Cliniques universitaires SAINT-LUC UCLouvain BRUXELLES

Laboratory tests in bleeding disorders

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BHS course 14/11/2026



General approach to laboratory testing

Pre-analytical phase:

- Blood collection
- Sample handling
- Patient specific factors

Typical evaluation:

- Complete blood count
- PT
- APTT
- Fibrinogen, (D-dimer)
- (PFA-100/200, von Willebrand factor)

Additional testing depending: on result:

- Specific clotting factor assays (clot based or chromogenic)
- von Willebrand factor
- Platelet function testing



• APTT: activated partial thromboplastin time; CBC: complete blood count; PFA: platelet function analyzer; PT: prothrombin time; VWF: von Willebrand factor

General approach to laboratory testing

1 Pre-analytical phase:

- Blood collection
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Typical evaluation:

- Complete blood count

4 3 PT

2 APTT

- Fibrinogen, (D-dimer)
- (PFA-100/200, von Willebrand factor)

Additional testing depending on result:

4 2 Specific clotting factor assays (clot based or chromogenic)

5 von Willebrand factor

- Platelet function testing

Clinical cases

- APTT: activated partial thromboplastin time; CBC: complete blood count; PFA: platelet function analyzer; PT: prothrombin time; VWF: von Willebrand factor

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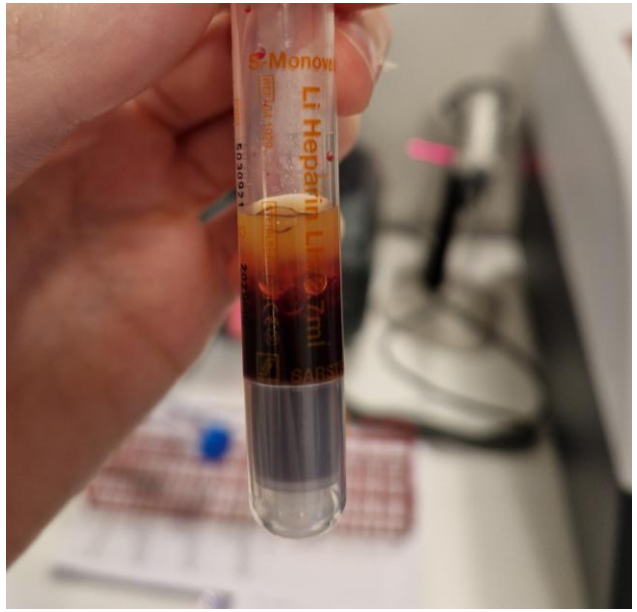
Clinical case 1

- Call of lab technician for a FVIII measurement
- Not possible to obtain a result, no coagulation
- Previous result 38 IU/dL (1 month ago) in a patient with a complicated history of both pulmonary embolism and mucocutaneous bleeding
- You order dilutions to see if there is assay interference (lupus anticoagulans)?
- Still no coagulation with dilution of 1/5 and 1/10, hypothesis: strong lupus anticoagulans?
- Following day: lupus anticoagulans: not possible to obtain a result (no coagulation in both screen, mix and confirm testing)
- Quid?



Clinical case 1

- One of the lab technicians proposes to search the collection tube
- After removing the label, we see that it is a heparin tube…



Clinical case 1

- Collection tubes changed colour 1 week before
  - Green citrate tubes → blue citrate tubes
  - Orange heparin tubes → green heparin tubes

- Error in selecting collection tube
- Error when decanting plasma for coagulation testing

Code EU	Type de S-Monovette®	Code ISO 6710
	Citrate 9NC / ESR 4NC	
	Sérum CAT / Sérum gel CAT	
	Héparinate le lithium LH	
	EDTA K3E	
	Fluore FE	
	Héparinate de lithium LH, Analyse de métaux	

Clinical case 1

The “Pre-analytical phase” describes all actions and laboratory procedures that occur prior to the analytical phase.

The pre-analytical phase is implicated in **60-70% of laboratory errors**!

Important to control this phase to provide qualitative laboratory results.



Clinical case 1

Pre-analytical phase: three broad categories

Sample collection

- Correct patient identification and sample labelling
- Phlebotomy technique
- Sample volume
- Sample collection tube

Sample handling

- Storage
- Mixing and centrifugation
- Transport conditions
- Delays in transport

Patient-specific factors

- Physiological variables
- Pathologies
- Stress, exercise,…
- Medication!



Clinical case 1

Sample collection

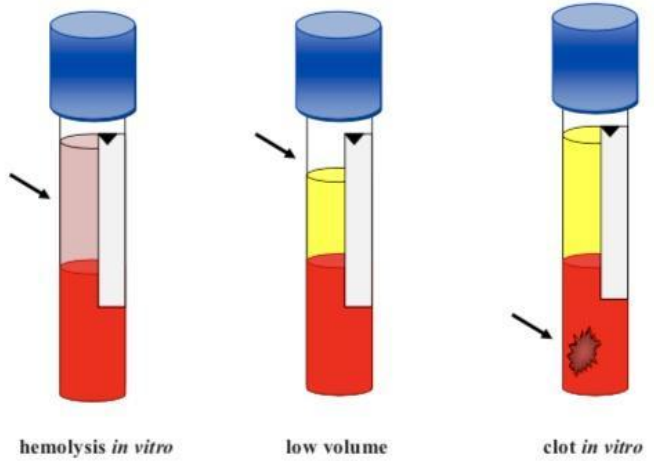
- Conventional straight needle
- Butterfly device: ok if discard tube is taken
- 19-22 gauge needle
- Tourniquet <1 minute
- Citrate tube (3,2%): 1 volume of citrate for 9 volumes of blood
- Citrate tube in second position
  - ✓ After blood cultures
  - ✓ After discard tube (neutral without additif)
  - ✓ After serum tube (without clot activator)



Clinical case 1

Sample collection

- Adequate filling of tubes
  - underfilling: too much citrate → prolonged routine assays
  - overfilling: not enough citrate/difficult to homogenise → clot formation risk
- Homogenization
- Transport at room temperature (exception: some fibrinolysis tests)
- Platelets are easily activated: system permitting slow manual drawing is preferred over vacuum system



Clinical case 1

Unacceptable samples

- Insufficiently filled tubes (or overfilled)
- Wrong anticoagulant (EDTA, heparin), delayed sending (>4h)
- Sampling on same side as heparin infusion
- Grossly hemolyzed, lipemic samples

General approach to laboratory testing

Pre-analytical phase:

- Blood collection
- Sample handling
- Patient specific factors

Typical evaluation:

- Complete blood count
- PT
- 2 APTT
- Fibrinogen, (D-dimer)
- (PFA-100/200, von Willebrand factor)

Additional testing depending on result:

- 2 Specific clotting factor assays (clot based or chromogenic)
- von Willebrand factor
- Platelet function testing



• APTT: activated partial thromboplastin time; CBC: complete blood count; PFA: platelet function analyzer; PT: prothrombin time; VWF: von Willebrand factor

Clinical case 2

- Patient of 82 years old
- Was shopping at IKEA and carried a heavy bag on his left arm
- Extended hematoma on left flank and arm
- At the emergency department a routine coagulation is executed:



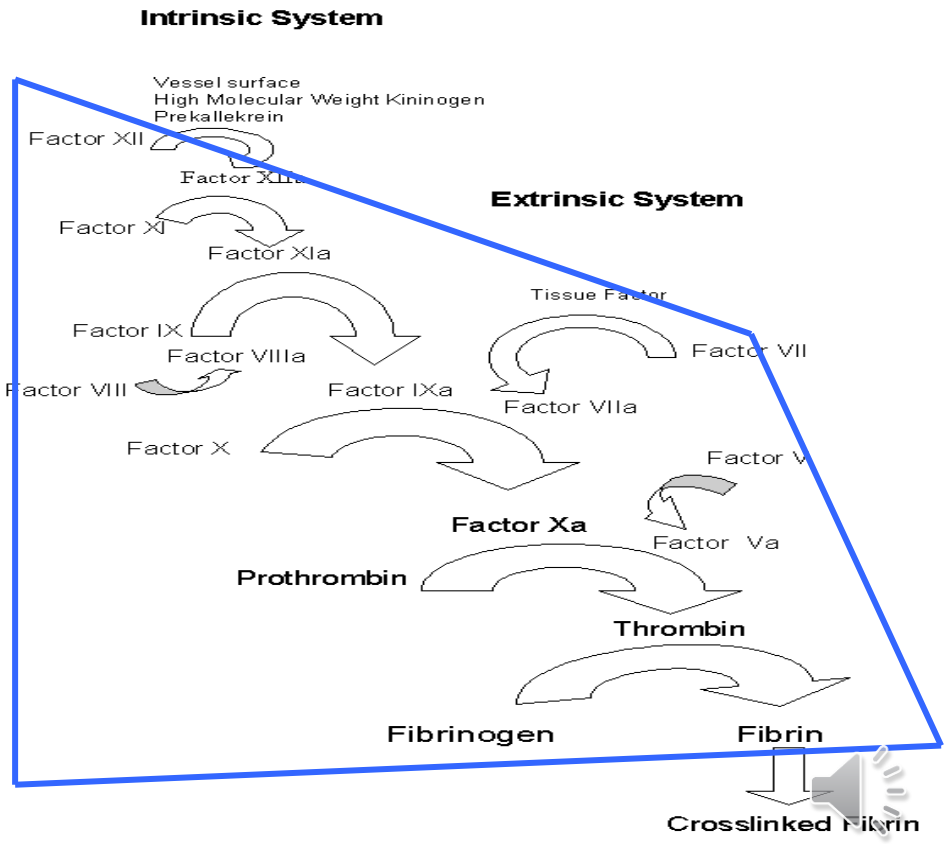
APTT: 70,9 s (25,1-36,5s)
PT: 13,10 s (9,35-14,30)
Fibrinogen: 259 mg/dL

