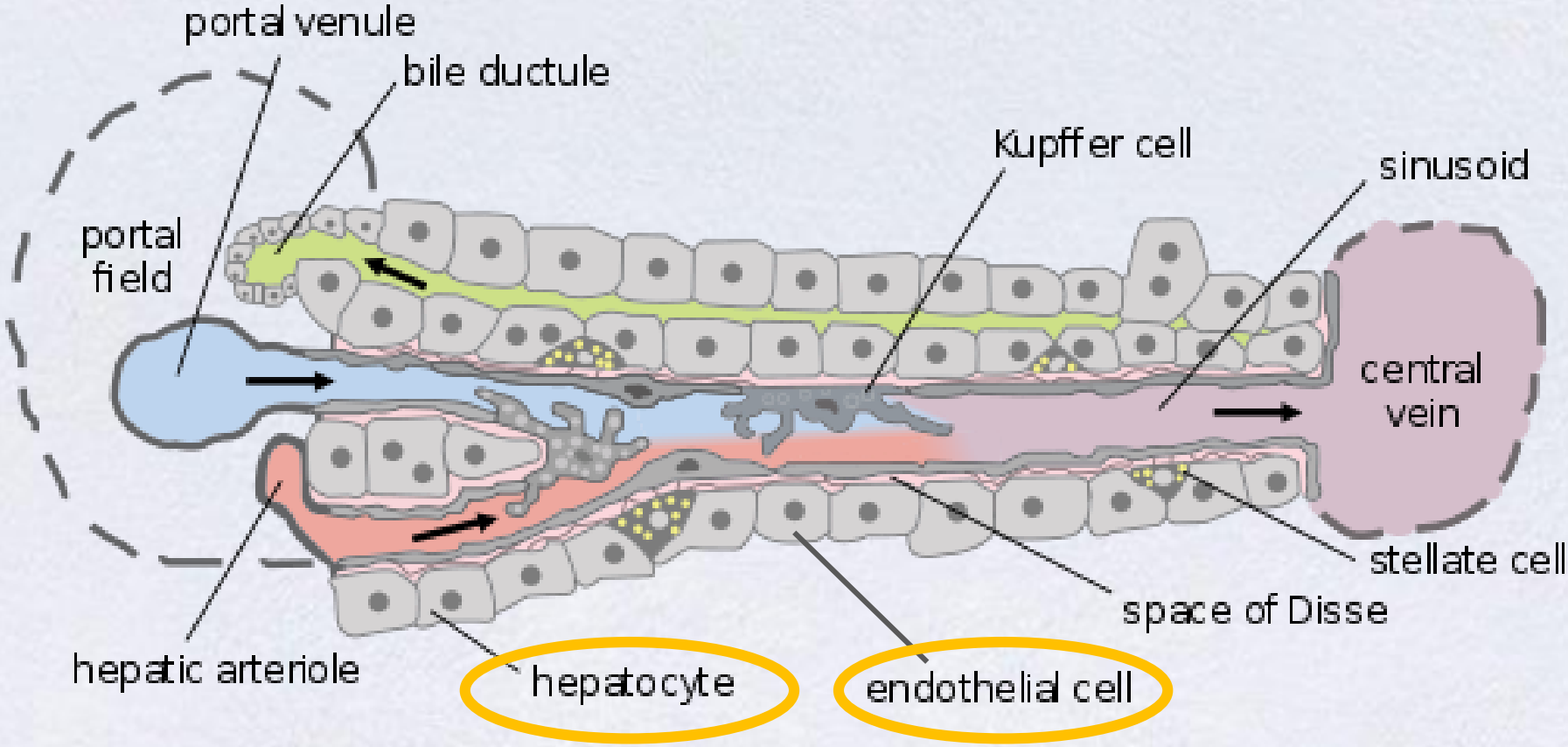
Discordance chromogenic versus coagulation assay:

post-translational modifications?

Gene therapy -> FVIII gene in hepatocytes

Physiological FVIII production site(s)?

Structure of a hepatic sinusoid in a lobule



Which cell produce FVIII in the liver?

