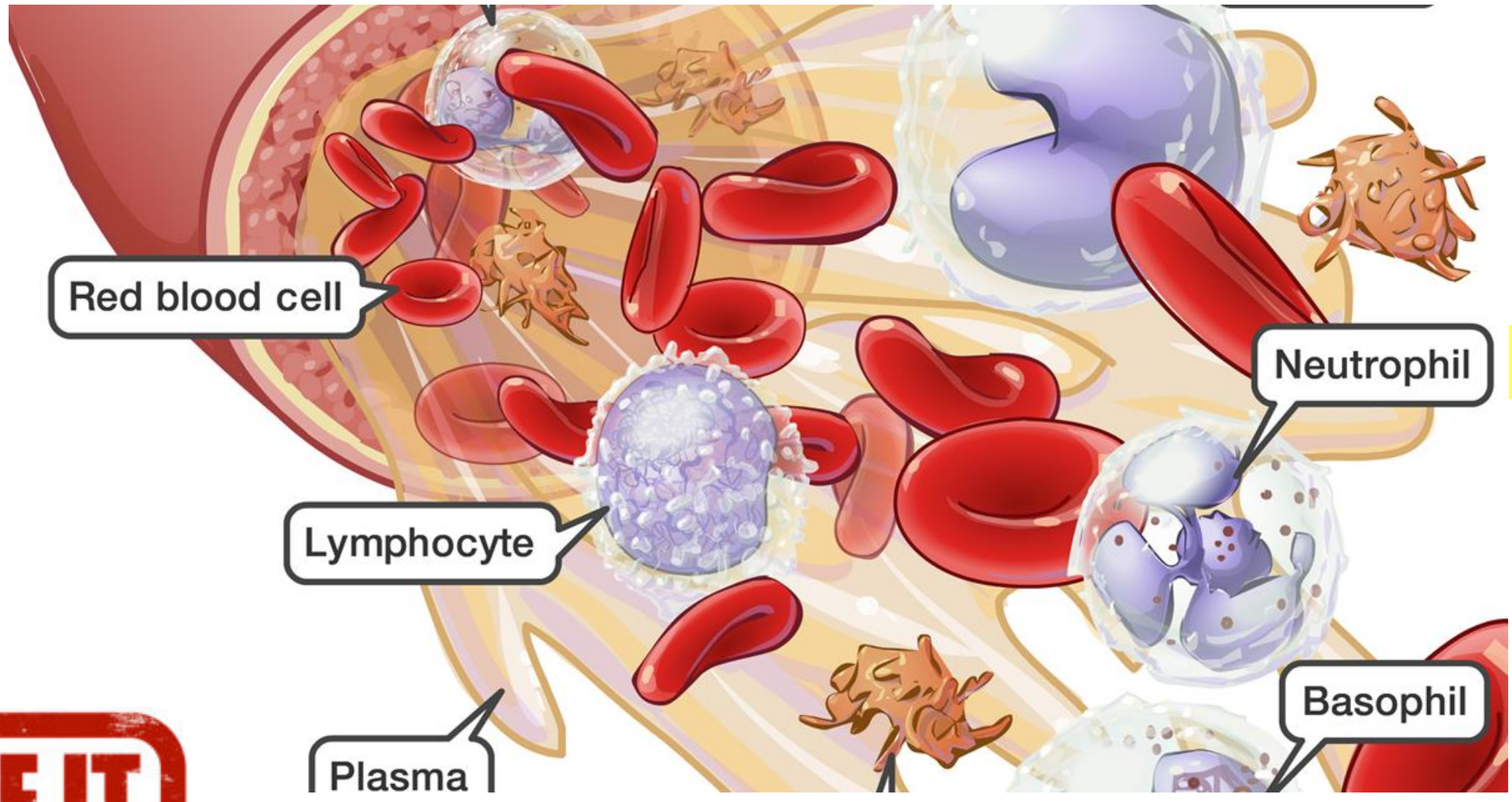
Pediatric Cancer & Hematologic Disorder
PedHemOnc-PMK

Understanding Laboratory in Benign Hematology



Pretest.

MAKE IT EASY

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Phramongkutklao Hospital

Lecture Scope

Cells at Work! はたらく細胞

Introduction / Case

Lab in RBC disorder

Lab in Plt disorder and Coagulopathy

Take home messages

Pediatric Cancer & Hematologic Disorder
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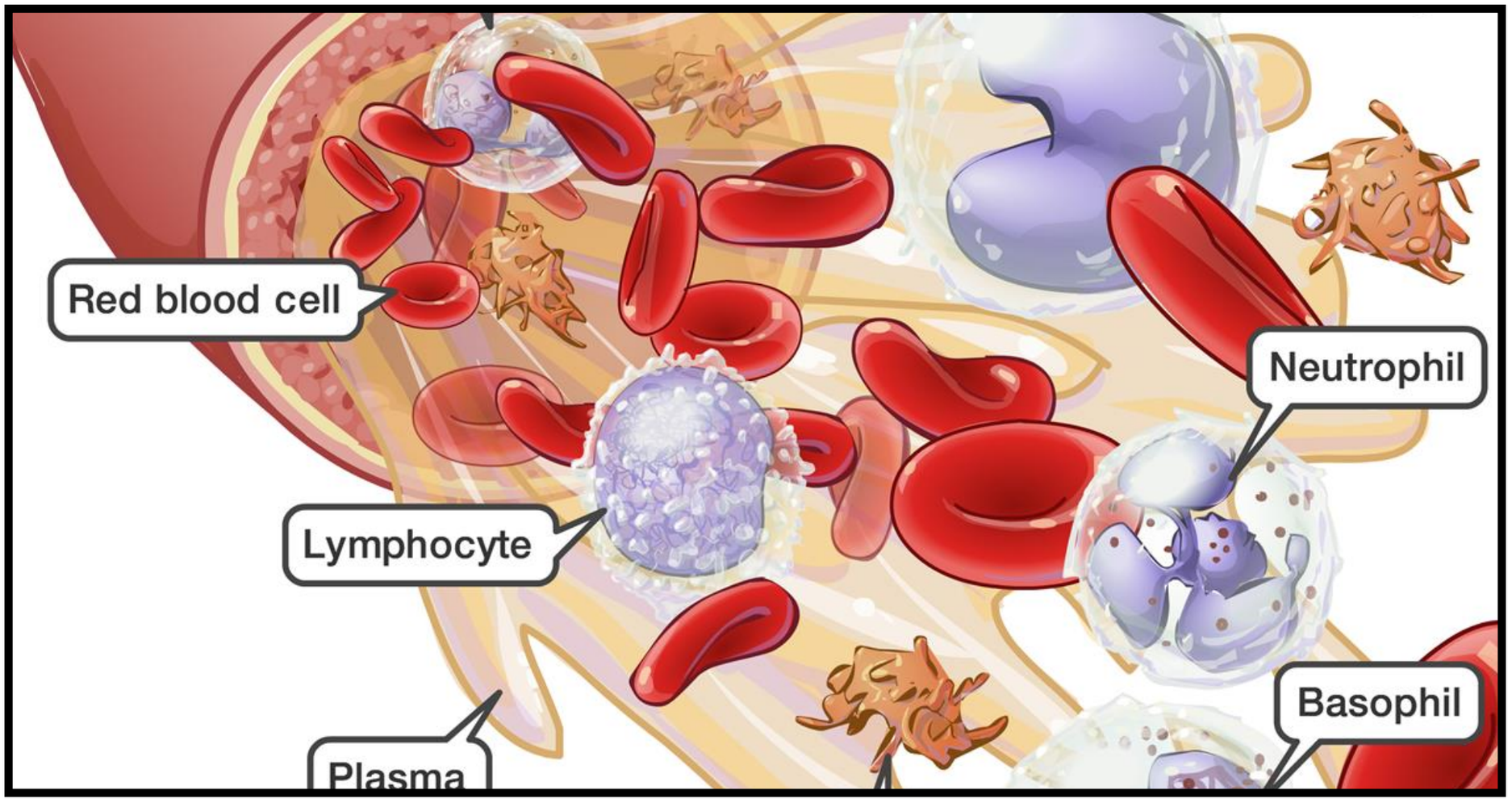
Introduction



- Cellular component : RBC, WBC, Platelet
- Plasma component : Coagulation factor

Blood Collection Tube

Range	Color Coding	Additive	Type	Blood Draw	Tube Size (mm)	Clinical Use
Serum Blood Collection Tubes	(Red)	Plain (No Additive) Clot Activator	Vacuum & Non - Vacuum	4 ml 4 ml 6 ml	13 x 75 13 x 75 13 x 100	Serum Biochemistry. Drug Monitoring & Serum Immunology Test
	(Yellow)	Clot Activator with Gel	Vacuum & Non - Vacuum	4 ml 6 ml	13 X 75 13 X 100	Serum Biochemistry. Drug Monitoring & Serum Immunology Test
Whole Blood Collection Tubes	(Lavender)	K3 EDTA K3 EDTA K2 EDTA	Vacuum & Non - Vacuum	2 ml 3 ml 2 ml	13 X 75 13 X 75 13 X 75	Hematology Test
	(Black)	ESR	Vacuum	2 ml	13 X 75	Sedimentation Rate Test
Plasma Blood Collection Tubes	(Grey)	Sodium Fluoride + Potassium Oxalate	Vacuum & Non - Vacuum	2 ml	13 X 75	Glucose Test (Analysis of Blood Sugar)
	(Light Blue)	Sodium Citrate (3.2%)	Vacuum & Non - Vacuum	2 ml	13 X 75	Coagulation Test
	(Green)	Lithium Heparin	Vacuum & Non - Vacuum	2 ml	13 x 75	Emergency Biochemistry & Plasma Biochemistry Test

- Coag factor

+ Coag factor

RBC Abnormalities

I. Membrane defects

- Primary membrane defects with specific morphologic abnormalities
  - **Hereditary spherocytosis**
  - **Hereditary elliptocytosis / pyropoikilocytosis**
  - **Hereditary stomatocytosis**
    - Over-hydrated stomatocytosis (increased RBC volume)
    - Dehydrated stomatocytosis/xerocytosis/desicytosis (decreased RBC volume)
    - Rh null
    - Normal osmotic fragility/normal volume
- Secondary membrane defects
  - **Abetalipoproteinemia / neuroacanthocytosis**

II. Enzyme defects

- *Energy potential defects*
  - **Phospho-fructokinase**
  - **Hexokinase**
  - **Pyruvate kinase**
  - **Glucose phosphate isomerase**
  - **Triose phosphate isomerase**
  - **Phosphoglycerate kinase**
  - **2,3-Diphospho-glyceromutase** (polycythemia, no hemolysis)
- *Reduction potential defects*
  - **6-Phosphogluconate dehydrogenase (6PGD)**
  - **G6PD**
  - **Glutathione reductase**
  - **Glutathione synthetase**
  - **2,3-Glutamyl-cysteine synthetase**
- *Abnormalities of erythrocyte nucleotide metabolism*
  - **Adenosine triphosphatase deficiency**
  - **Adenylate kinase deficiency**
  - **Pyrimidine 5'-nucleotidase (P5N) deficiency**
  - **Adenosine deaminase excess**

III. Hemoglobin defects (see Chapter 10)

- Heme
  - **Congenital erythropoietic porphyria**
- Globin
  - Qualitative **Hemoglobinopathies** (*e.g.*, HbS, C, M)
  - Quantitative (**α- and β-thalassemia**)

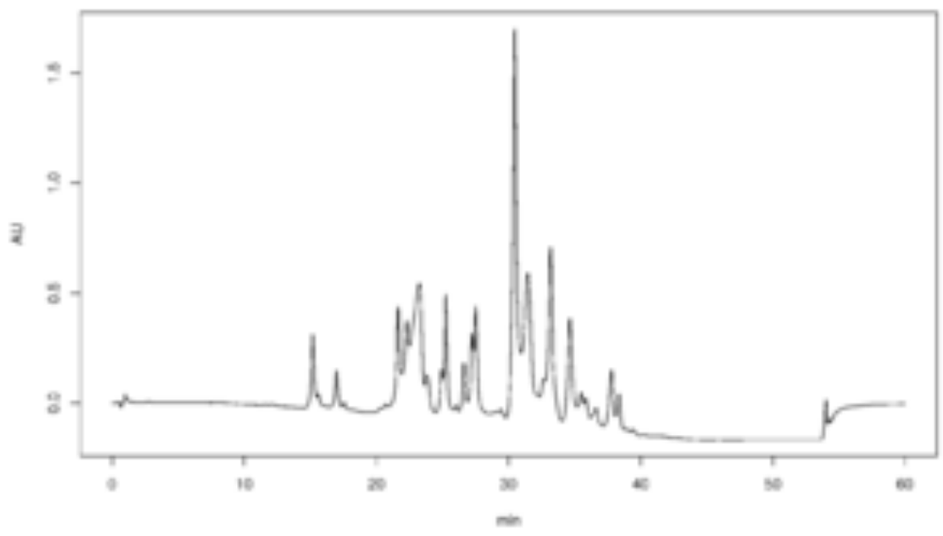
IV. Congenital dyserythropoietic anemias (see Chapter 10)