- Image from Tiede et al. JTH 2021; 19, 637-644,

Clinical case 2

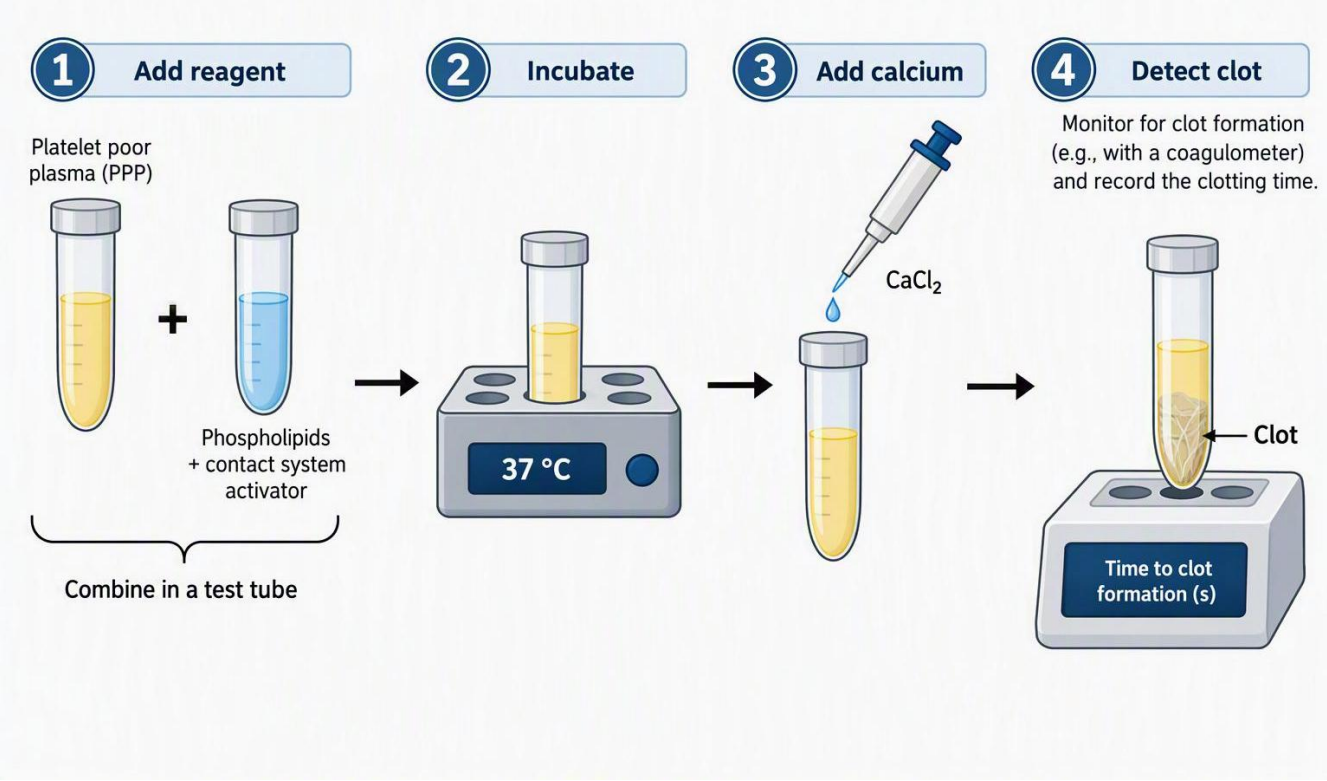
Activated partial thromboplastin time (APTT)

APTT



Clinical case 2

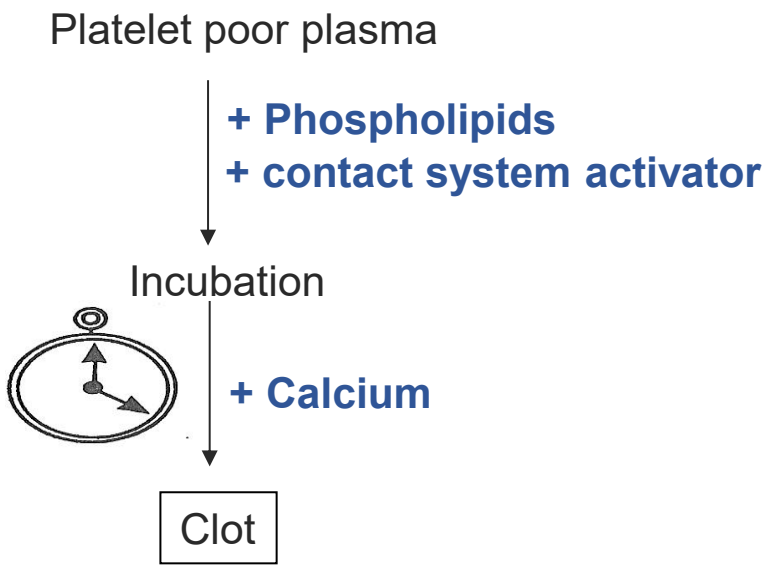
APTT



Clinical case 2

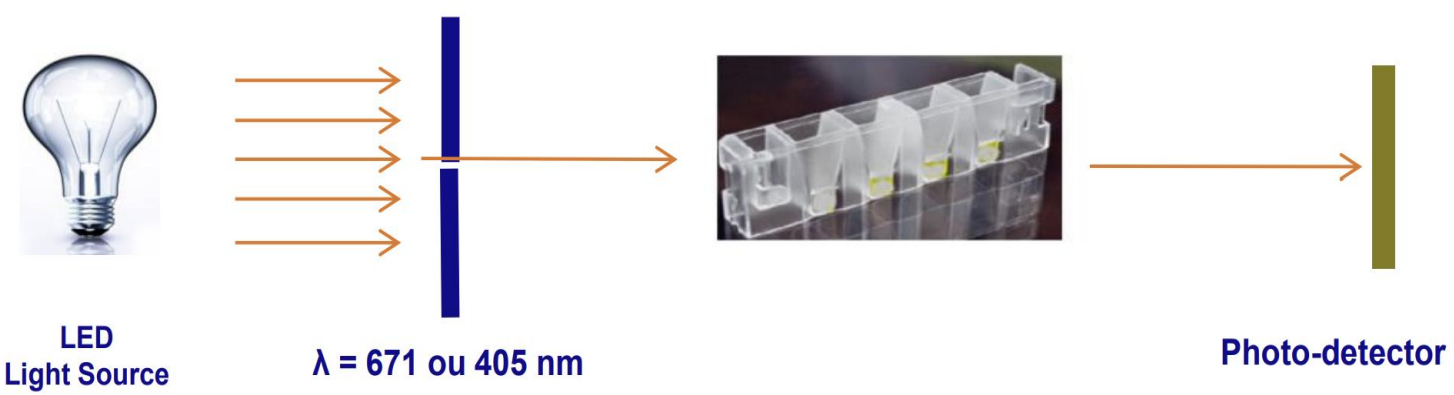
APTT

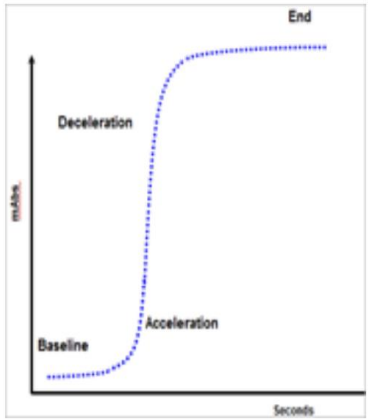
- Monitors coagulation in intrinsic and common coagulation pathways.
- Type of assay: Activators (such as micronized silica, ellagic acid, kaolin) plus phospholipids and calcium are added to plasma and the clotting time is recorded. Incubation phase is needed (activation of the contact phase is very slow)
- Choice of reagent: sensitive to factor deficiencies; weak/modest sensitivity to lupus anticoagulans