- hepatocytes (not LSEC)
  - tissues: mRNA and FVIII:Ag (Wion et al, 1985; Zelechowska et al, 1985)
  - in vitro culture: FVIII:Ag (Ingerslev et al, 1998; Biron-Andreani, 2004)
- Liver sinusoidal endothelial Cells (not hepatocytes)
  - tissues: immunohistochemistry (Stel et al, 1983; Van der Kwast et al, 1983)

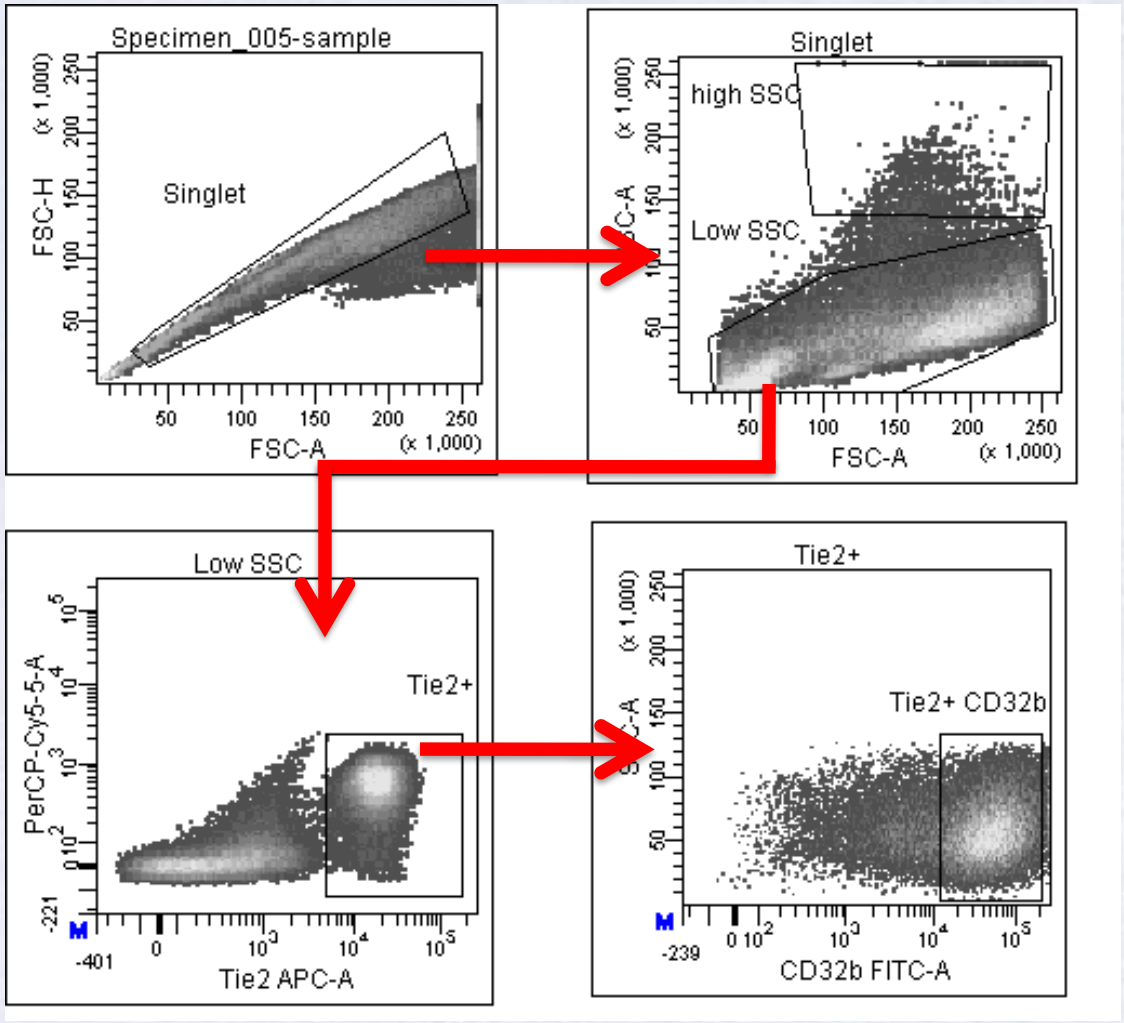
Determination of the cell type producing FVIII in the human liver

How to identify the liver cell type producing FVIII?