- **Type I**
- **Type II**
- **Type III**
- **Type IV**

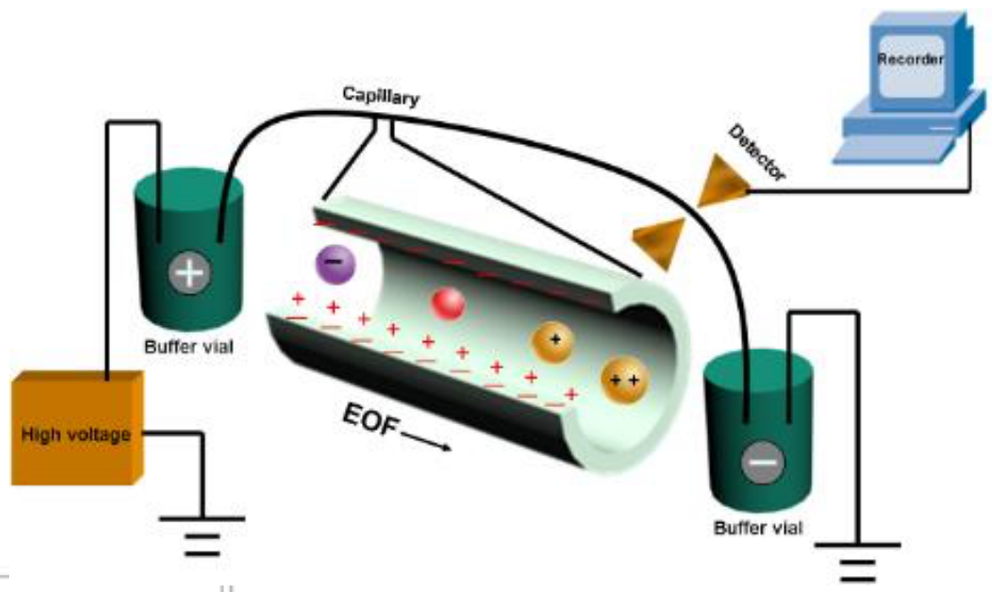
Ref : Lanzkowsky 6th edition

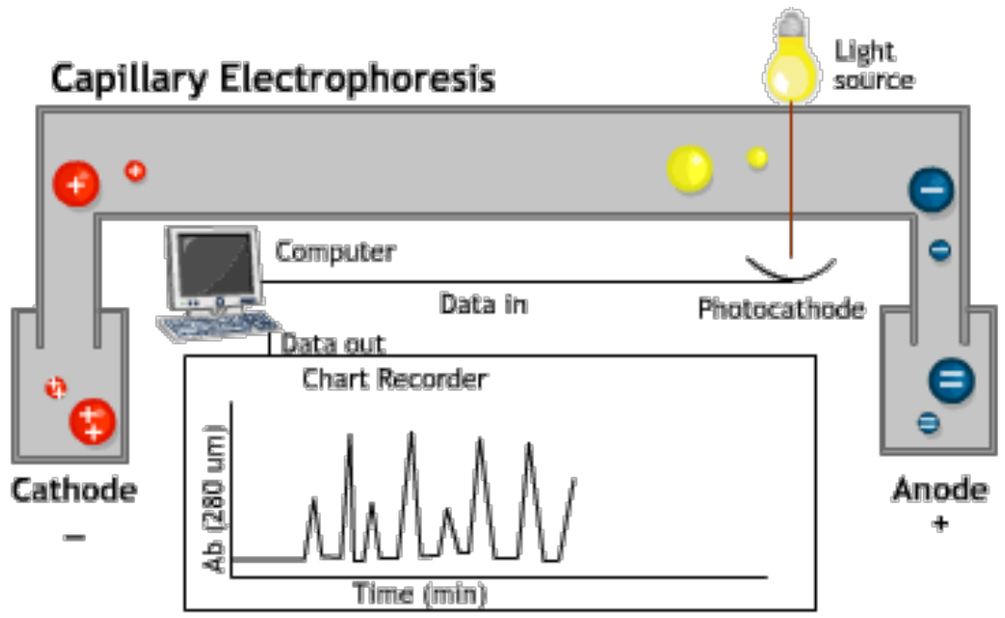
Hemoglobin typing

HPLC



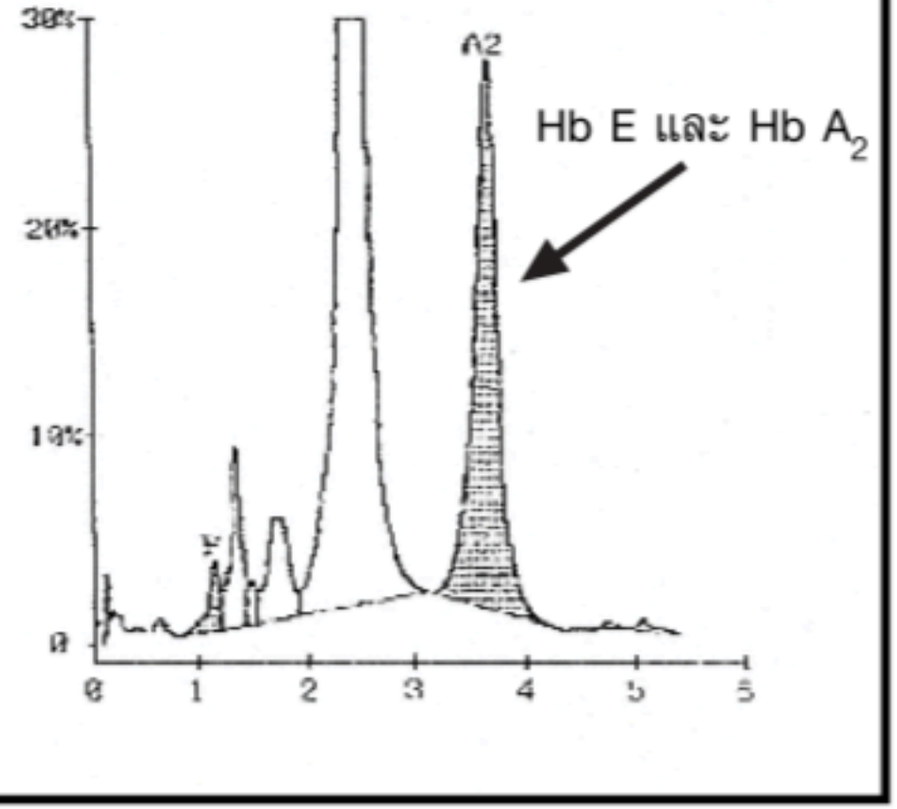
Capillary Electropheresis



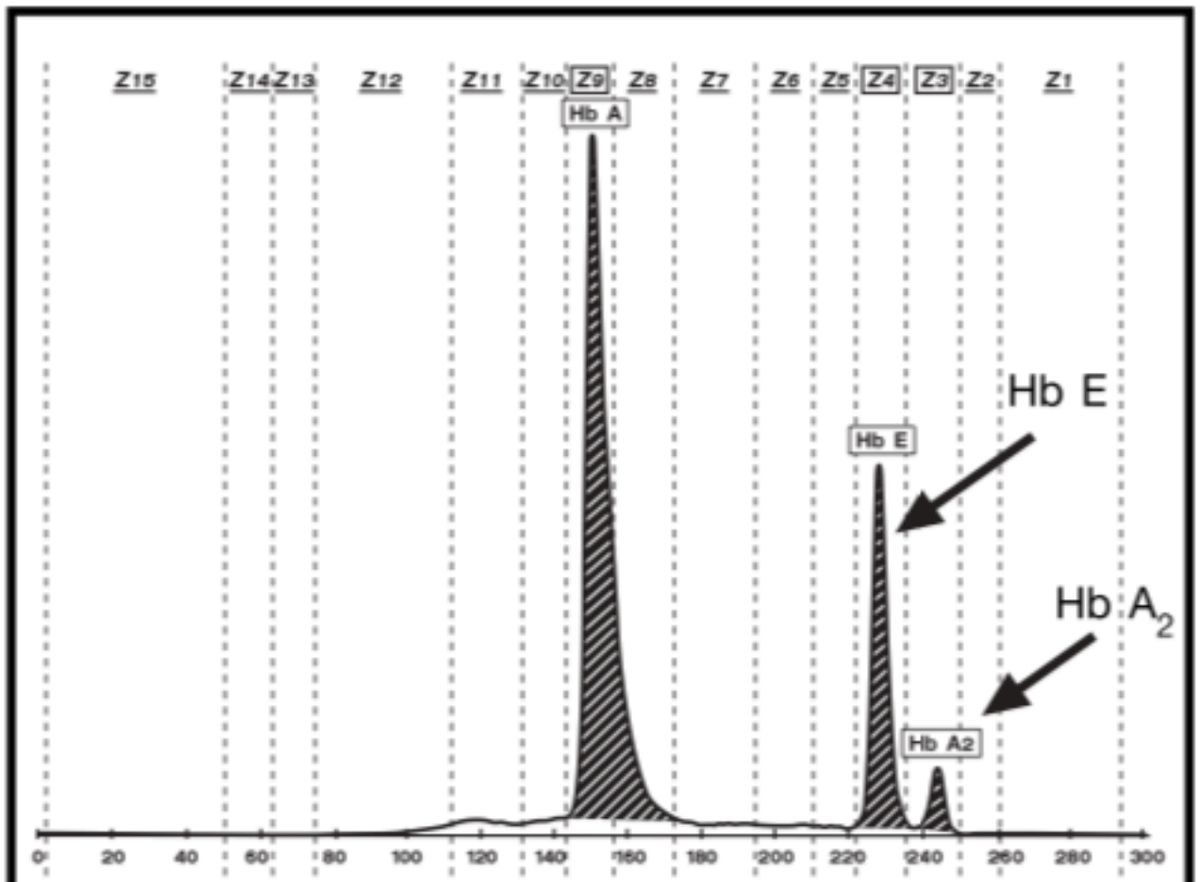


Hemoglobin typing

ANALYTE ID	%	TIME	AREA
F	1.3	1.12	32713
P2	3.4	1.32	84862
Unknown 1	0.7	1.44	17745
P3	4.1	1.67	101871
Ao	62.4	2.43	1548798
A2	28.1	3.60	697043
	TOTAL AREA		2392232
F	1.3%	A2	28.1%



High Pressure Liquid Chromatography



Hemoglobin Electrophoresis

Fractions	%	Ref. %	Ref. g/dl
Hb A	72.5		
Hb E	23.7		
Hb A2	3.8		

Capillary Electrophoresis

Hemoglobin typing

Must know!!!			
Hb A	**α2 β2**	***Hb H***	**β4**
Hb A2	**α2 δ2**	***Hb Bart's***	**ɣ4**
Hb F	**α2 ɣ2**		

Normal : (αα,αα)(β,β)

A. Decrease production : ปริมาณ *Hb*

B. Abnormal production *(amino acid)*

Variant Hb Ex; Hb E, Hb Cs

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Case #1

%Hb Bart's H Present	HbF 0.4%	HbA_2 1.5%	Hb-typing : A_2ABart's H
แปลผล : Hemoglobin H disease			

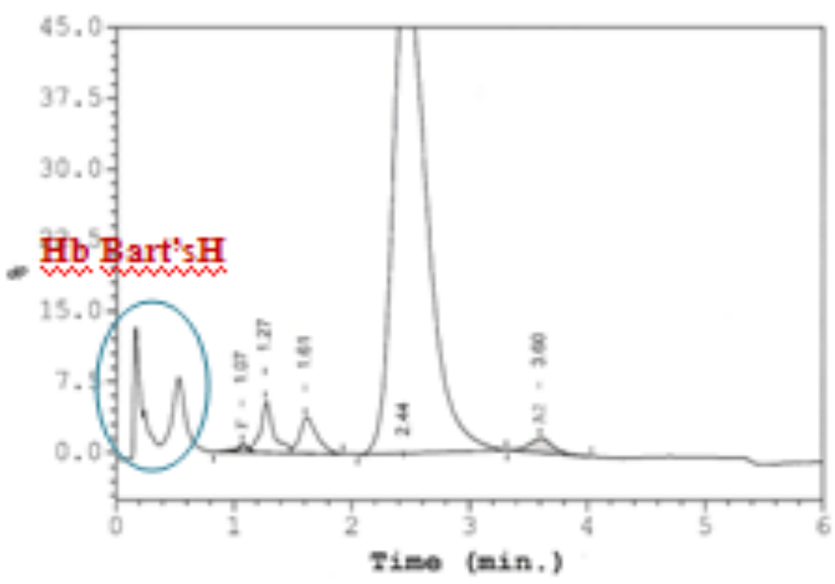
Peak Name	Calibrated Area%	Area%	Retention Time (min.)	Peak Area
F	0.4	---	1.07	7544
P2	---	3.6	1.27	63280
P3	---	3.3	1.61	57175
Ao	---	90.8	2.44	1578815
A2	1.5*	---	3.60	32266

Total Area :1,739,080

F Concentration = 0.4 %
A2 Concentration =1.5* %

Analysis comments:

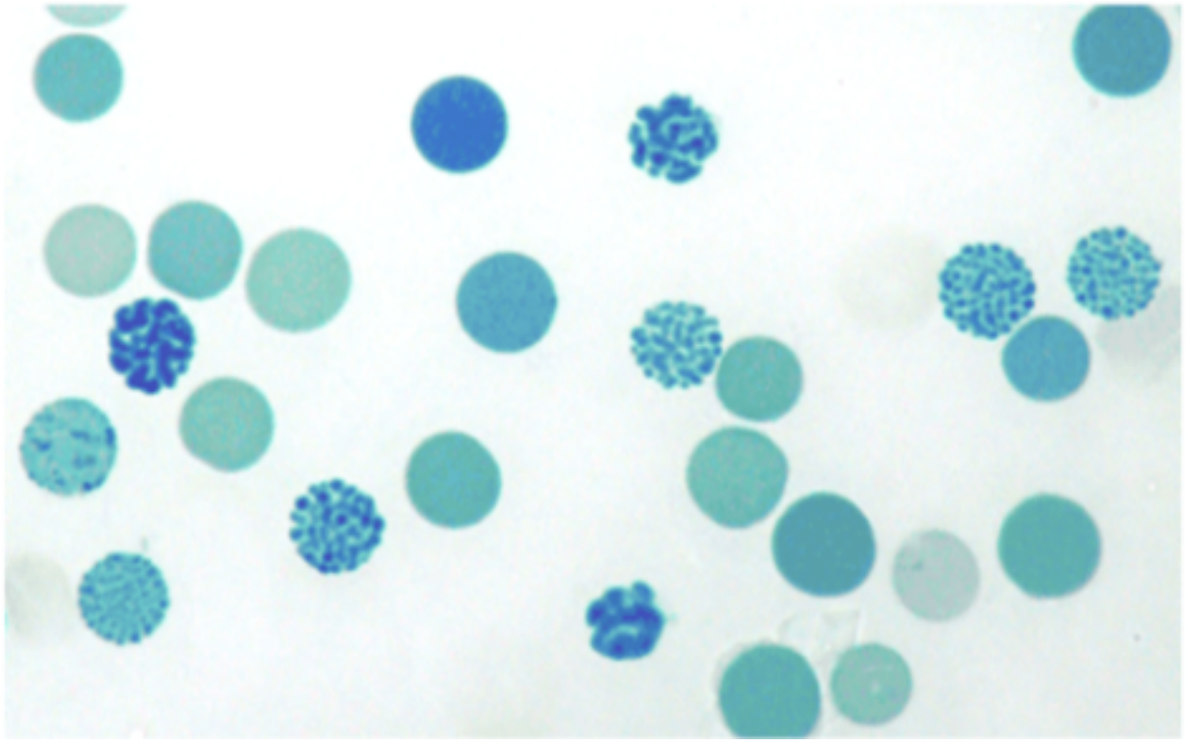
*Values outside of expected ranges

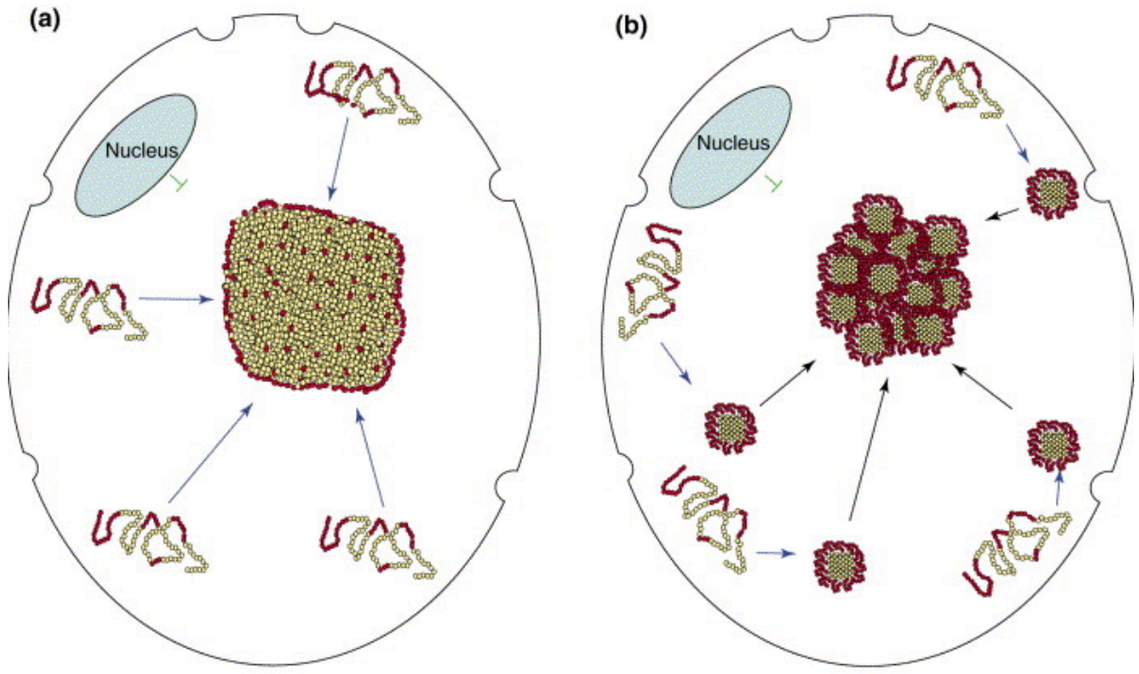


Genotype β / β

α-Thal1 / α-Thal2

Inclusion Body





ย้อมด้วยสี supravital stain (1% Methylene blue) หยดบน Slide 1 หยด พร้อมเลือดติดปลายไม้จิ้มฟันทิ้งไว้ 1-2 ชั่วโมง แล้วนำไปดูด้วยกล้องจุลทรรศน์

Abnormal : จะพบ HbH inclusion body มากกว่า 50%

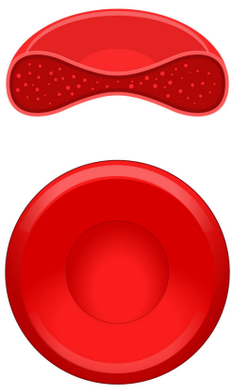
ข้อควรระวัง : อายุน้อยกว่า 6 เดือน หรือมีภาวะ Beta trait ร่วมด้วย

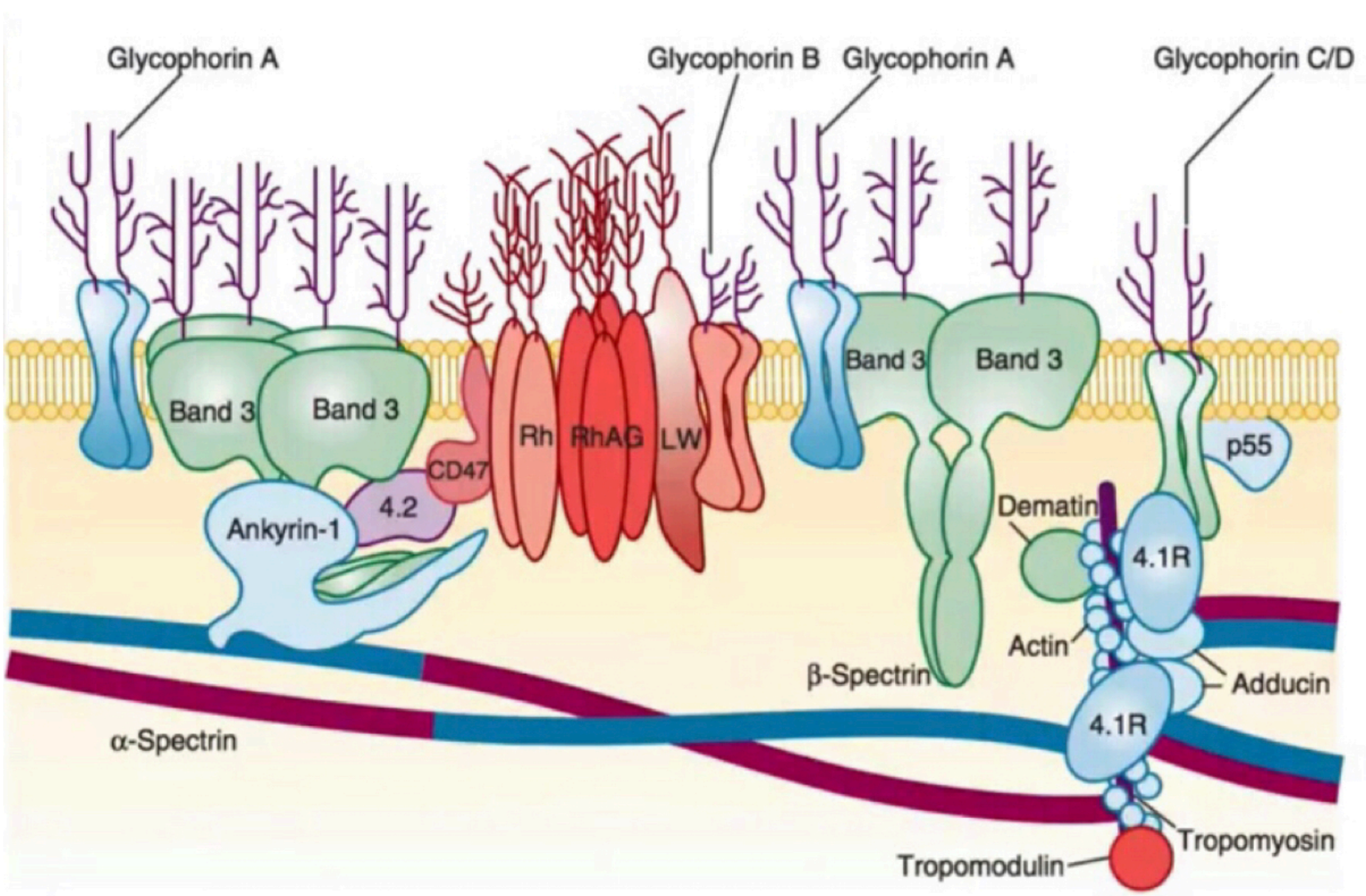
Inclusion Body

Common RBC Inclusions	Cartoon Image	Inclusion	May be associated with
Howell Jolly Bodies		DNA	Hyposplenism Asplenism Severe hemolytic anemia
Heinz Bodies	 *Supravital stain*	Hemoglobin	G6PD deficiency Oxidant drugs Unstable hemoglobin
Pappenheimer Bodies		Iron deposits	Thalassemia Sideroblastic anemia Hemolytic anemia Post-splenectomy
Hemoglobin H Inclusion	 *Supravital stain*	Hemoglobin	Hemoglobin H disease
Basophilic Stippling		Ribosomes	Lead poisoning Thalassemia Sickle cell anemia MDS