Clinical case 2

Clot detection: optical method



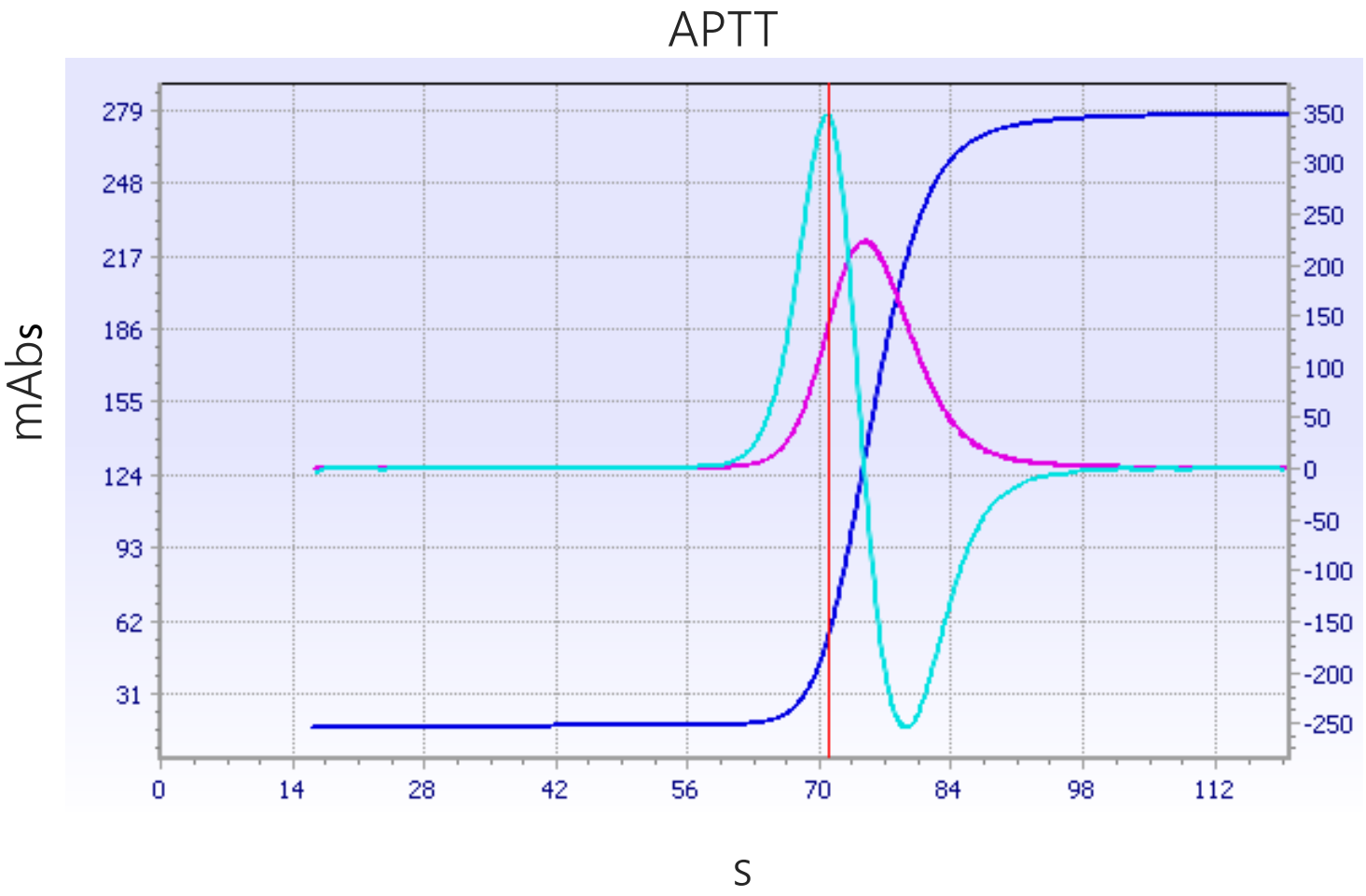


Clinical case 2

Clot detection: mechanical method: change in movement of magnetic bead



Clinical case 2



APTT on ACL-TOP 750 (Werfen)

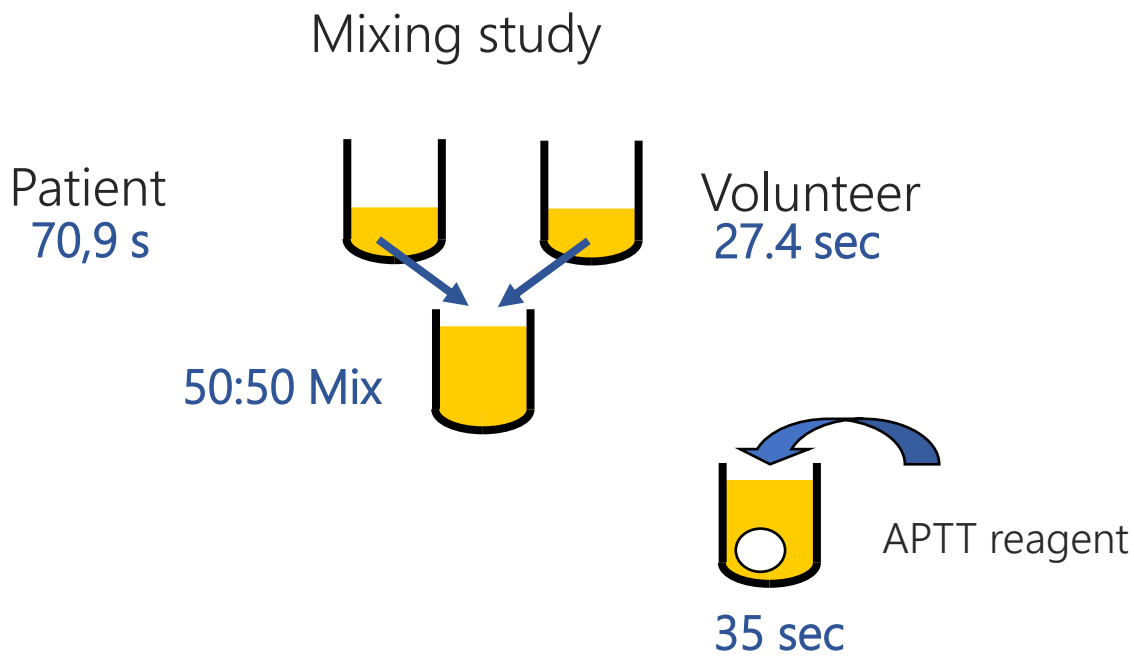
- Optical method
- Peak of 2nd derivative

- Image from Tiede et al. JTH 2021; 19, 637-644,

Clinical case 2

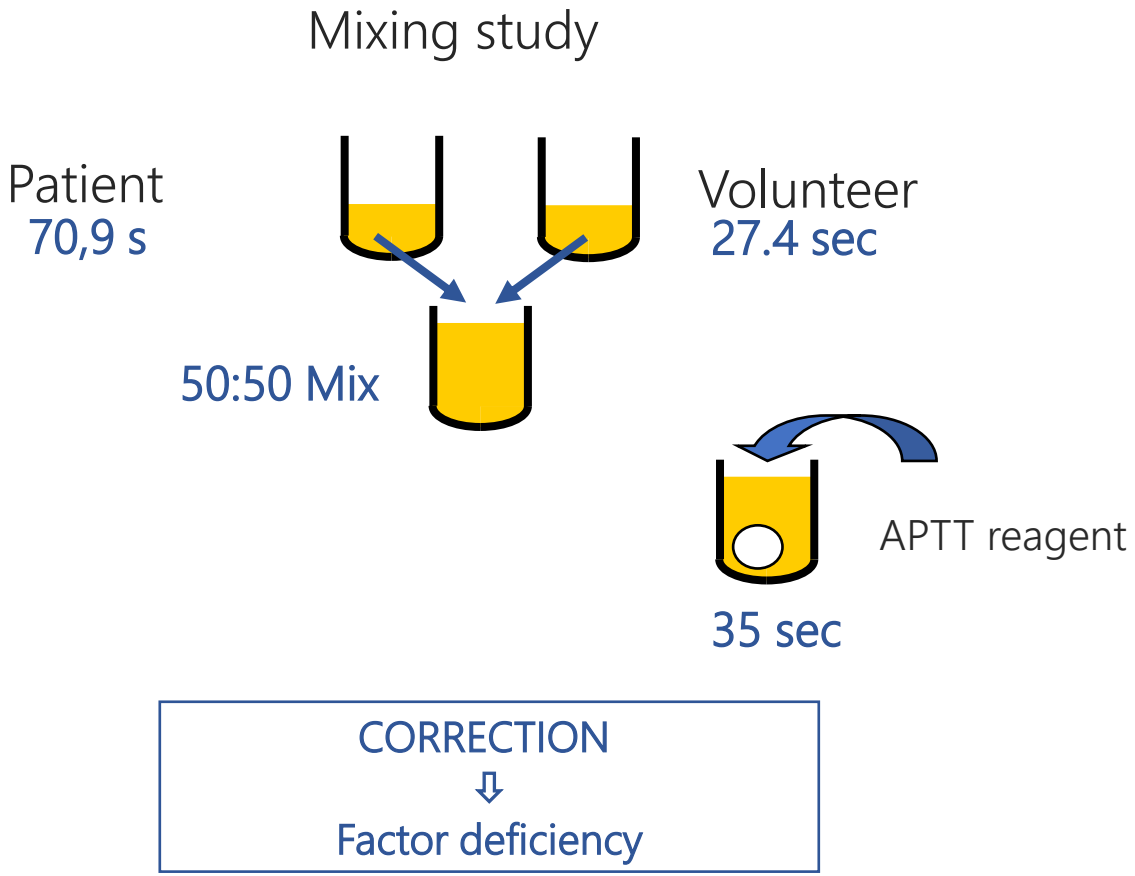
The patient has a isolated prolonged APTT:

We need a mixing study



- Image from Tiede et al. JTH 2021; 19, 637-644,

Clinical case 2

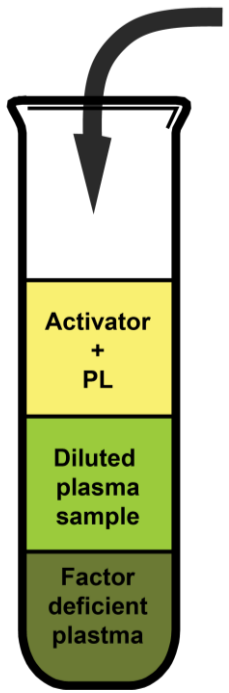


CAVE: often correction for INHI FVIII/IX
→ Antibodies are time/temperature dependent

- Image from Tiede et al. JTH 2021; 19, 637-644,

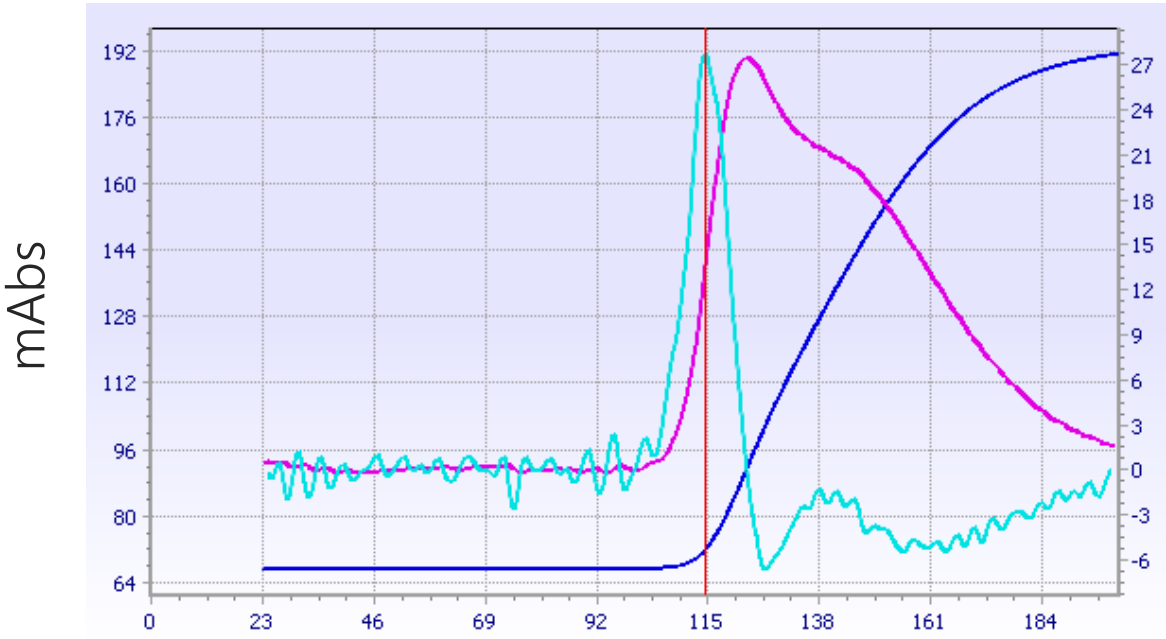
Clinical case 2

Next step: we look for a deficiency in one of the intrinsic coagulation factors



Perform
APTT

FVIII OSA (based on APTT)