- **level of FVIII mRNA**
- **sensitive FVIII FUNCTIONAL assays**
  (allows inhibition with specific antibodies)
- **cell purification: FACS sorting**
- **control of purity by mRNA transcriptome**
  (massive parallel sequencing)

Shahani JTH 2014; 12: 36

Isolation strategy



"Low SSC" gate validated to contain most of CD32b+/Tie2+ cells

CD32b+ cells fully contained in Tie2+ (2 markers for increased purity)

FVIII/VWF/albumin production

Cell type	FVIII:C	VWF:Ag	Albumin
	(LOD) nU/cell	(LOD) nU/cell	(LOD) fg/cell
Hepatocyte	< (0.3)	< (0.8)	190 (1.6)
Hepatocyte	< (0.15)	< (0.4)	100 (0.8)
Hepatocyte	< (0.03)	0.5 (0.07)	265 (0.6)
Hepatocyte	< (0.03)	0.5 (0.08)	100 (0.7)
LSEC	1.1 (0.2)	1.8 (0.6)	2 (1.1)
LSEC	2.8 (0.3)	2.8 (0.8)	61.6 (0.8)
LSEC (commercial)	0.55 (0.2)	13.6 (0.7)	< (1.3)
HUVEC (passage 1)	< 0.2	88 (0.5)	nd
CHO-rFVIII	0.3 (0.03)	< (0.08)	< (0.1)

Which cell produce FVIII in the liver?

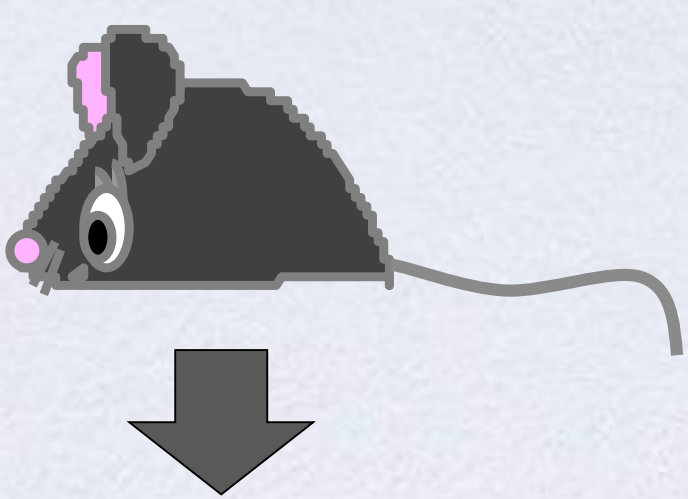
+ hepatocytes (not LSEC)
  - tissues: mRNA and FVIII:Ag (Wion et al, 1985; Zelechowska et al, 1985)
  - in vitro culture: FVIII:Ag (Ingerslev et al, 1998; Biron-Andreani, 2004)

+ Liver sinusoidal endothelial Cells (not hepatocytes)
  - tissues: immunohistochemistry (Stel et al, 1983; Van der Kwast et al, 1983)
  - **purified cells (Shahani et al, 2014)**

Who is right?

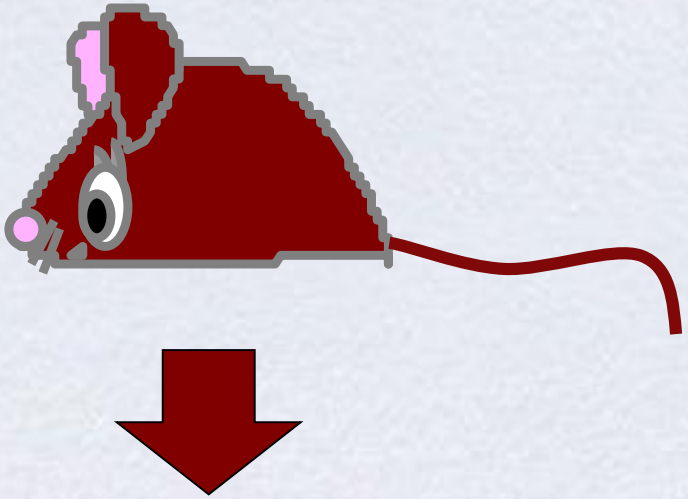
Inactivation of the FVIII gene in different cell types

hepatocytes



FVIII: normal

endothelial cells



FVIII: undetectable

Gene therapy for hemophilia A

Discordance chromogenic versus coagulation assay:

post-translational modifications?

Gene therapy -> FVIII gene in hepatocytes

Physiological FVIII production site(s)?