RBC Membrane Defect

Vertical linkage

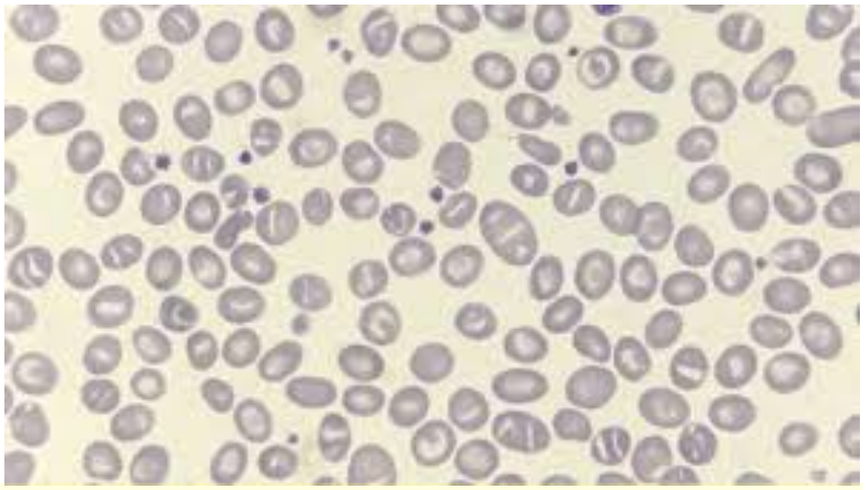




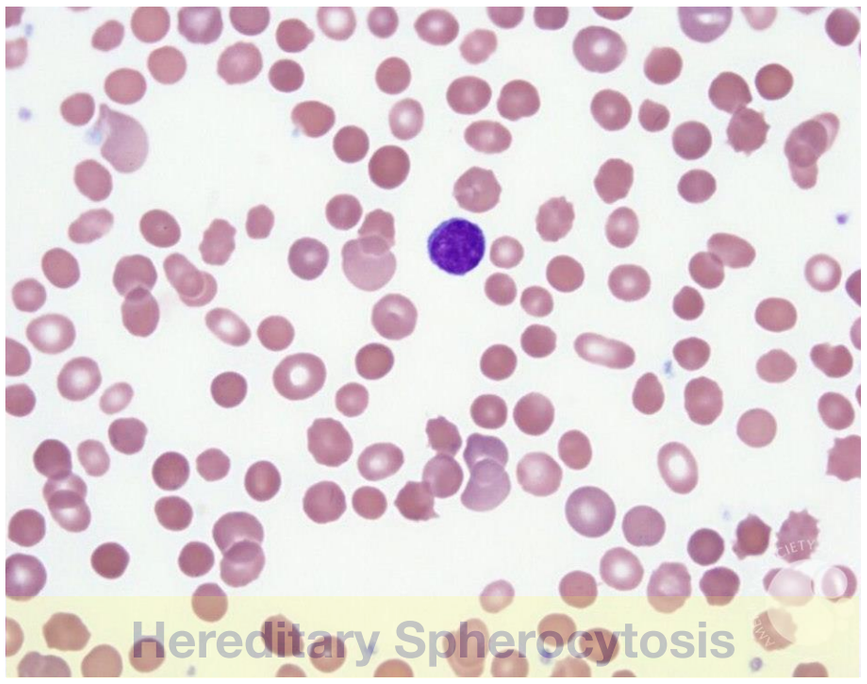
Horizontal linkage

RBC Membrane Defect

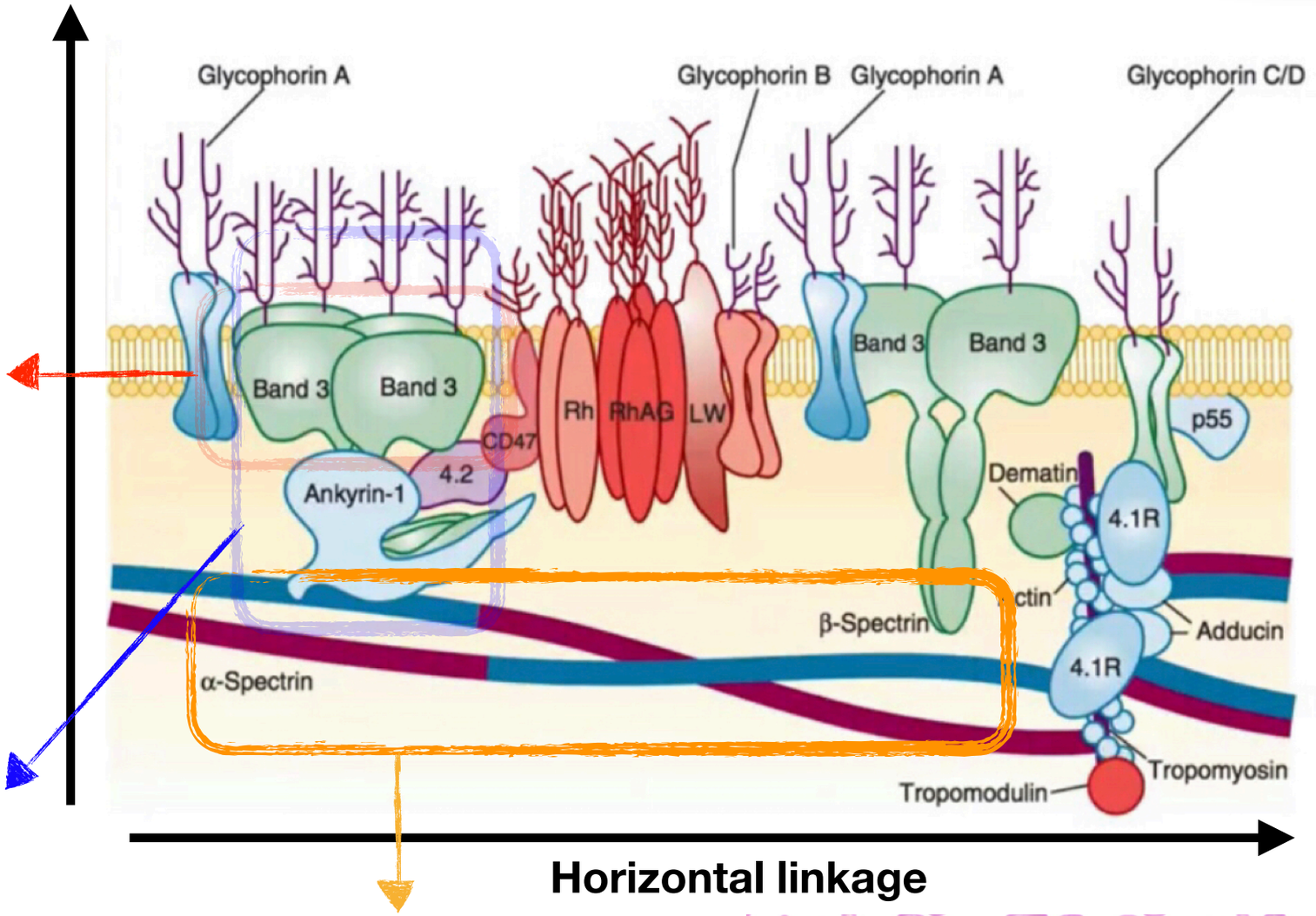
Vertical linkage



Southeast Asian Ovalocytosis

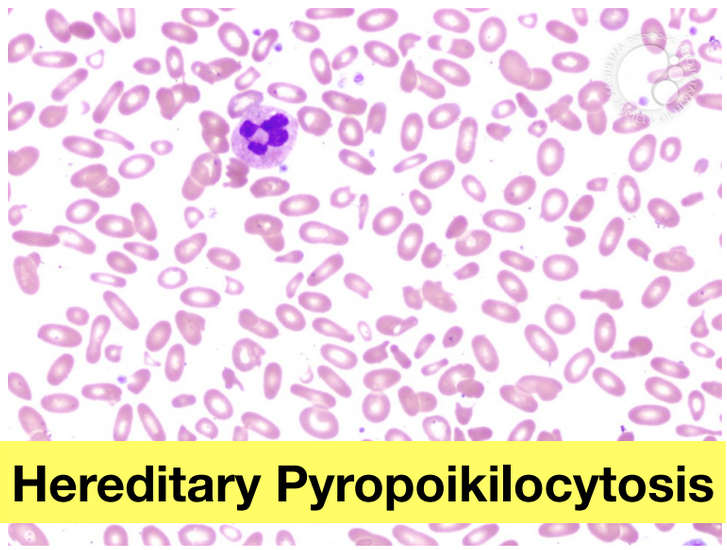


Hereditary Spherocytosis



Horizontal linkage

Hereditary Elliptocytosis



Hereditary Pyropoikilocytosis

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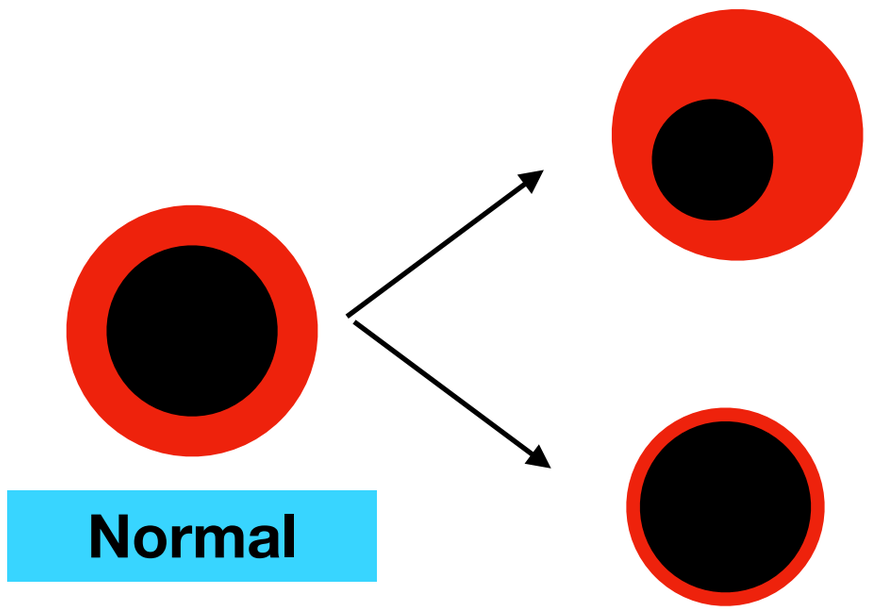
Osmotic Fragility test