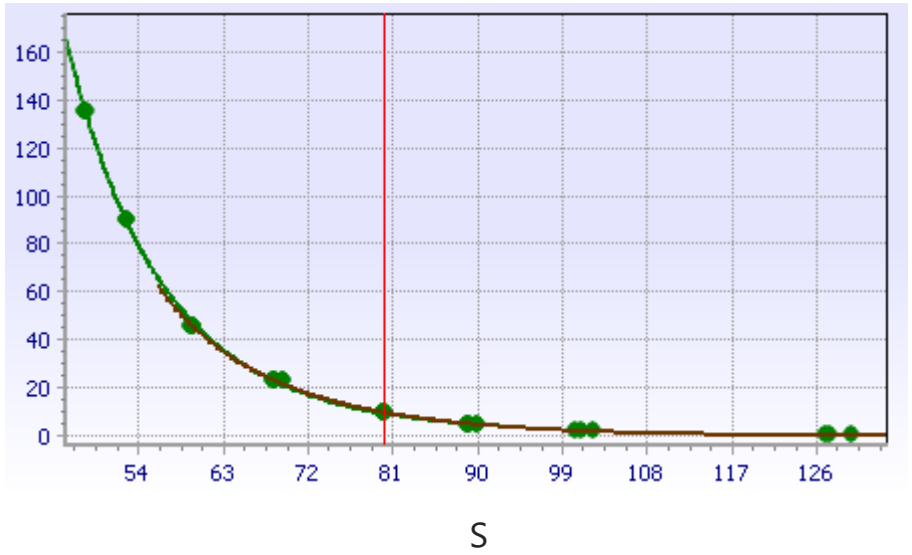


114,5 sec

Clinical case 2

Next step: we look for a deficiency in one of the intrinsic coagulation factors

Calibration curve FVIII OSA



114,5 s corresponds to <1% FVIII

Calibration curve is traceable to WHO standards

Clinical case 2

Unmeasurable APTT in a patient without bleeding history

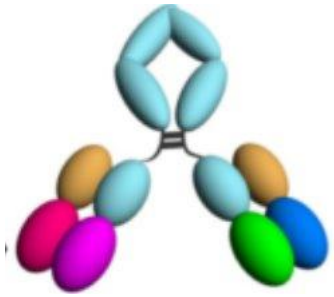
→ Look for FVIII inhibitors

Nijmegen-Bethesda: 12,4 BU

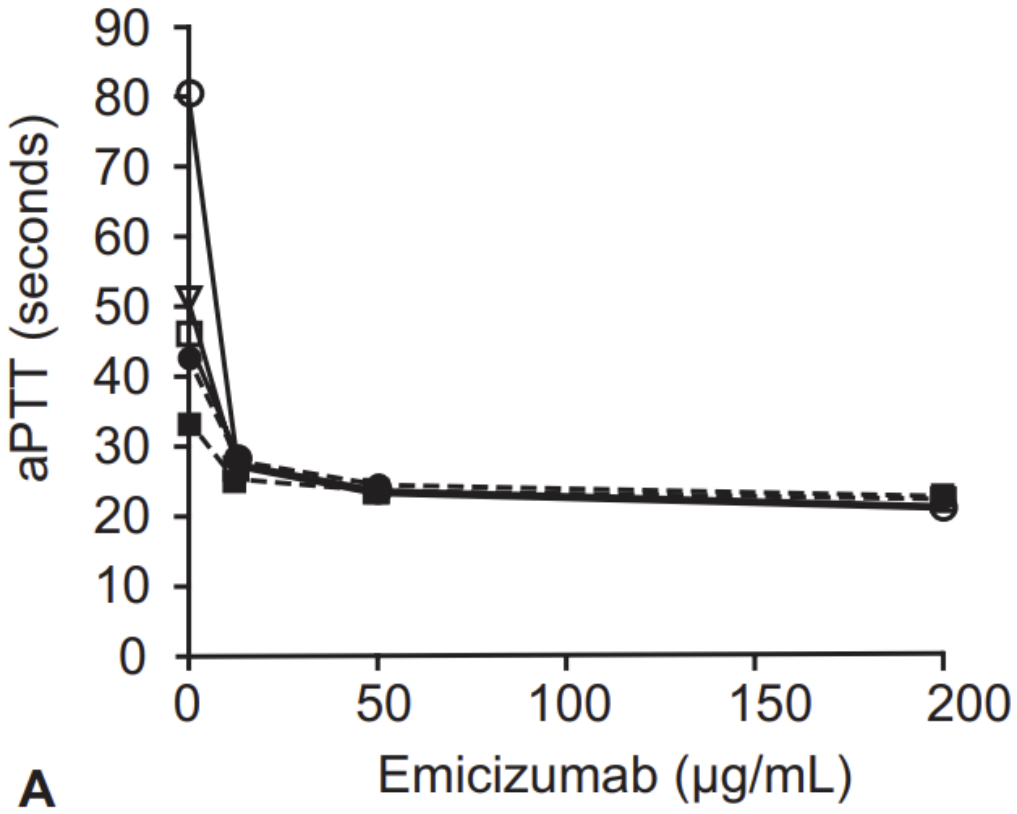
Acquired Hemophilia A confirmed

Clinical case 2

- Patient is started on emicizumab (Hemlibra®)
- A follow-up FVIII is requested 5 days later
- APTT = 24,0 s (25,1-36,5s), how to interpret APTT?
- How do we measure endogeneous FVIII?



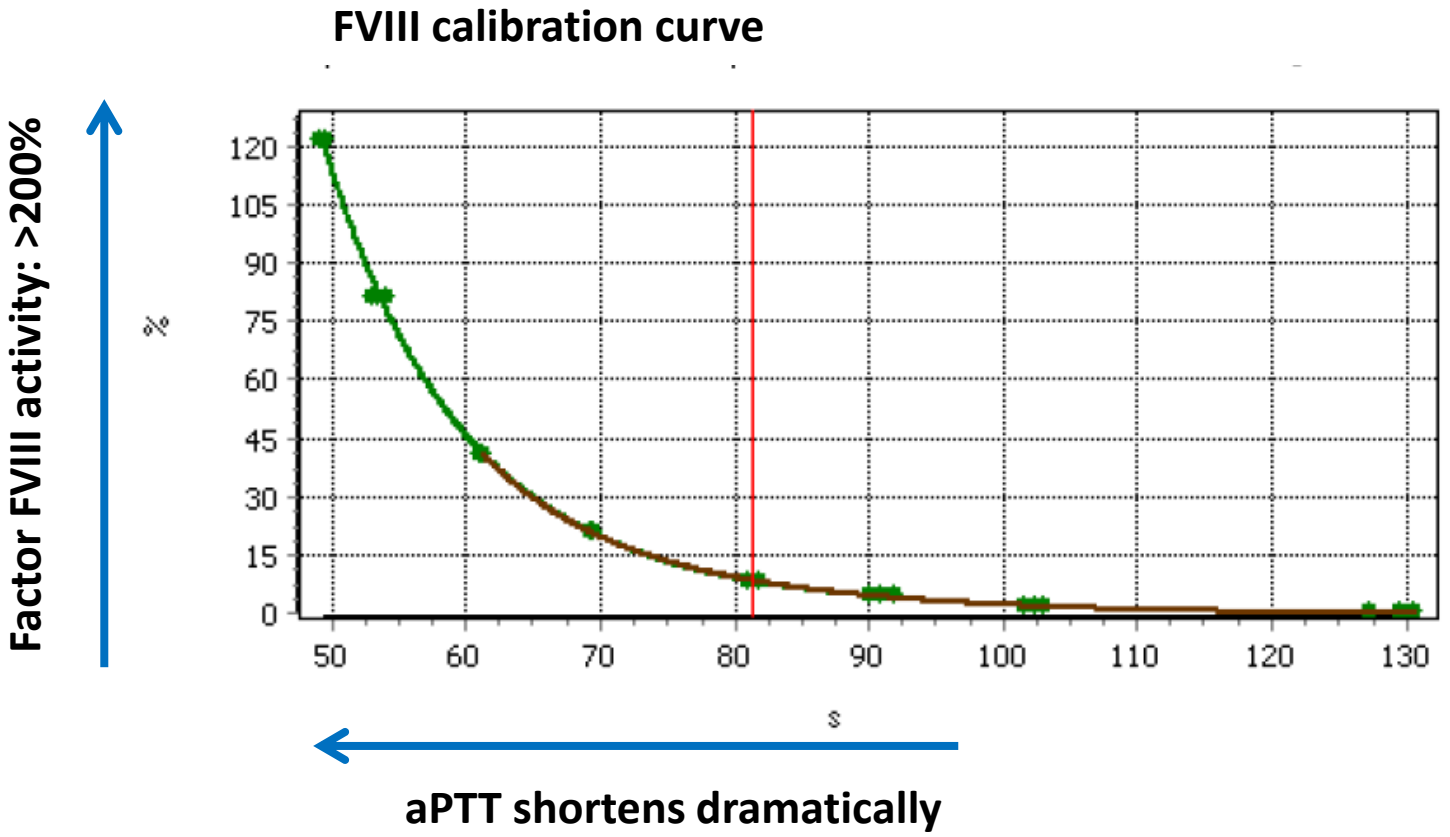
Clinical case 2



- Unlike FVIII emicizumab does not require preactivation to be functional
  → Shortens aPTT
- Short or normal aPTT does not reflect effective hemostasis
- Interference with all aPTT-based assays