endothelial cells

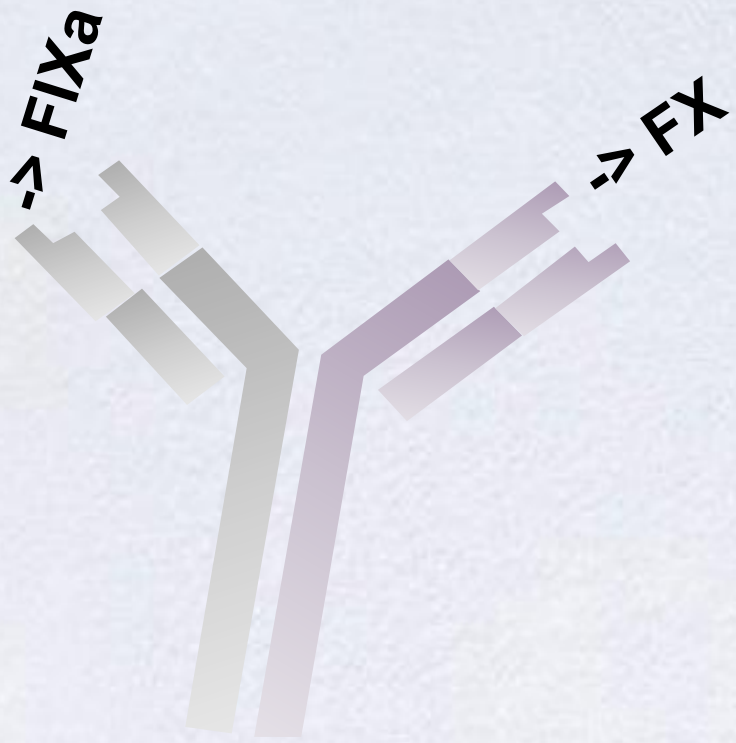
Patiënt met ernstige hemofilie A (FVIII < 1%)

- APTT = 24.4 sec
- FVIII one stage assay: 348,6%
- FVIII chromogene test A: 25 %
- FVIII chromogene test B: onmeetbaar