One tube OF test



Normal

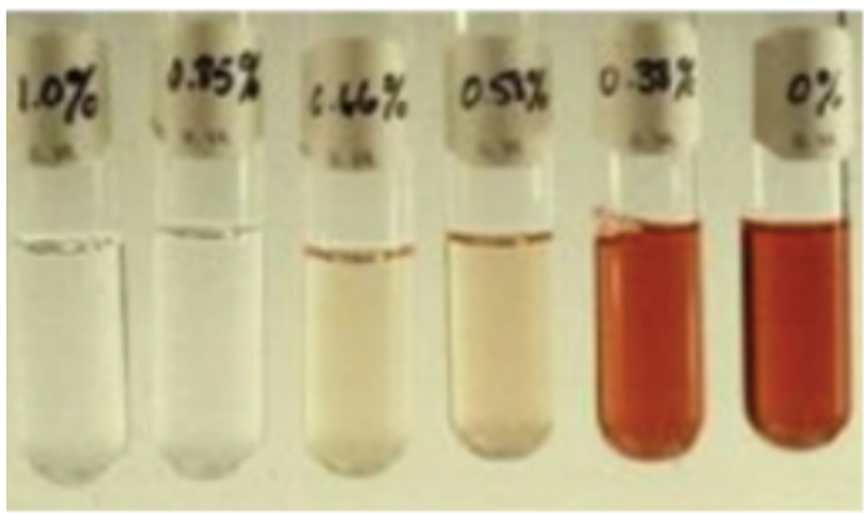
Thal trait

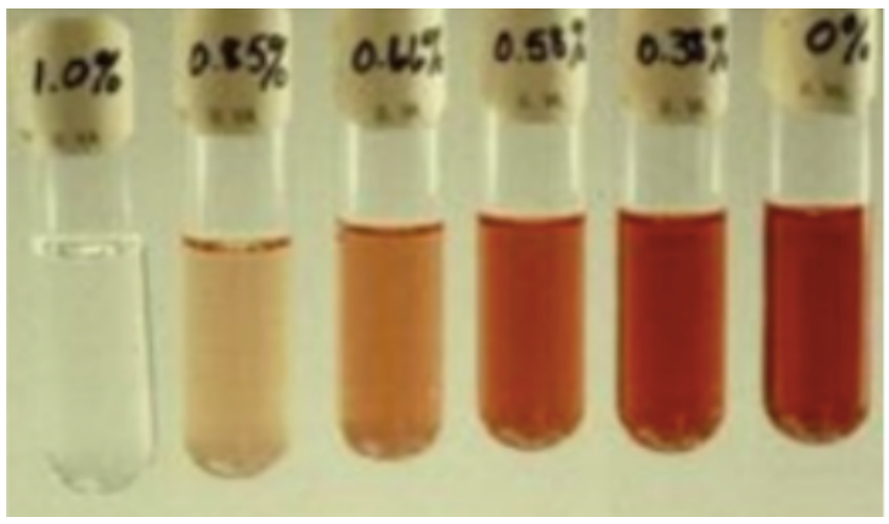
Positive
RBC แตกยากกว่าปกติ

HS, AIHA

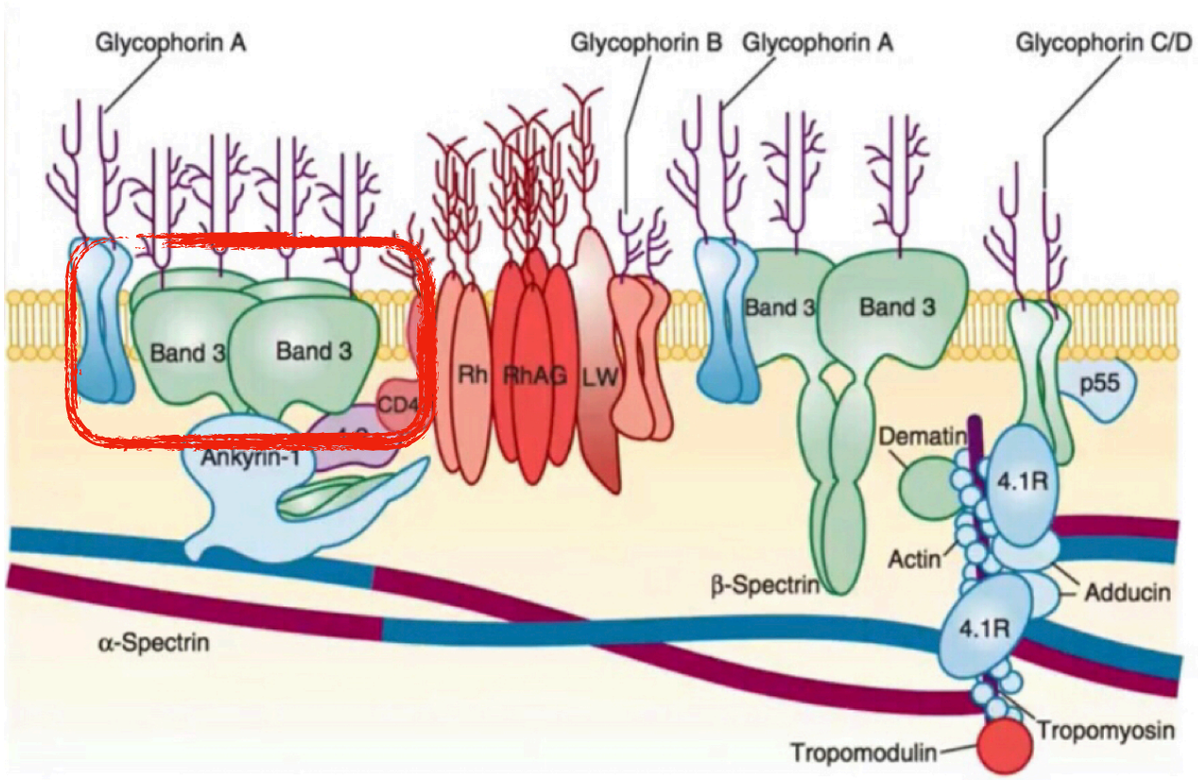
Positive
RBC แตกง่ายกว่าปกติ

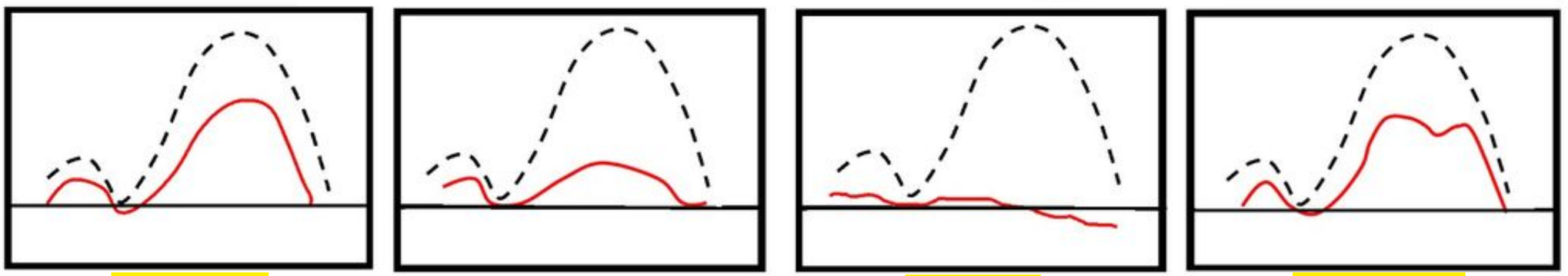
Incubated OF test



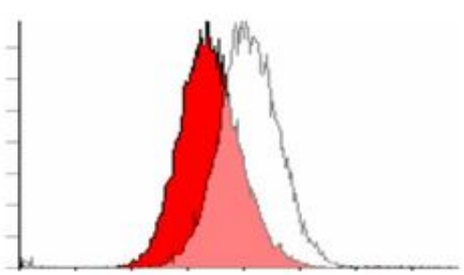


Flow Eosin-5-Maleimide





Ankyrin | **Band3** | **Spectrin**



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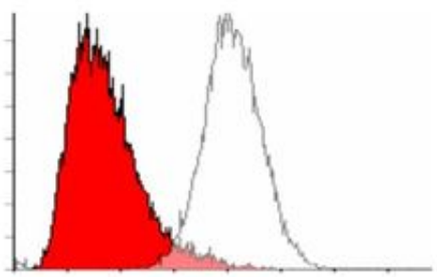

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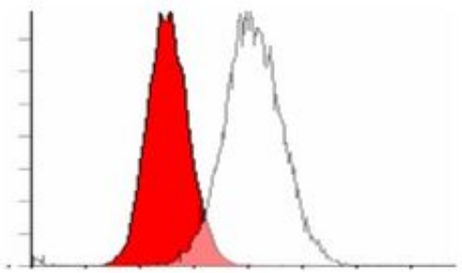

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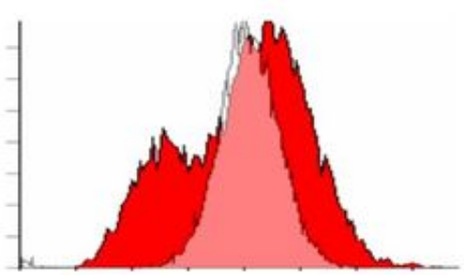
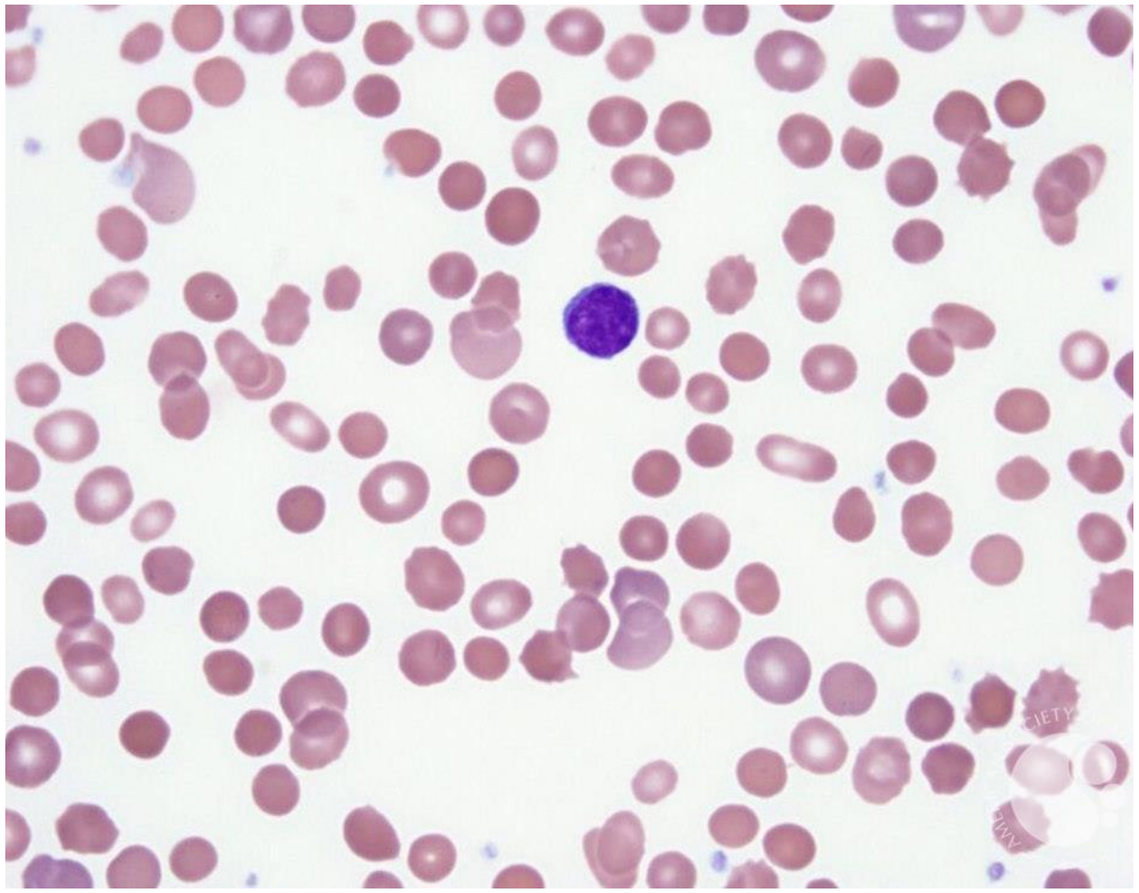
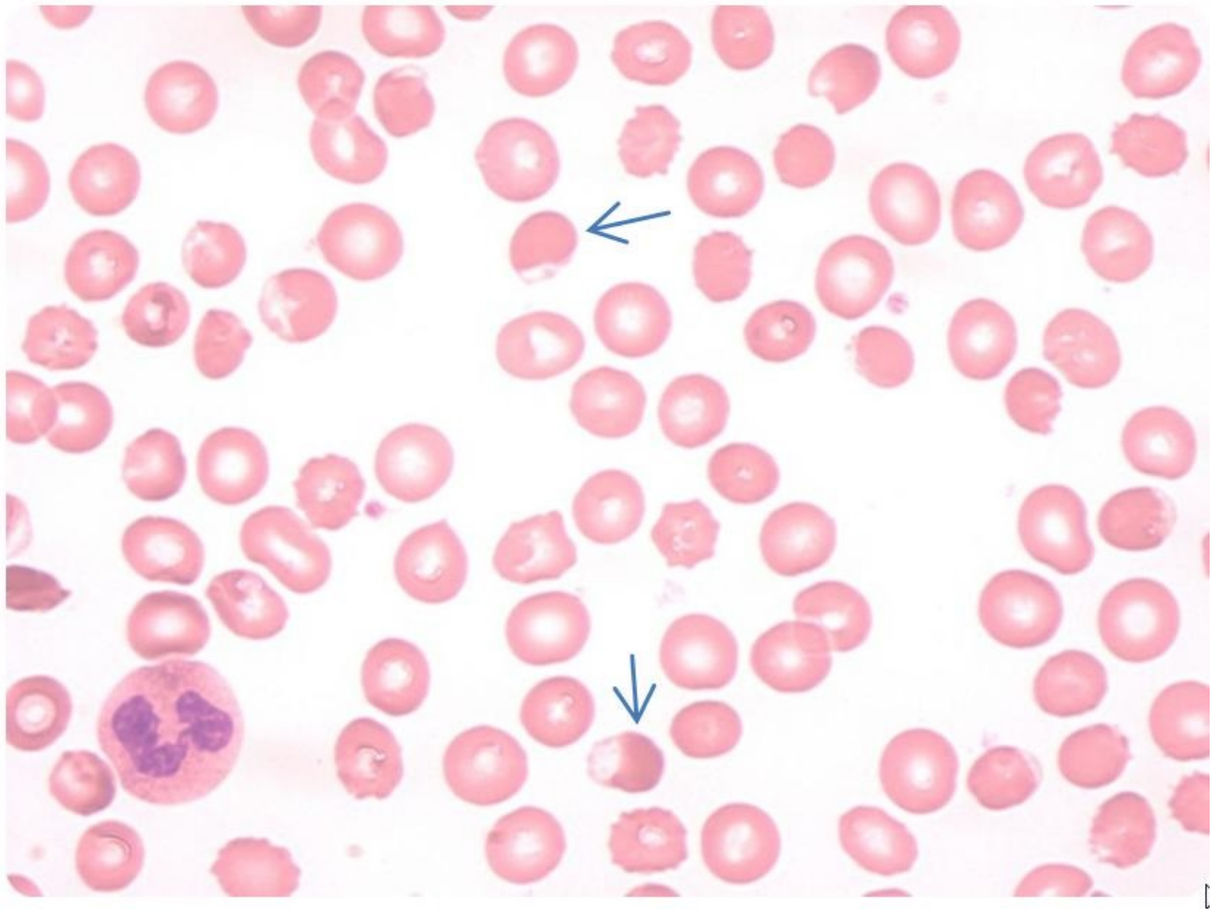


Hereditary Elliptocytosis

RBC Abnormalities



Diagnosis ???



Diagnosis ???

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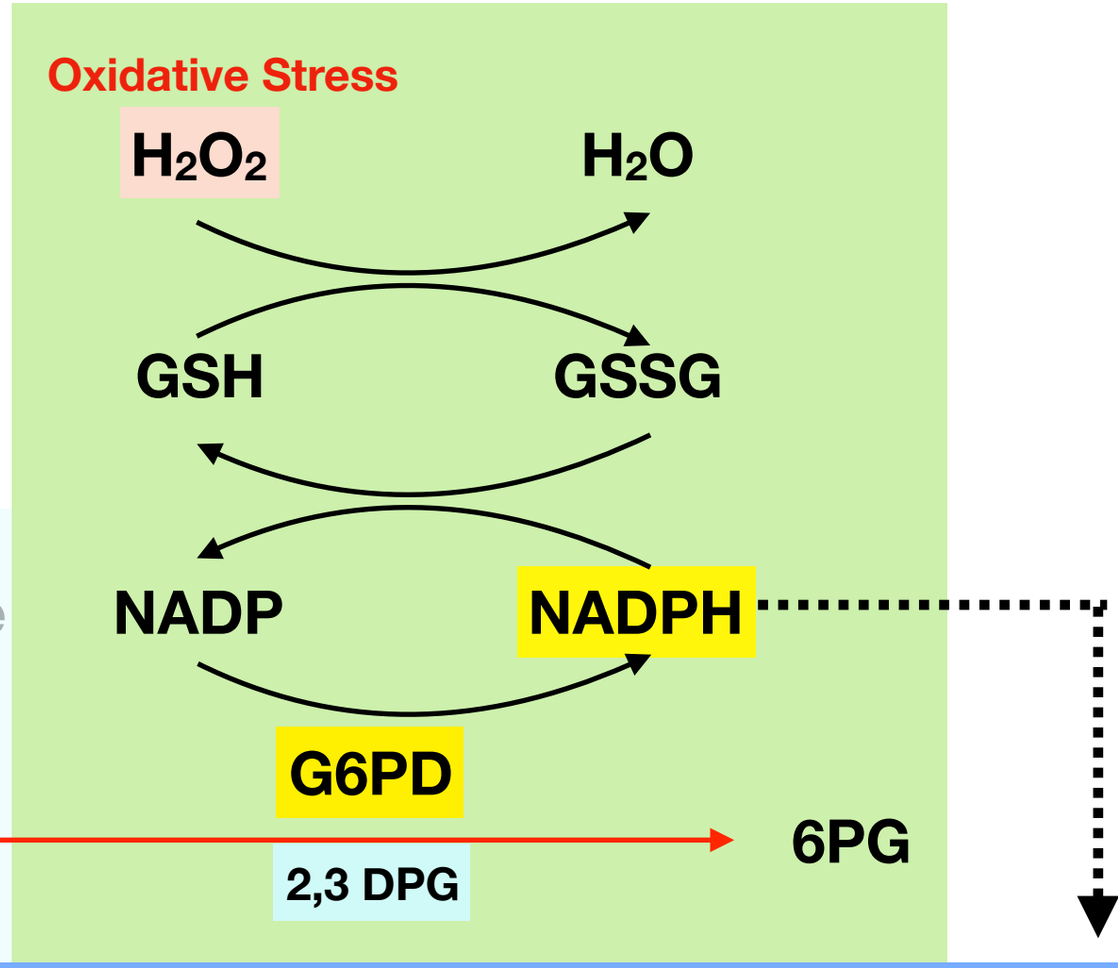
G6PD Level