Adamlewicz et al. Coag and Fibrin 2019

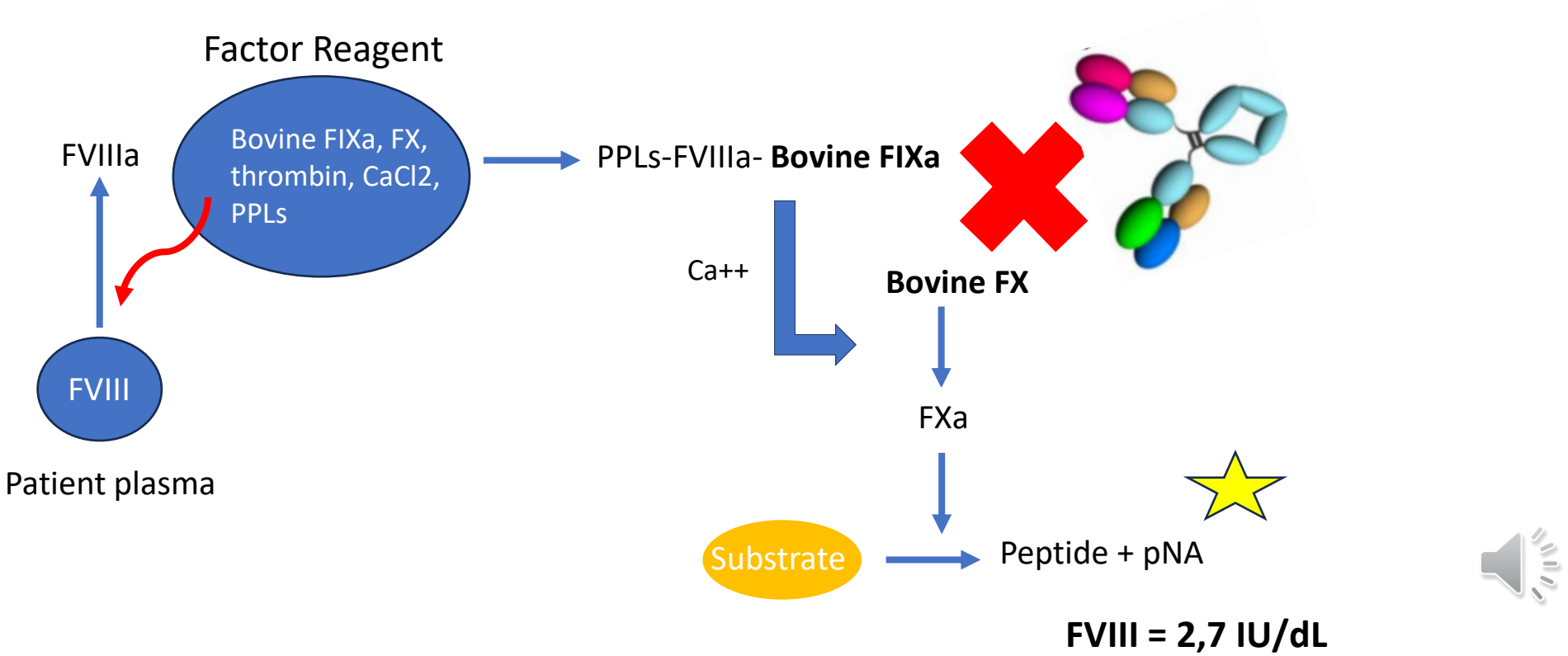
Clinical case 2



APTT-based factor assays are not suitable for patients treated with emicizumab

Clinical case 2

In our patient we have to use a chromogenic substrate assay (CSA) with bovine reagents to measure endogeneous FVIII



General approach to laboratory testing

Pre-analytical phase:

- Blood collection
- Sample handling
- Patient specific factors

Typical evaluation:

- Complete blood count
- PT
- APTT
- Fibrinogen, (D-dimer)
- (PFA-100/200, von Willebrand factor)

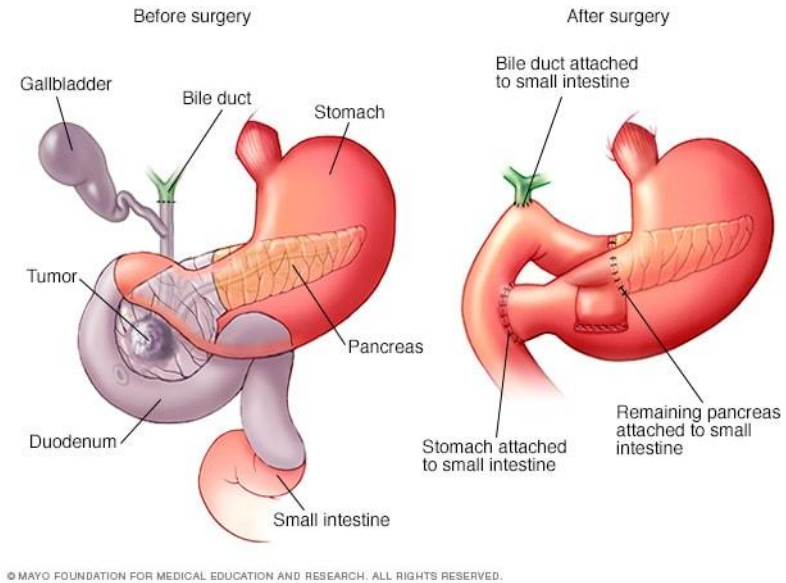
Additional testing depending on result:

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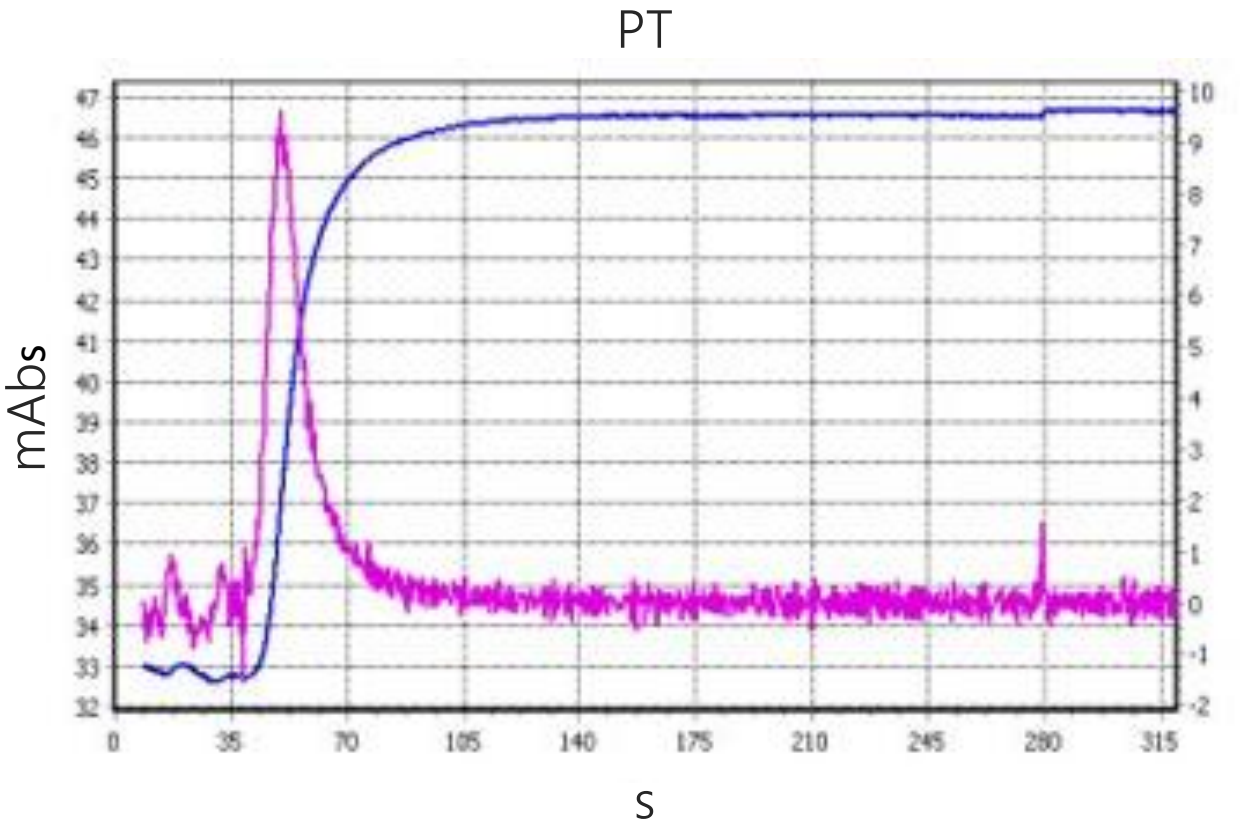
Clinical case 3

- 74-year-old man; tendency to bruise easily
- Pancreaticoduodenectomy (Whipple procedure) in 2014.
- On Eliquis® (apixaban) for the past 2 years for atrial fibrillation.
- Laboratory results:
  - PT: 45.00 s (INR: 3.5)
  - aPTT: normal
  - Platelet count: normal
  - Hemoglobin: 11.7 g/dL