misleidende informatie

! op spoed
! op de operatie kamer

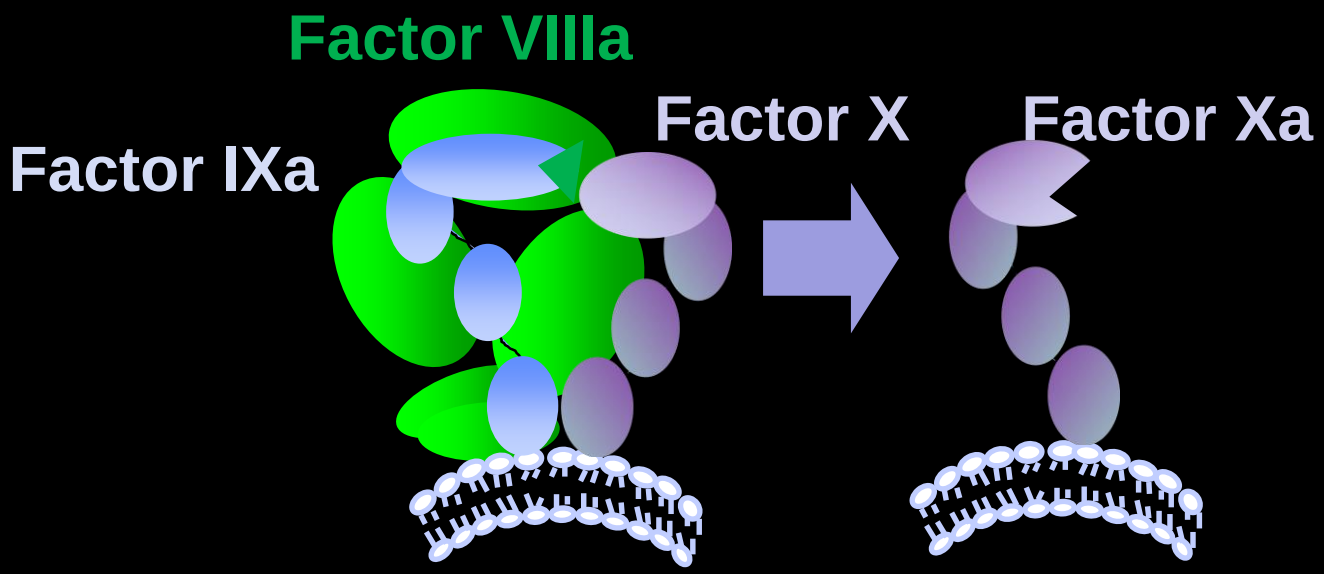
Emicizumab



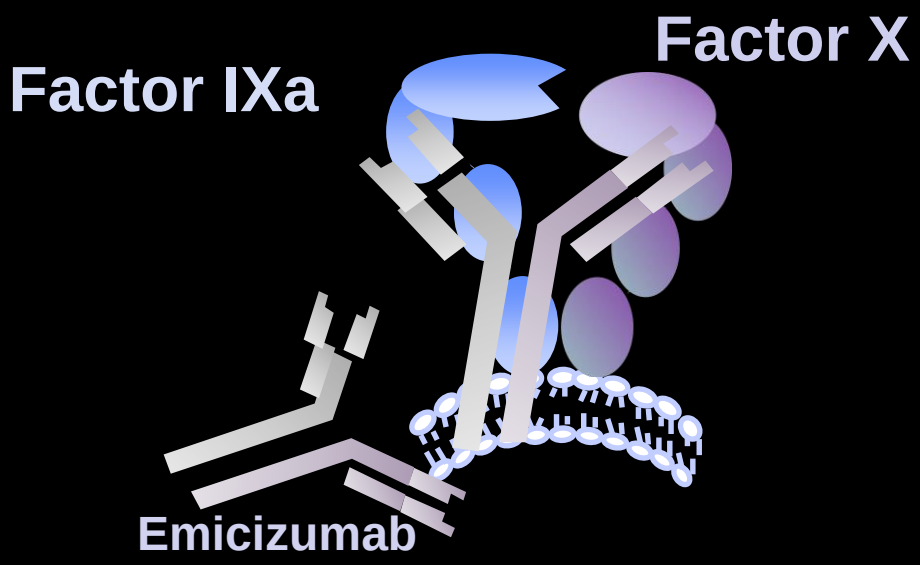
Bispecifiek antilichaam:

één keten -> FIXa

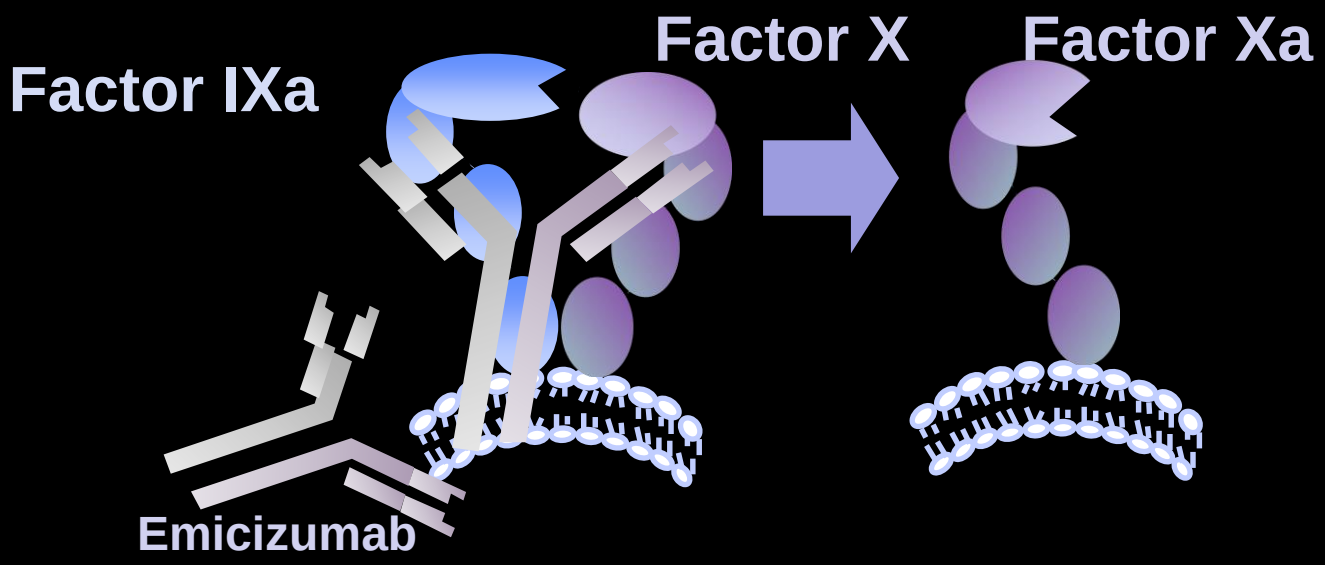
één keten -> FX


Factor VIIIa
Factor IXa
Factor X
Factor Xa

Tekort and FVIII en Emicizumab

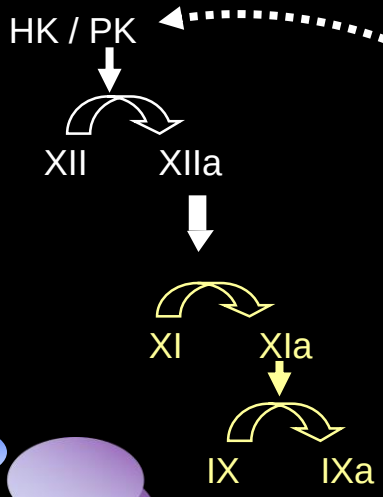


Tekort and FVIII en Emicizumab



APTT FVIII tekort

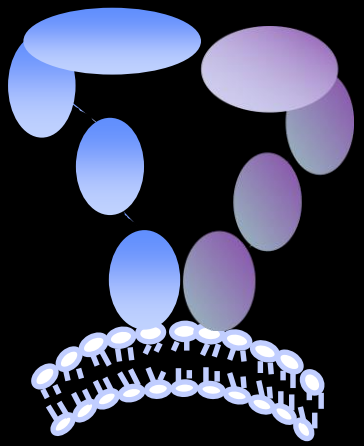
Contact Activation pathway



+ niet fysiologische oppervlakken
(glas, silica, kaolin)