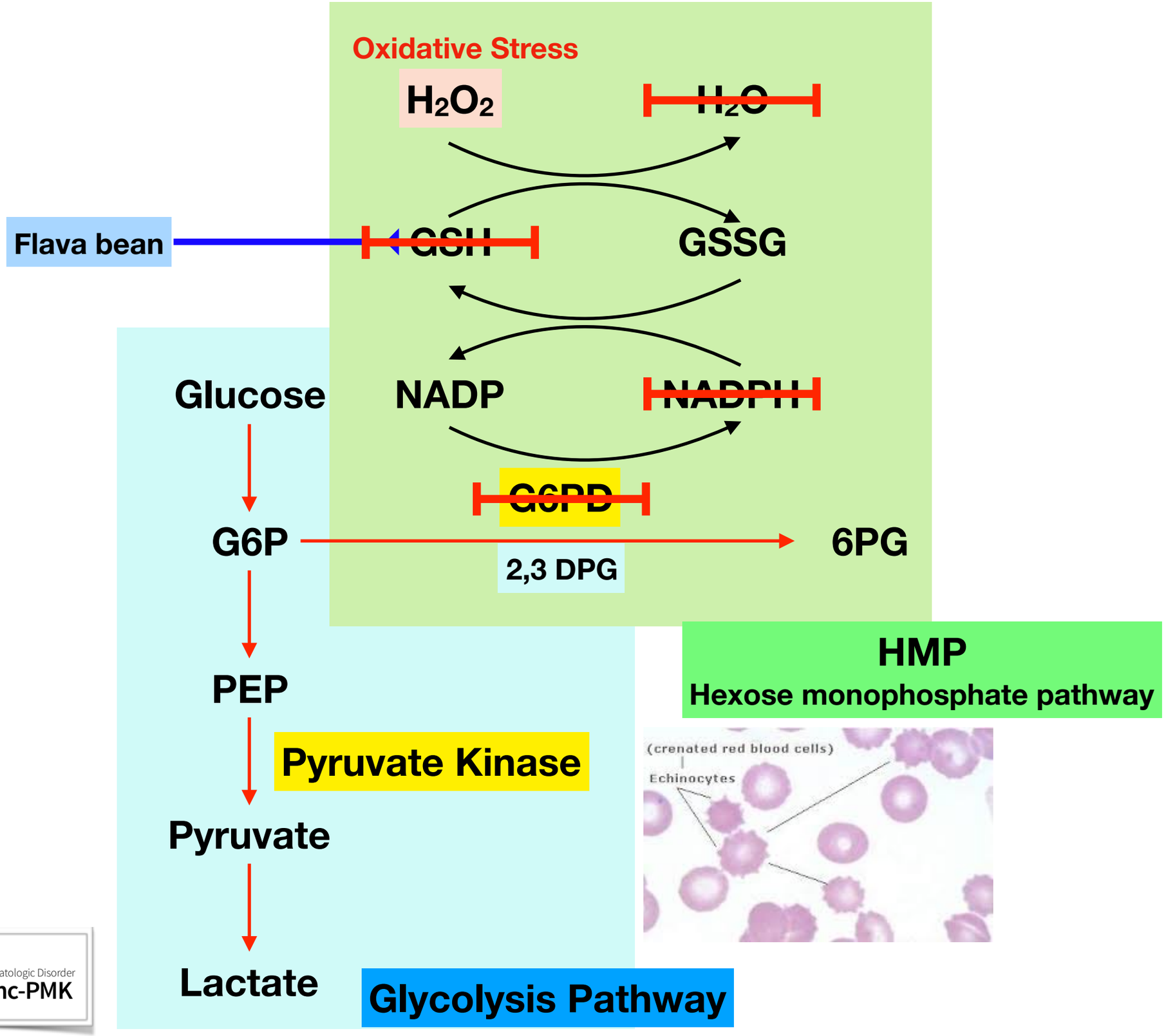


NADPH จะดูดกลืนแสงความยาวคลื่น 340 nm แล้ววัดได้ด้วยเครื่อง spectrometry ถ้า NADPH มาก ก็จะมีการดูดกลืนแสงมาก และนำค่าการดูดกลืนแสงมาคำนวน enzyme “G6PD” หน่วย IU/ml. RBC

ค่าปกติ ชาย 159-297 IU/ml.RBC
หญิง 197-331 IU/ml.RBC

Quantitative Method

G6PD Level



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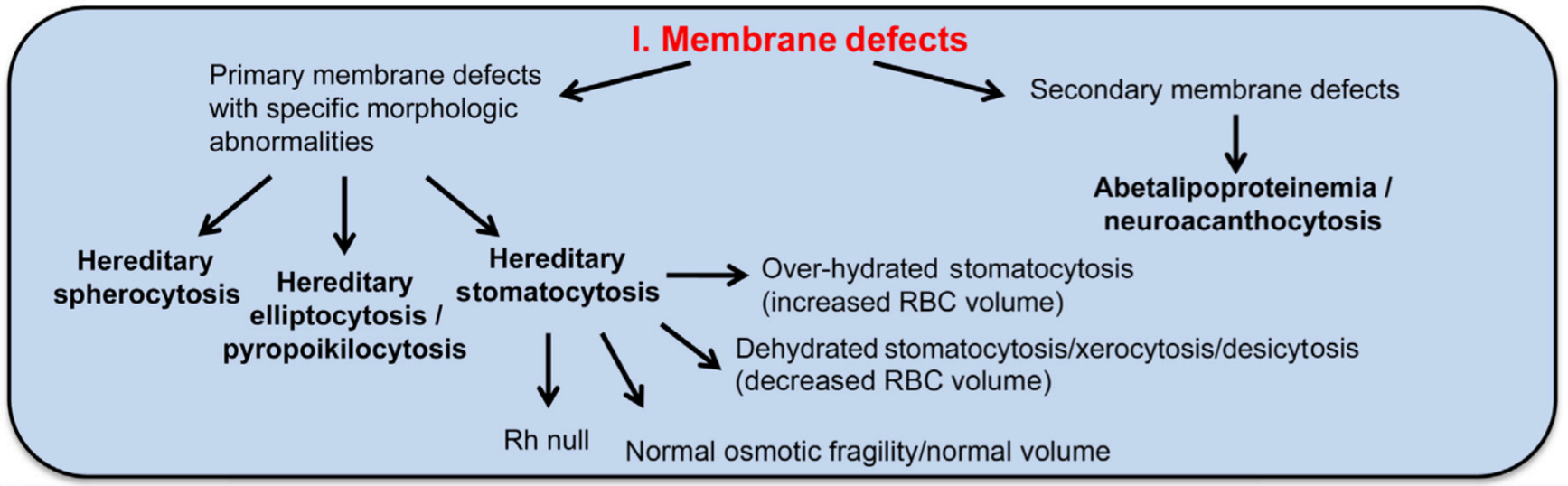
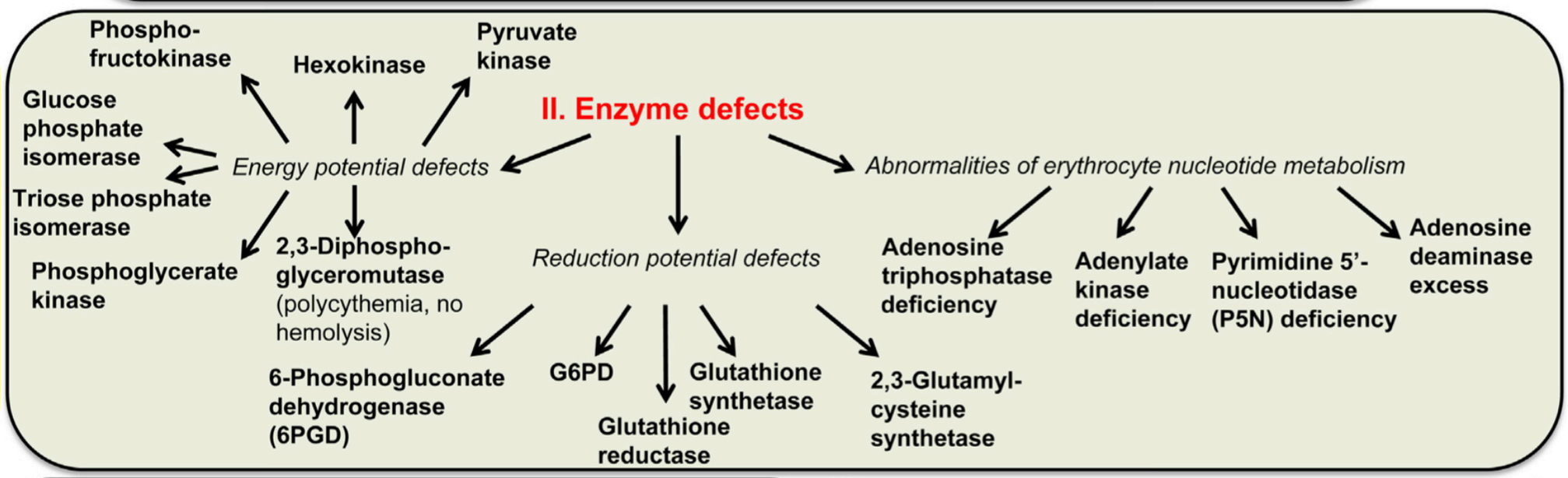
RBC Abnormalities

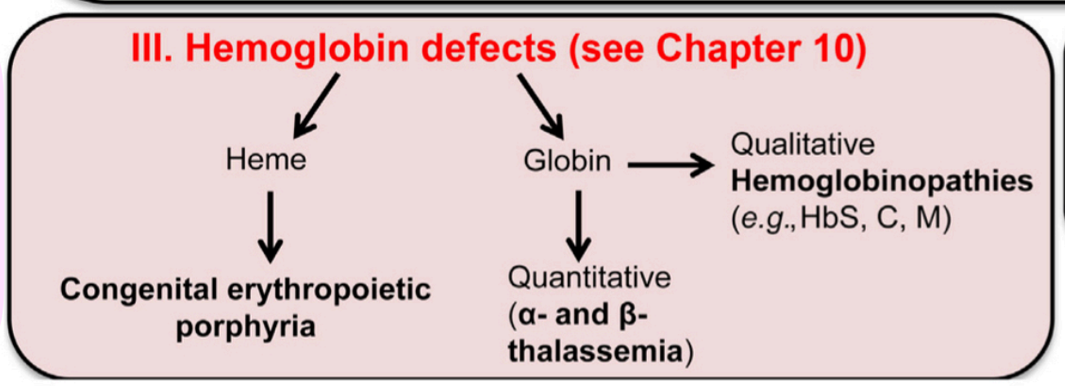
- PBS
- Flow for EMA
- OF test

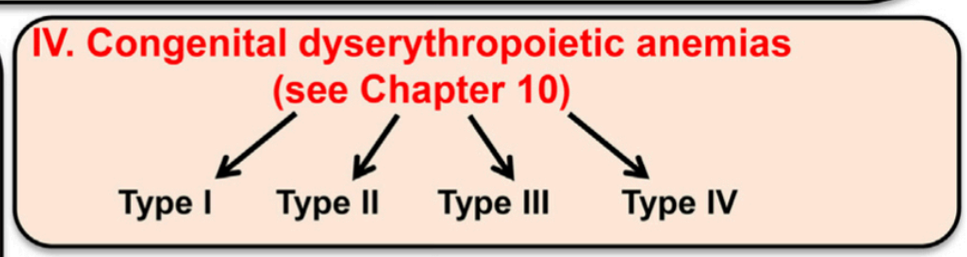


- G6PD level
- PK level



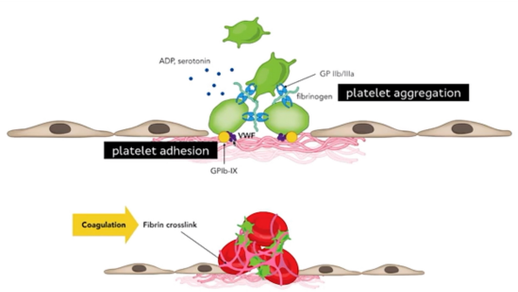
- PBS
- Hb typing
- Inclusion body

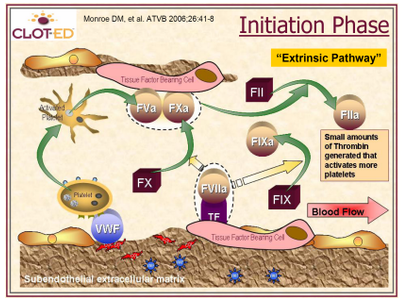


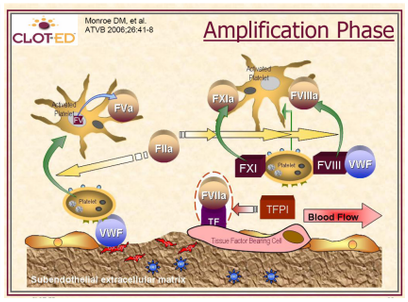


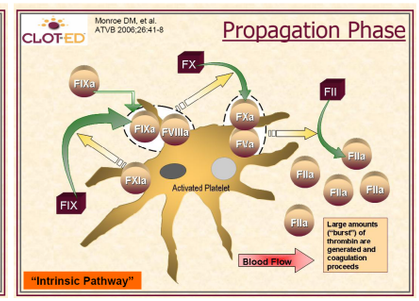
Ref : Lanzkowsky 6th edition

Normal Hemostasis









Vessel Injury

(1)