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Clinical case 3

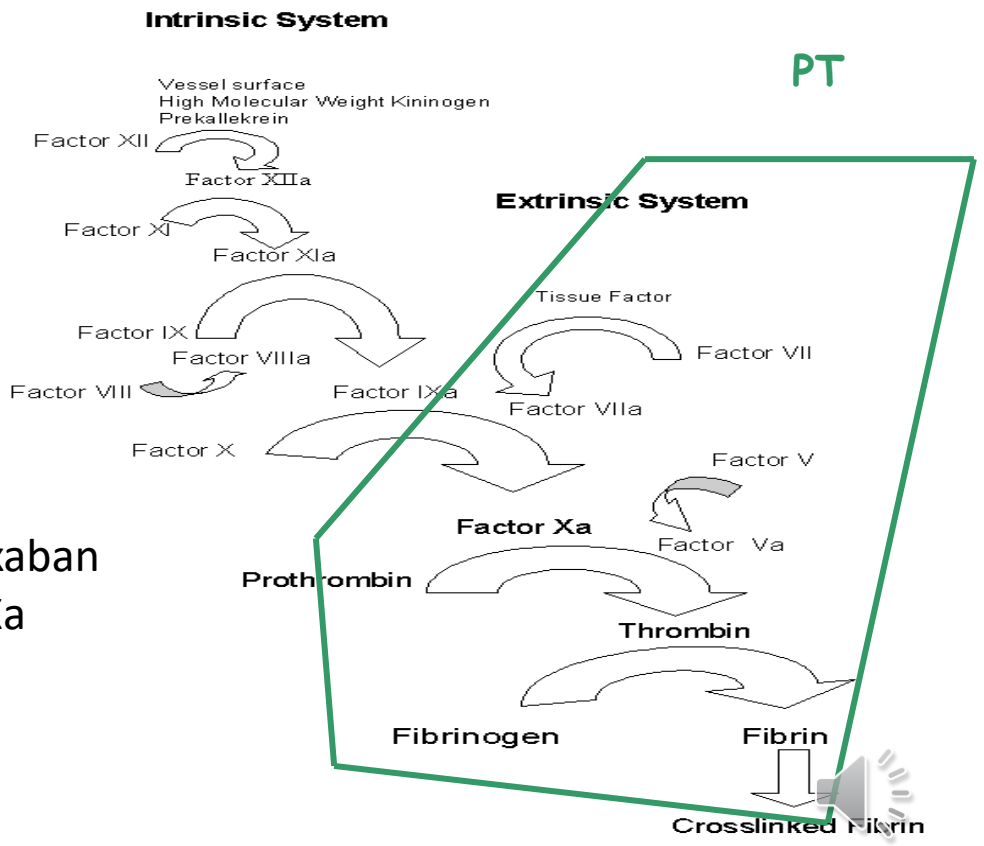


Isolated prolonged PT of 45 s
Optical method: peak of the first derivative

- Image from Tiede et al. JTH 2021; 19, 637-644,

Clinical case 3

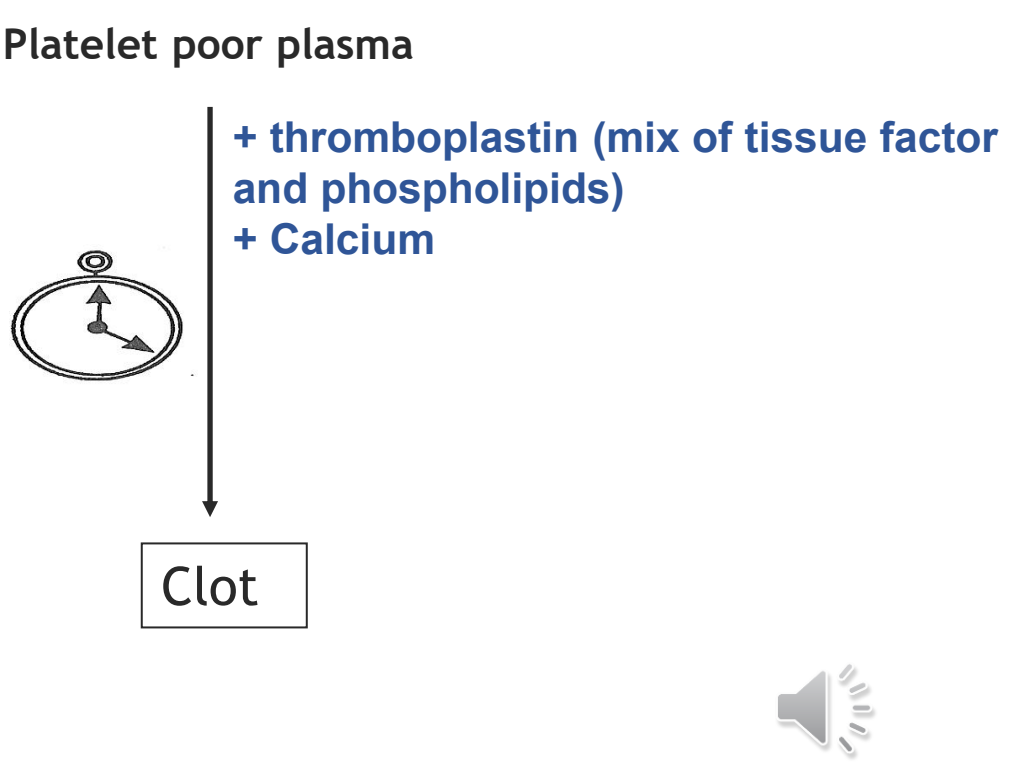
Prothrombin Time (PT)



Rivaroxaban
Anti-FXa

Clinical case 3

- Monitors coagulation in extrinsic and common coagulation pathways.
- Application: factor deficiencies, monitoring of vitamin K antagonists, liver disease,…
- Type of assay: Tissue factor and phospholipids are added to plasma and the clotting time is recorded.



Clinical case 3

Interpretation of a prolonged PT with normal APTT:

Factor VII deficiency

- Congenital = rare; acquired inhibitor = very rare
- Acquired = Start of VKA treatment, vitamin K deficiency, liver disease, … (short half-life)

DOAC?

A lot of causes of mildly prolonged PT

Clinical case 3

- Always consider a pre-analytical issue:
  - Assess for clots in the collection tube
  - If doubt, ask a control sample
- You can check the DOAC concentration (if suspicion of toxic level)
  - Anti-Xa calibrated for heparin is not suitable for assessing DOAC concentrations
  - Use anti-Xa calibrated with apixaban (idem for rivaroxaban, edoxaban)



- Van Blerk IJLH 2016; Douxfils et al JTH 2017

Clinical case 3

- No clot detected (always consider a pre-analytical issue).
- Apixaban: 350 ng/mL (2 × 5 mg; expected peak concentration: 91-321 ng/mL), minor overdose.
- Does this explain the prolonged prothrombin time of 45.00 s (INR 3,3)?

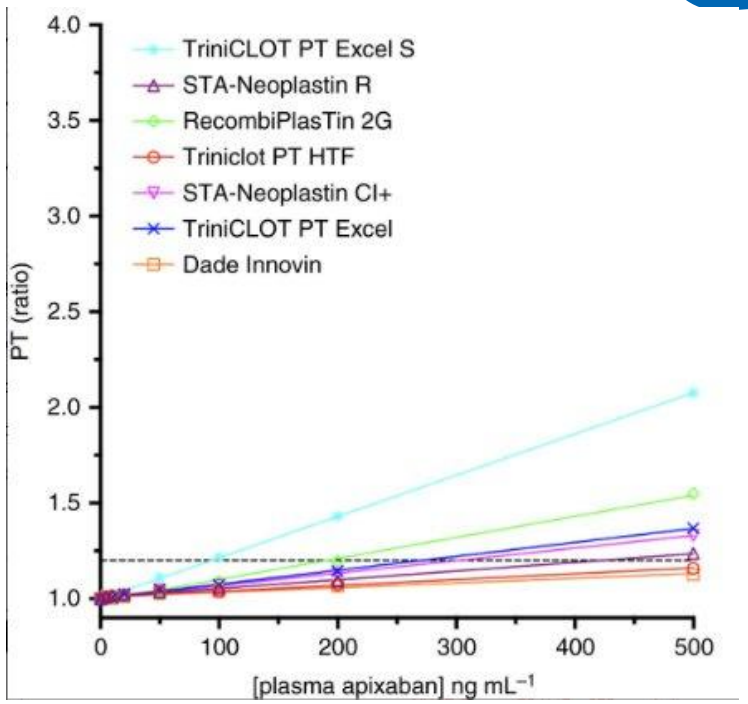


Table 1. Effect of apixaban on PT results

Reagent	*N*	41A	94A	225A
Innovin	53	1.01 (0.7%)	1.02 (0.7%)	1.04 (1.1%)
Neoplastin CI Plus	47	1.02 (1.1%)	1.06 (1.3%)	1.15 (1.4%)
Neoplastin R	22	1.02 (0.9%)	1.05 (1.2%)	1.13 (1.0%)
RecombiPlasTin 2G	58	1.05 (1.7%)	1.12 (1.6%)	1.32 (2.2%)
Thromborel S	14	1.01 (1.4%)	1.03 (1.2%)	1.09 (2.0%)
Global result	195	1.02 (2.5%)	1.05 (5.6%)	1.15 (16.0%)

- Van Blerk IJLH 2016; Douxfils et al JTH 2017

Clinical case 3

Interpretation of a prolonged PT with normal APTT:

Factor VII deficiency

- Congenital = rare; acquired inhibitor = very rare
- Acquired = Start of VKA treatment, **vitamin K deficiency**, liver disease, … (short half-life)



General approach to laboratory testing

Pre-analytical phase:

- Blood collection
- Sample handling
- Patient specific factors

4

Typical evaluation:

- Complete blood count
- PT
- APTT
- Fibrinogen, (D-dimer)
- (PFA-100/200, von Willebrand factor)

4

Additional testing depending on result:

- Specific clotting factor assays (clot based or chromogenic)
- von Willebrand factor
- Platelet function testing



• APTT: activated partial thromboplastin time; CBC: complete blood count; PFA: platelet function analyzer; PT: prothrombin time; VWF: von Willebrand factor