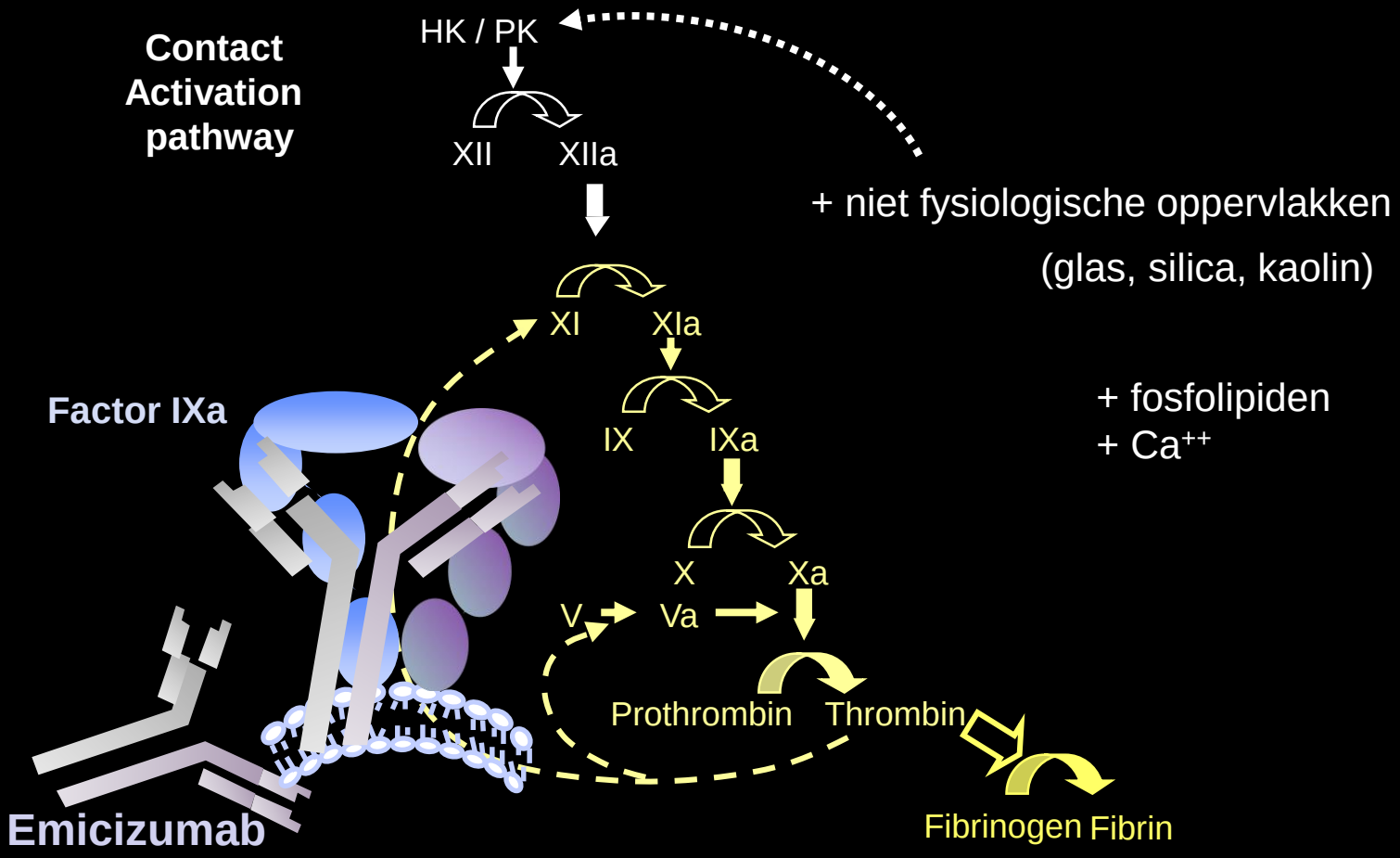
+ fosfolipiden
+ Ca^{++}

Factor IXa

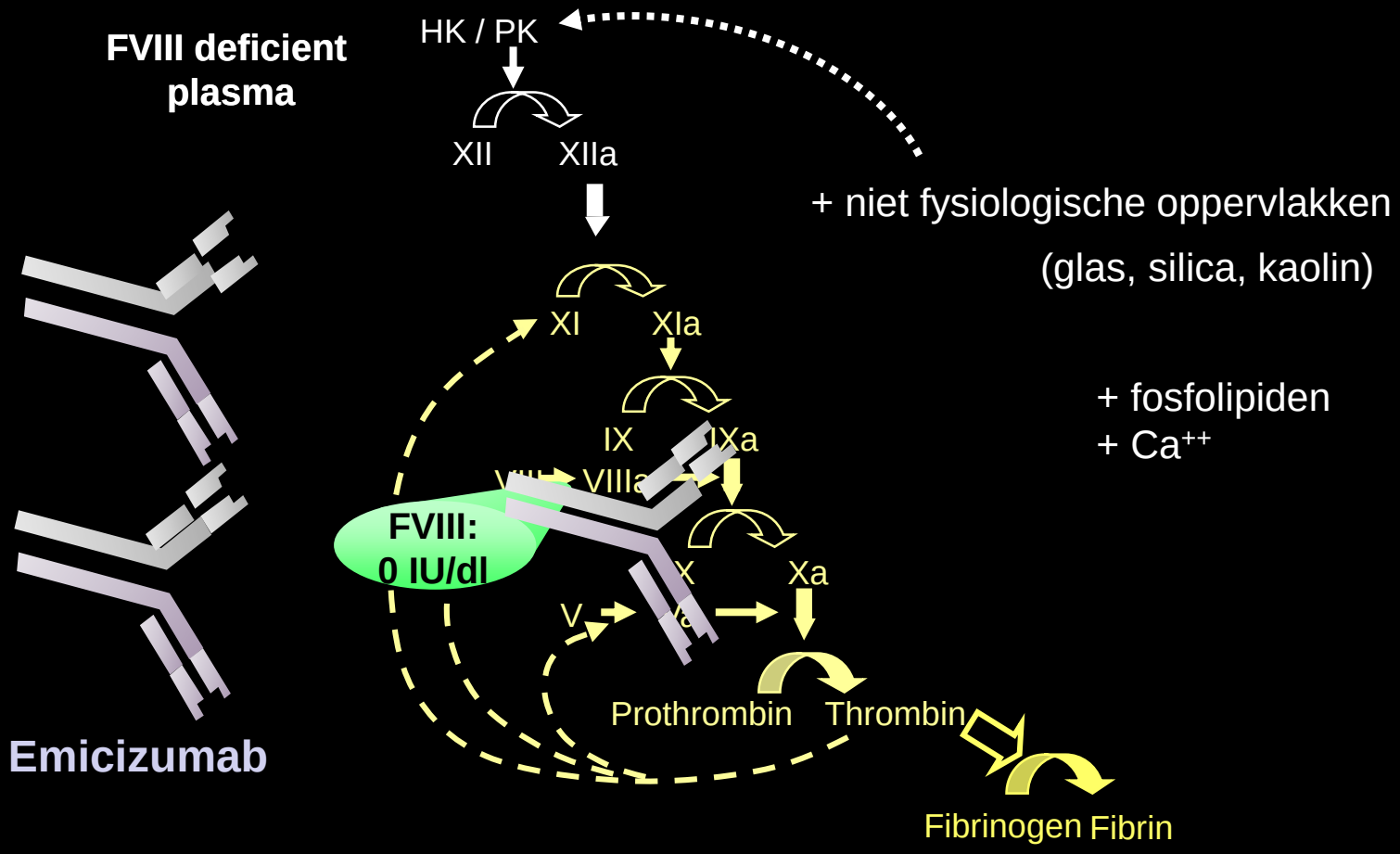


Phospholipids

APTT FVIII tekort Emicizumab



Meting van FVIII met een stollingstest (APTT)



Patiënt met ernstige hemofilie A (FVIII < 1%)