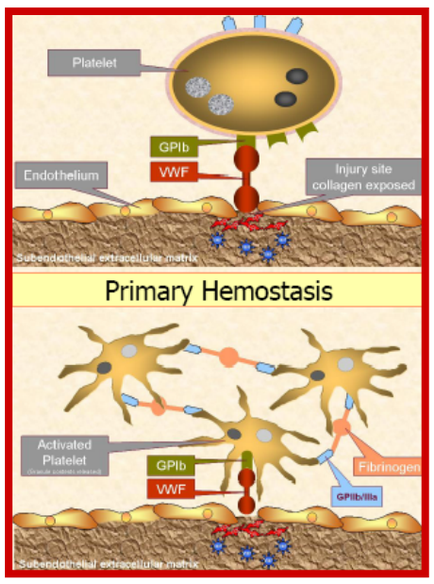
Adhesion

Platelet

Activation

Aggregation

Platelet plug



+

Fibrin plug

(2)

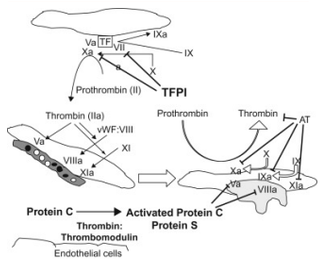
Coagulation Systems

Stabilize with **FXIII**

(3)

Inhibition of Coagulation Systems

TM,PC,PS, AT,TFPI



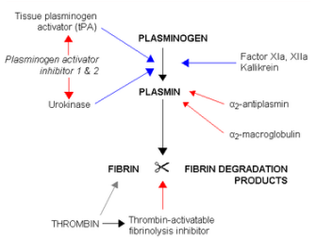
(4)

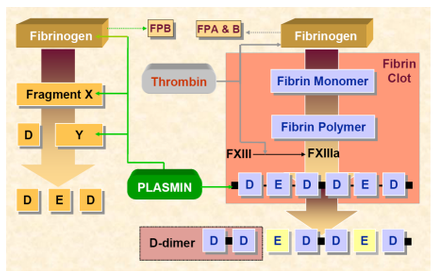
Fibrinolysis Pathway

(5)

Inhibition of Fibrinolysis Pathway

TAFI,PAI-1,2 a2AP,a2MG

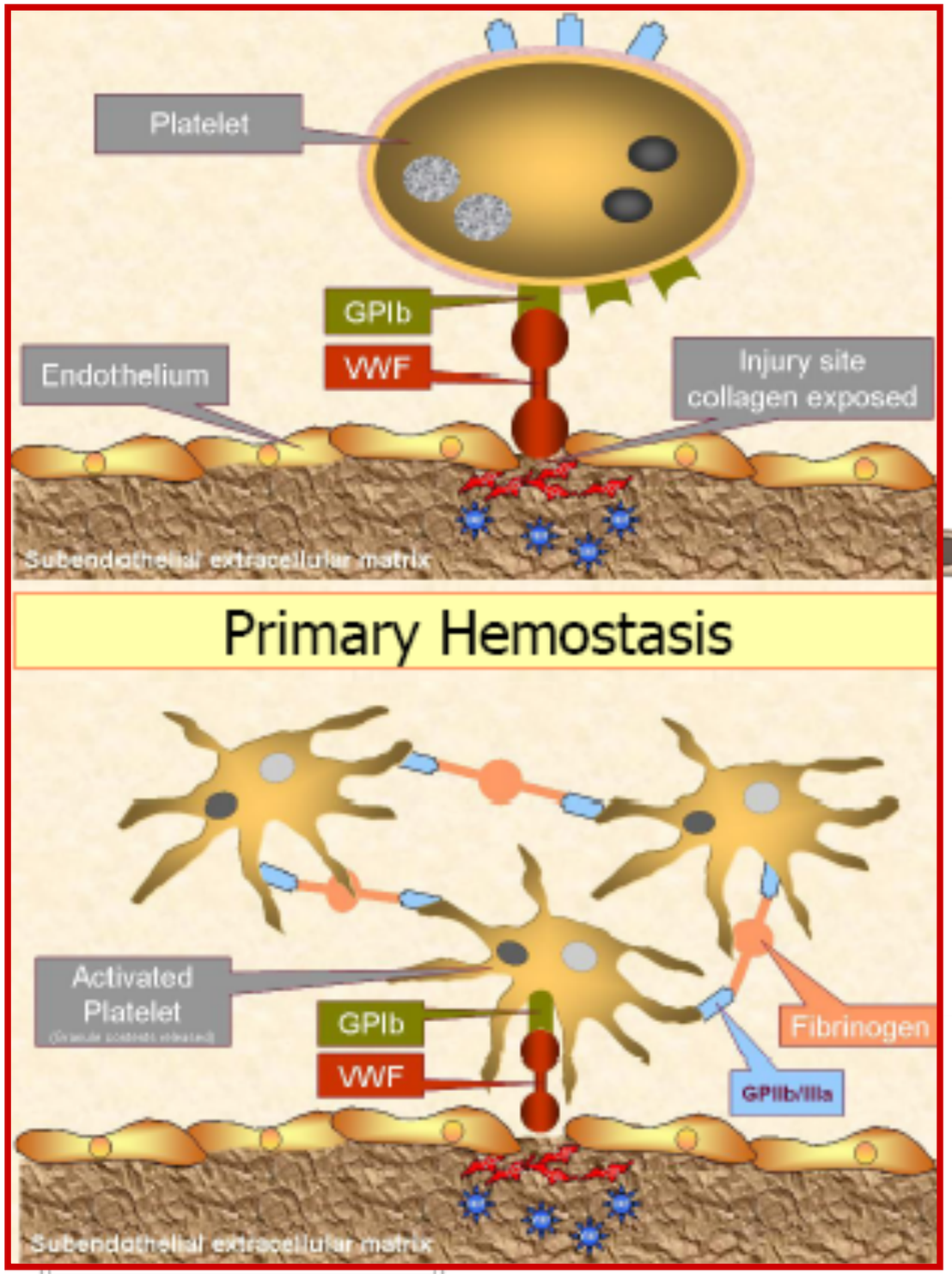


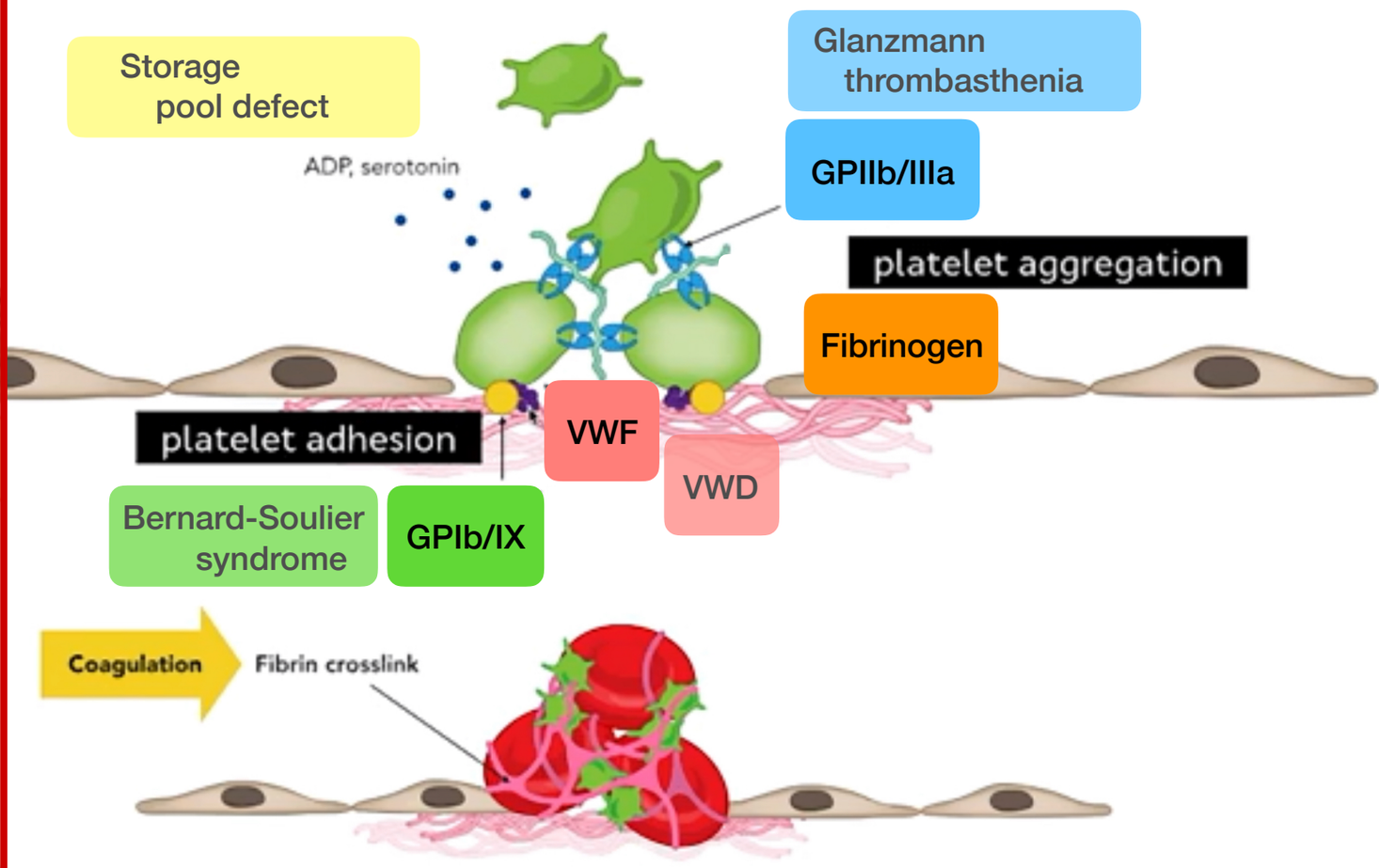


Fibrin degradation

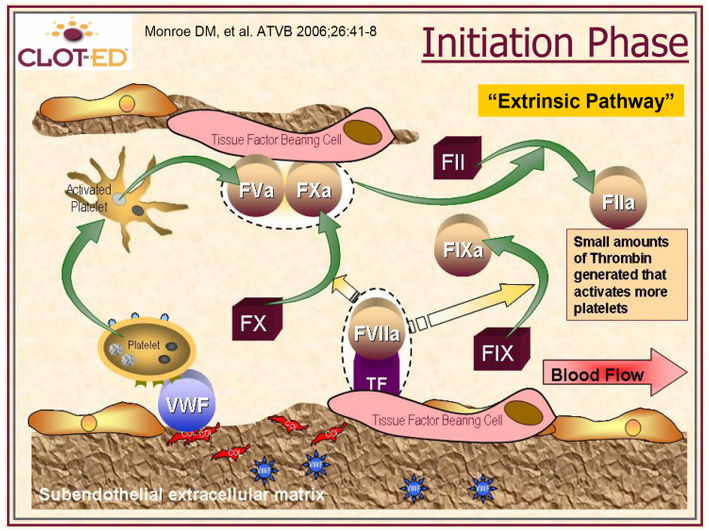
Pediatric Cancer & Hematologic Disorder
PedHemOnc-PMK