Clinical case 4

- 16-year-old man; abundant bleeding after nasal septum surgery
- No anticoagulant medication
- Laboratory results:
  - PT: 20.60 s (INR: 1.73)
  - aPTT: normal
  - Platelet count: normal
  - Hemoglobin: 12.2 g/dL



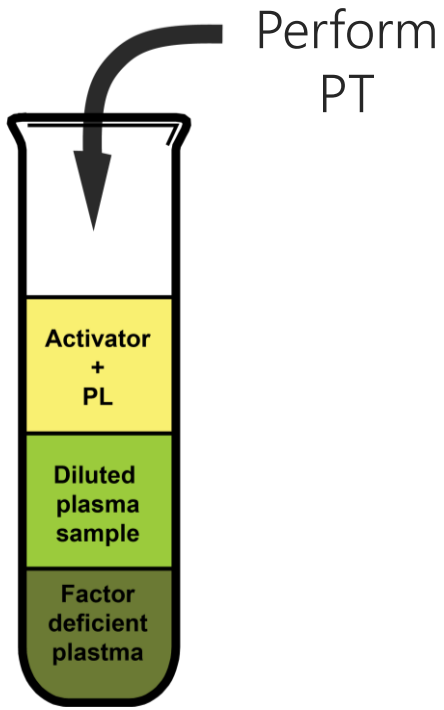
Clinical case 4

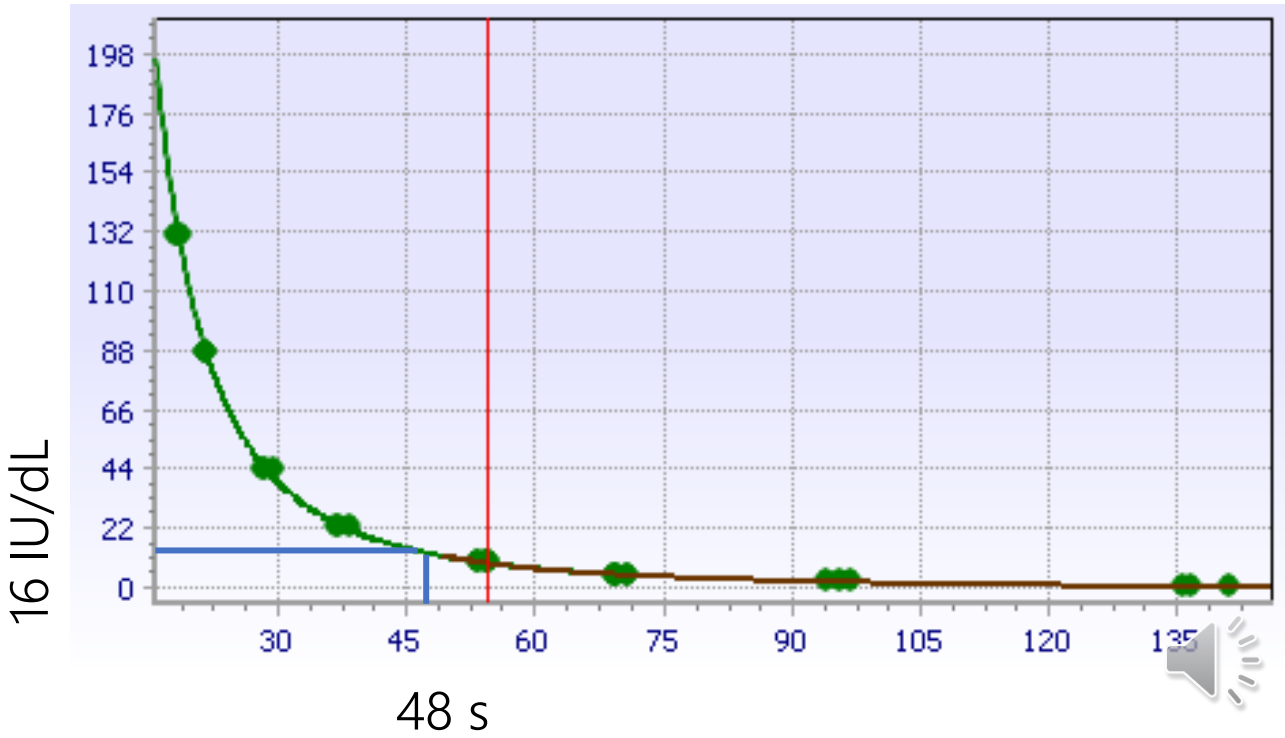
- Isolated prolonged prothrombin time
- No previous record of normal PT
- No anticoagulant medication, no improvement after vitamin K substitution, no hepatic insufficiency



Clinical case 4

FVII OSA: based on the PT





Clinical case 4

Interpretation of a prolonged PT with normal APTT:

Factor VII deficiency

- **Congenital** = rare; acquired inhibitor = very rare
- Acquired = Start of VKA treatment, DOAC, vitamin K deficiency, liver disease, … (short half-life)

Always rule out acquired causes of FVII



General approach to laboratory testing

Pre-analytical phase:

- Blood collection
- Sample handling
- Patient specific factors

Typical evaluation:

- Complete blood count
- PT
- APTT
- Fibrinogen, (D-dimer)
- (PFA-100/200, von Willebrand factor)

Additional testing depending on result:

- Specific clotting factor assays (clot based or chromogenic)
- von Willebrand factor
- Platelet function testing

5



• APTT: activated partial thromboplastin time; CBC: complete blood count; PFA: platelet function analyzer; PT: prothrombin time; VWF: von Willebrand factor

Clinical case 5

- 5-year old patient
- As an infant, whenever he was picked up, areas subjected to greater pressure would develop bruises
- No significant hemostatic challenge
- Recently, he fell when running and sustained a bleeding wound to the hand, which stopped with local compression and application of cold



Clinical case 5

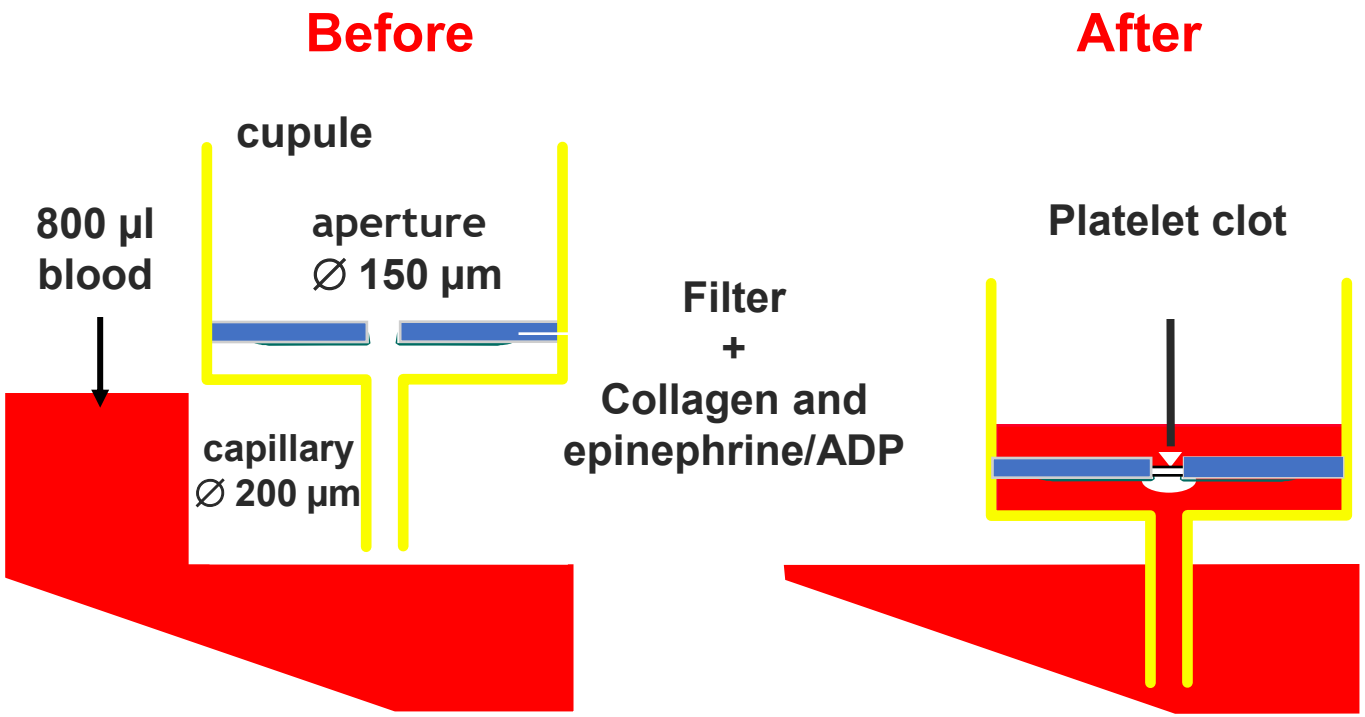
Laboratory results

- Hb: 11,0 g/dL (10,2-12,7 g/dL)
- Hct: 34%
- PLT: 265*10^3/µL
- APTT: 40,3 s (24,0-39,2 s)
- PT: 12,9 s (INR: 1,10)
- Fibrinogen: 240 mg/dL
- PFA-EPI >300 s; PFA-ADP >300 s



Clinical case 5

PFA-EPI and ADP >300 s



Highly dependent on hematocrit, platelet count and von Willebrand factor

No interpretation when:

- Hct <25%
- Platelet count <100.000/µL

Pathological when prolonged: severe platelet dysfunction (Glanzmann, Bernard-Soulier), von Willebrand disease and anti-platelet treatment

- Hct: hematocrit

Clinical case 5

Two types of cartridges: collagen +

- Epinephrine (von Willebrand disease, platelet disorder, aspirin)
- ADP (von Willebrand disease, platelet disorder)

PFA : epinephrine	PFA : ADP	Think of…
↗	Normal	Aspirin Platelet disorder
↗	↗	vW disease Platelet disorder