- **voor behandeling**
  - **APTT = 133 sec**
  - **FVIII onmeetbaar**
- **Na Emicizumab**
  - **APTT = 24.4 sec**
  - **FVIII stollingstest: 348,6%**

Patiënt met ernstige hemofilie A (FVIII < 1%)

- APTT = 24.4 sec
- FVIII one stage assay: 348,6%
- **FVIII chromogene test A: 25 %**
- **FVIII chromogene test B: onmeetbaar**

FVIII chromogene testen

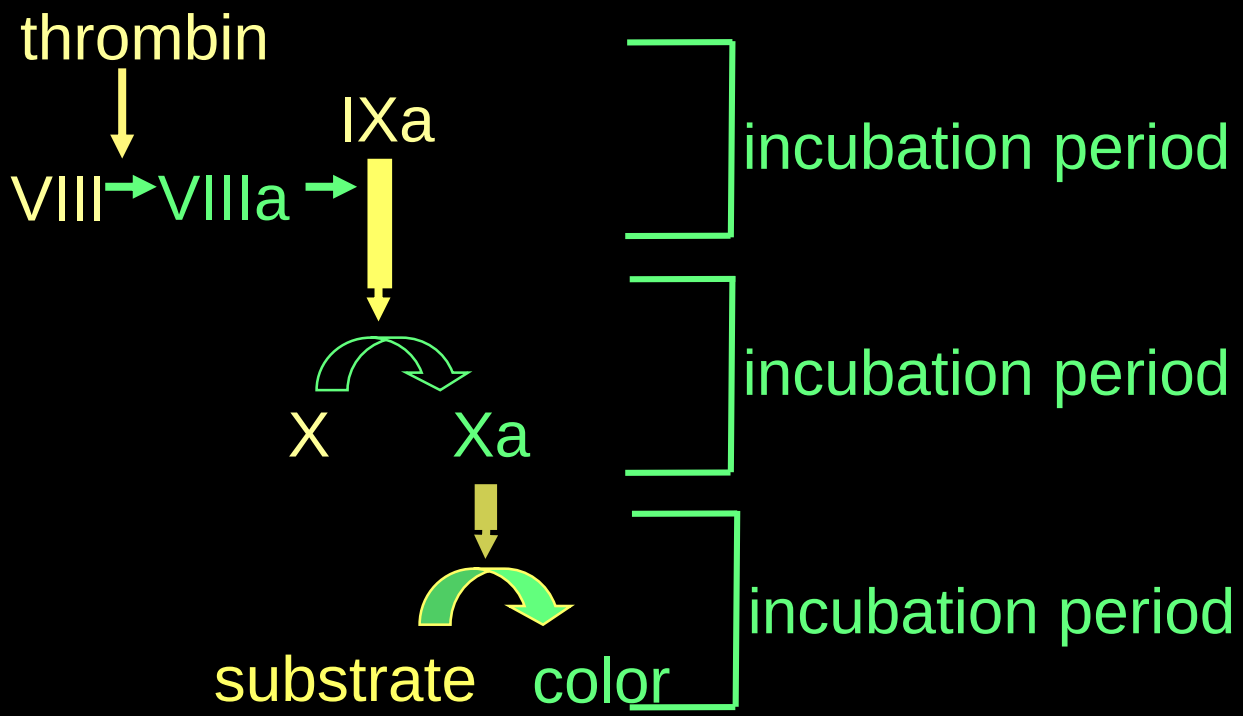
FVIII chromogenic assay

test sample (FVIII)

+ a little thrombin
+ phospholipids
+ FIXa, FX

+ Ca^{++}

+ chromogenic substrate for FXa



FVIII chromogene testen

Boviene reagenten

FIXa and FX

Siemens
Werfen
Stago

Humane reagenten

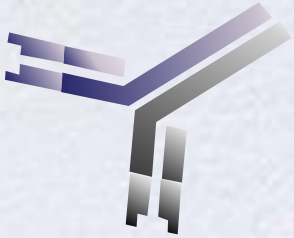
FIXa and FX

Hyphen

FVIII chromogene testen

Boviene reagentia

FIXa and FX



geen activiteit

Humane reagentia

FIXa and FX

FVIII-like activiteit

Patiënt met ernstige hemofilie A (FVIII < 1%)

- APTT = 24.4 sec
- FVIII one stage assay: 348,6%
- **FVIII chromogene test A: 25 %**
  humane reagentia
- **FVIII chromogene test B: onmeetbaar**
  boviene reagentia

Communicatie met de clinici!

welke product?

welke test?

DEAL “FVIII” aanvragen