Clinical case 5

Laboratory results

- APTT: 40,3 s (24,0-39,2 s)
- PT: 12,9 s (INR: 1,10)
- Fibrinogen: 240 mg/dL
- PFA-EPI >300 s; PFA-ADP >300 s
- Von Willebrand antigen: 35 IU/dL
- Von Willebrand activity: 10 IU/dL
- Ratio: 0,4
- FVIII: 35 IU/dL



Clinical case 5

Quantitatif (antigen) :

Automated immunological assays

Qualitative (activity) :

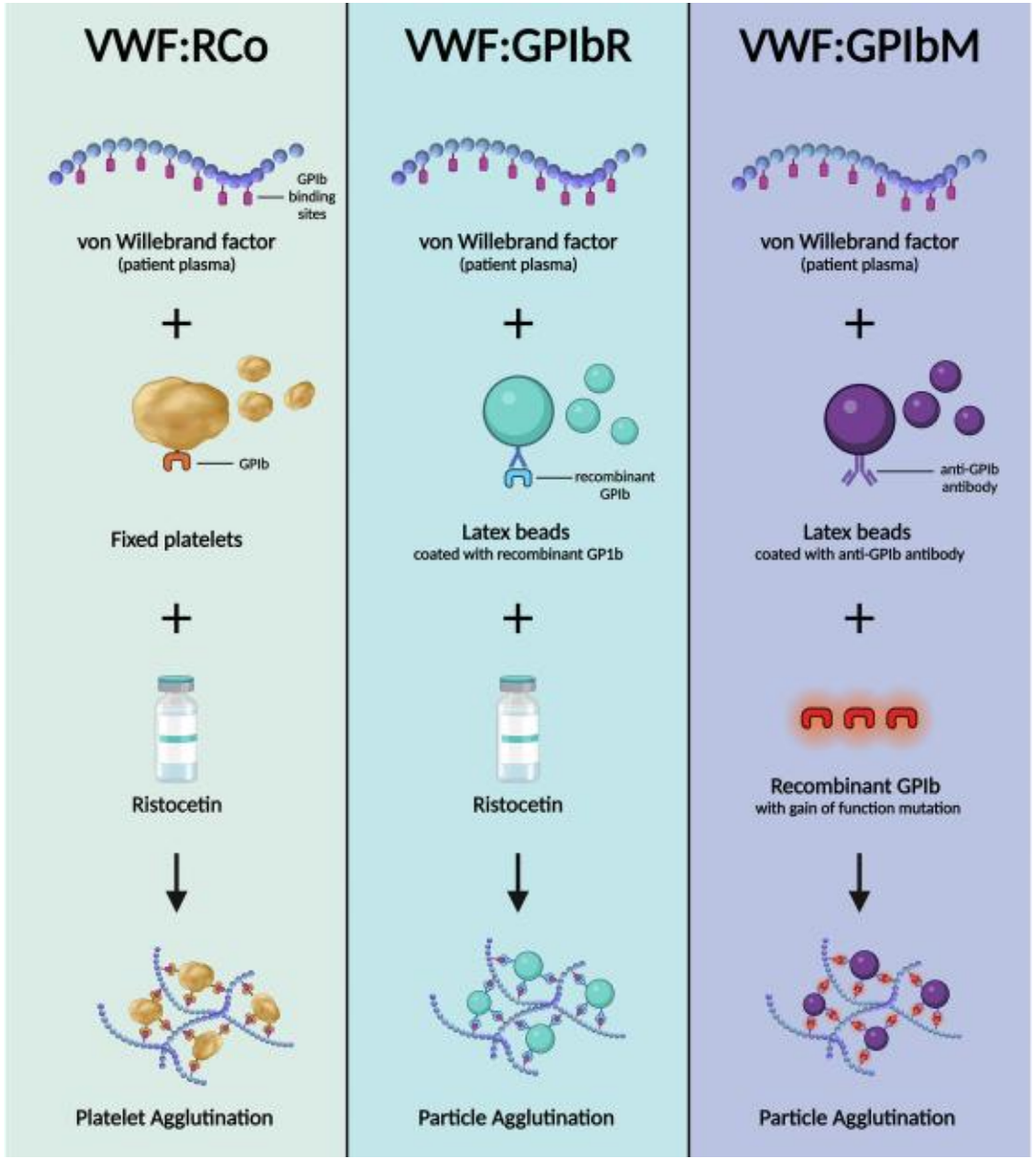
(Ristocetin) cofactor activity
→ platelet affinity

Collagen binding assay
→ collagen affinity

Results

- Reference : 50 – 150 % (variable in function of blood group)
- Activity/antigen ratio= +/- 1
- If ratio < 0.7 : Qualitatif problem

- RIPA: ristocetin induced platelet aggregation



Different types of VWF activity assays

- Fixed platelets: high CV and poor sensitivity
- GPIbR
  - Latex particles
  - Chimiluminescence (not in image)
- GPIbM (no need for ristocetin)

ASH ISTH NHF WFH 2021 guidelines suggest using newer assays of platelet-dependent VWF function over the VWF:RCo whenever possible

- Abou-Ismail et al. JTH 2023;21:204–214

Clinical case 5

Laboratory results

- APTT: 40,3 s (24,0-39,2 s)
- PT: 12,9 s (INR: 1,10)
- Fibrinogen: 240 mg/dL
- PFA-EPI >300 s; PFA-ADP >300 s
- Von Willebrand antigen: 35 IU/dL
- Von Willebrand activity: 10 IU/dL
- Ratio: 0,4 → Type II VWD, add multimers and RIPA
- FVIII: 35 IU/dL

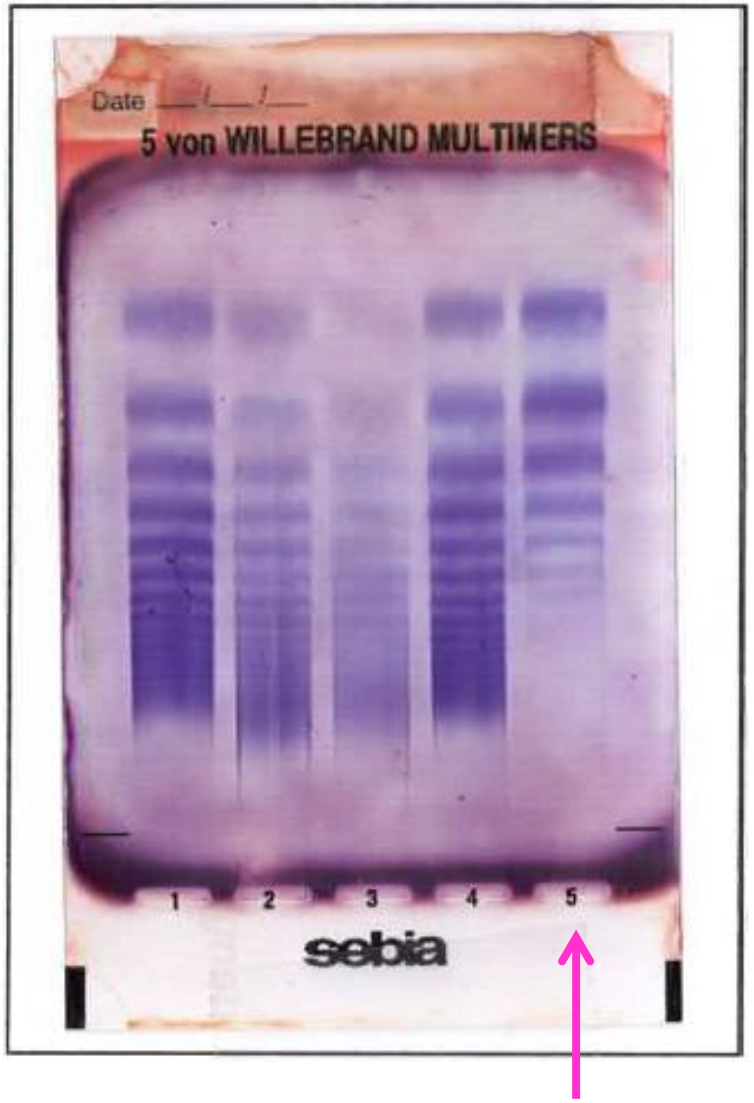


- RIPA: ristocetin induced platelet activation

Clinical case 5

Multimer analysis

Loss of high molecular weight multimers
→ Type IIA or type IIB



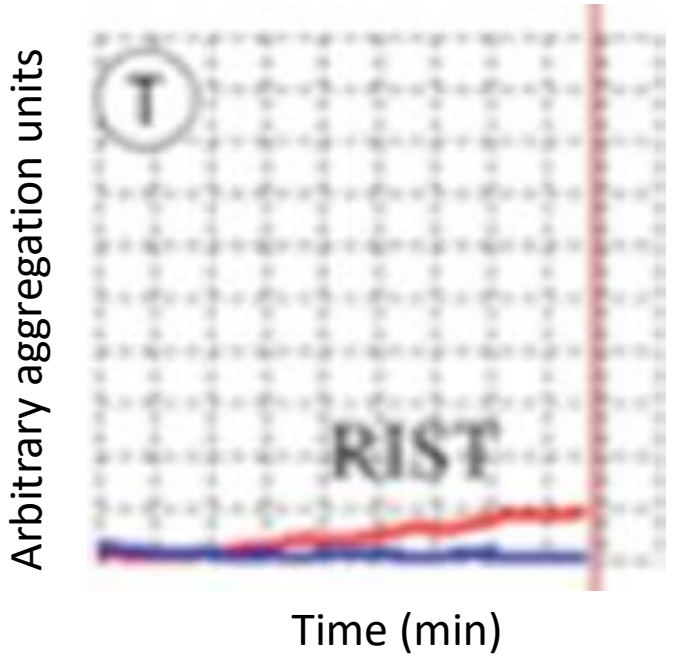
- RIPA: ristocetin induced platelet aggregation

Clinical case 5

RIPA

No aggregation with low ristocetin concentration

→ Type 2A



• RIPA: ristocetin induced platelet aggregation

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Thank you very much for your attention