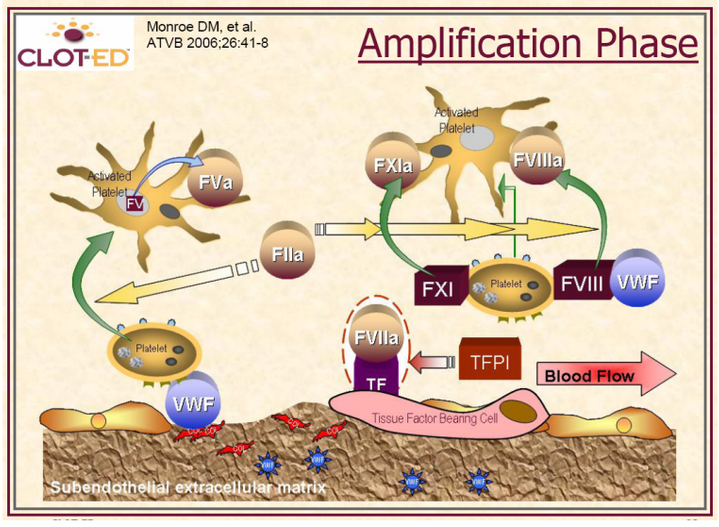
Primary Hemostasis

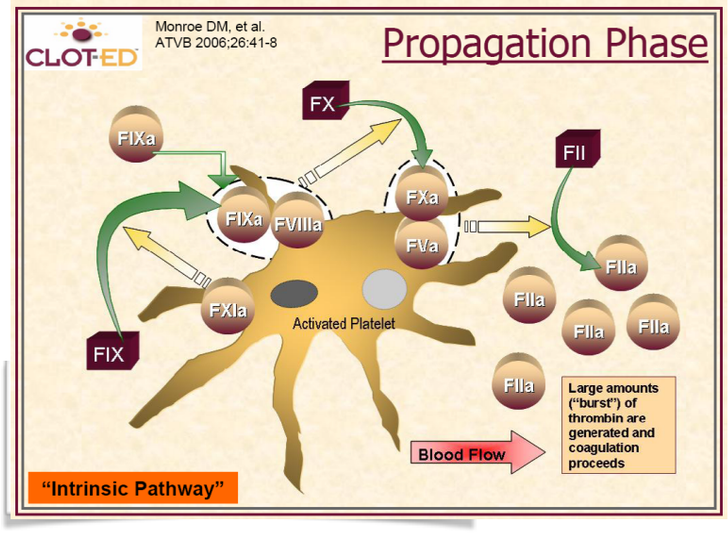




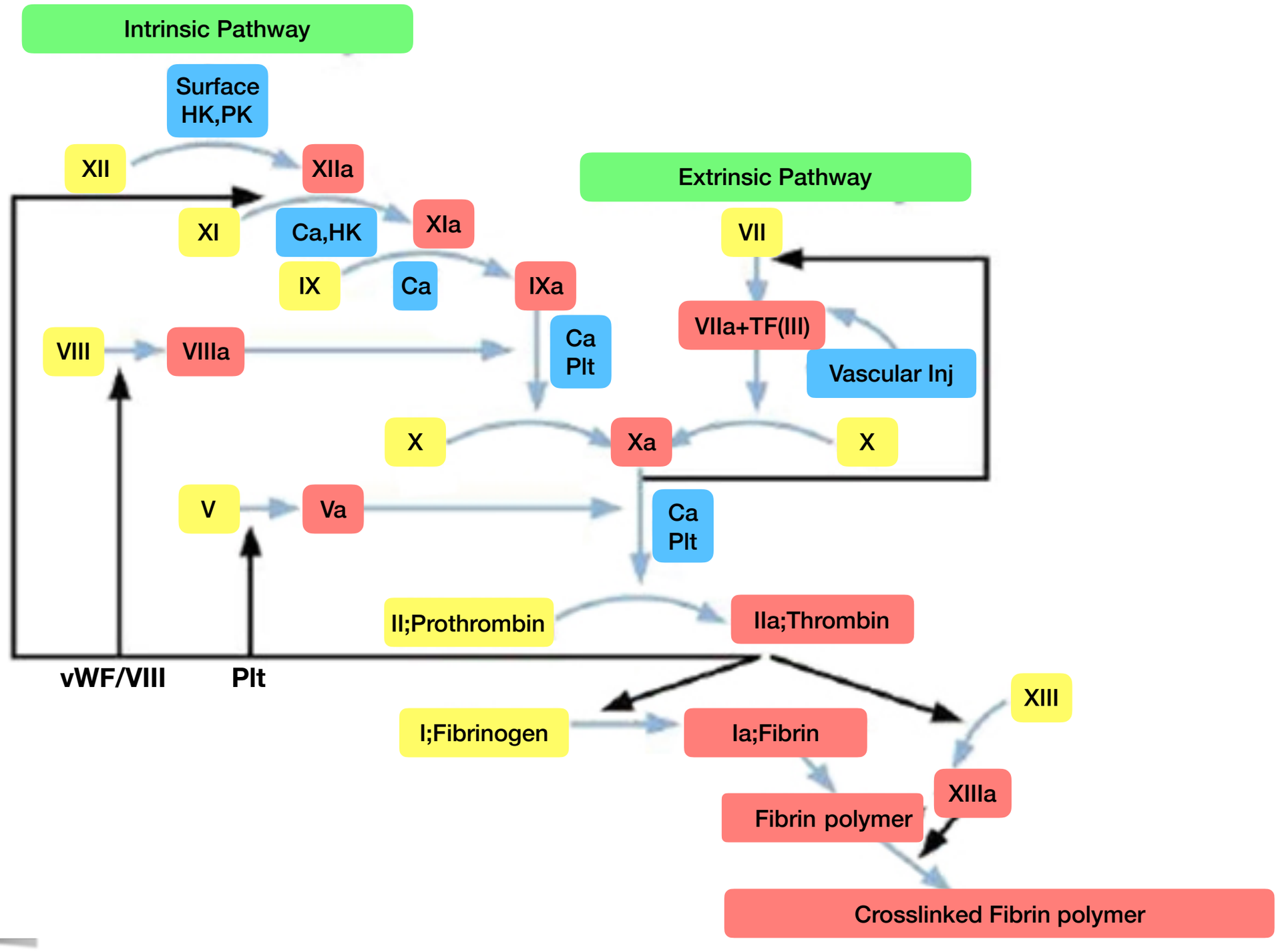
Secondary Hemostasis







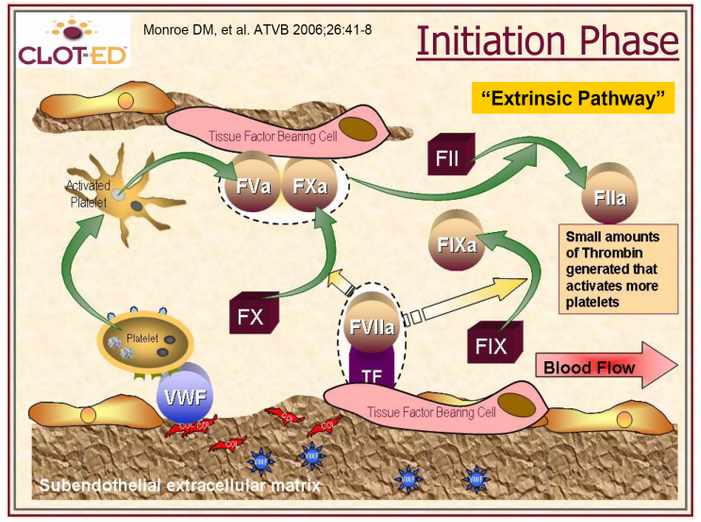
Water-fall Model of Coagulation



Cell-based Model : Summary

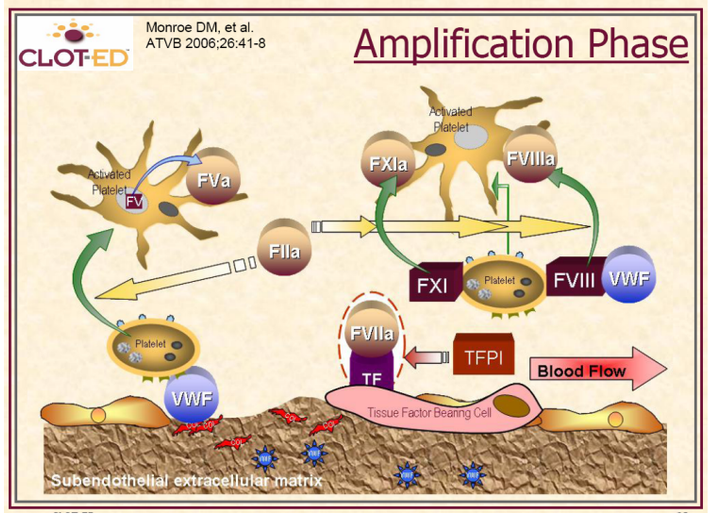
Initiation เกิดบนเซลล์

- เริ่มจาก TF + FVII
- Key: TF/FVIIa complex
- ผลที่ได้คือ thrombin จำนวนน้อย



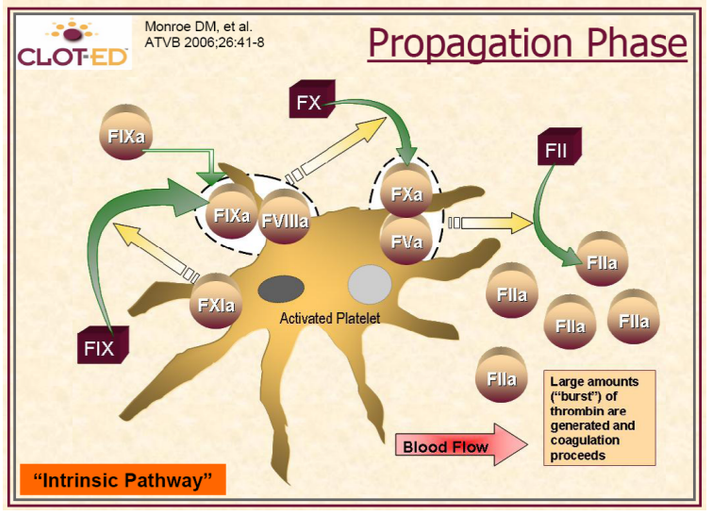
Amplification เกิดบน Plt

- เริ่มจาก FII, V, VIII, IX
- Key: VIIIa/IXa tenase complex
- ผลที่ได้คือ FVa, FXa



Propagation เกิดบน plt

- เริ่มจาก FV, FX
- Key: FXa/Va tenase complex
- ผลที่ได้คือ thrombin จำนวนมาก



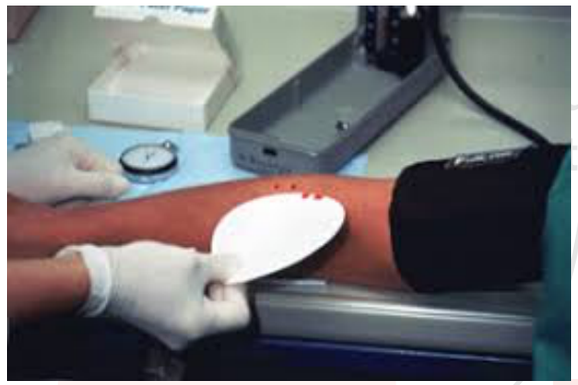
Lab in Hemostasis

Lab in Hemostasis

CBC+PBS

Platelet number and morphology

Bleeding time



Normal < 9 mins

Vessel Injury

Adhesion

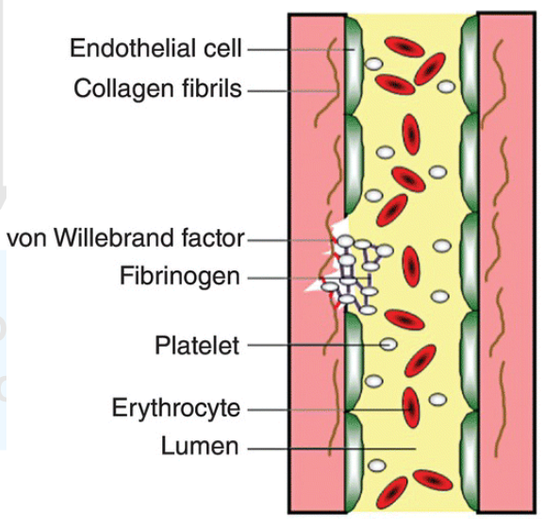
Platelet

PFA-100

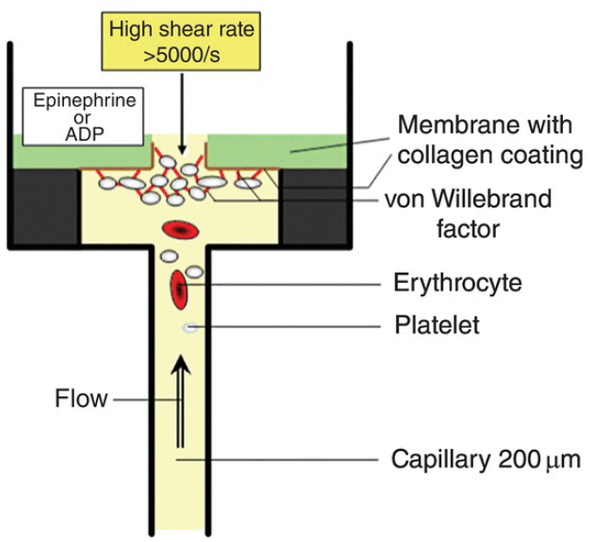
Membrane closure time
response to
ADP and Epinephrine

Required… Hct 25-50
Plt > 50k

In vivo haemostasis



PFA-100®



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Lab in Hemostasis

Platelet Aggregation Study

Vessel Injury

Adhesion (1) → Platelet Aggregation Study

Need to control the affect factor
Detect : Adhesion - Activation - Aggregation

Platelet —**Activation**→ **Aggregation** → **Platelet plug**

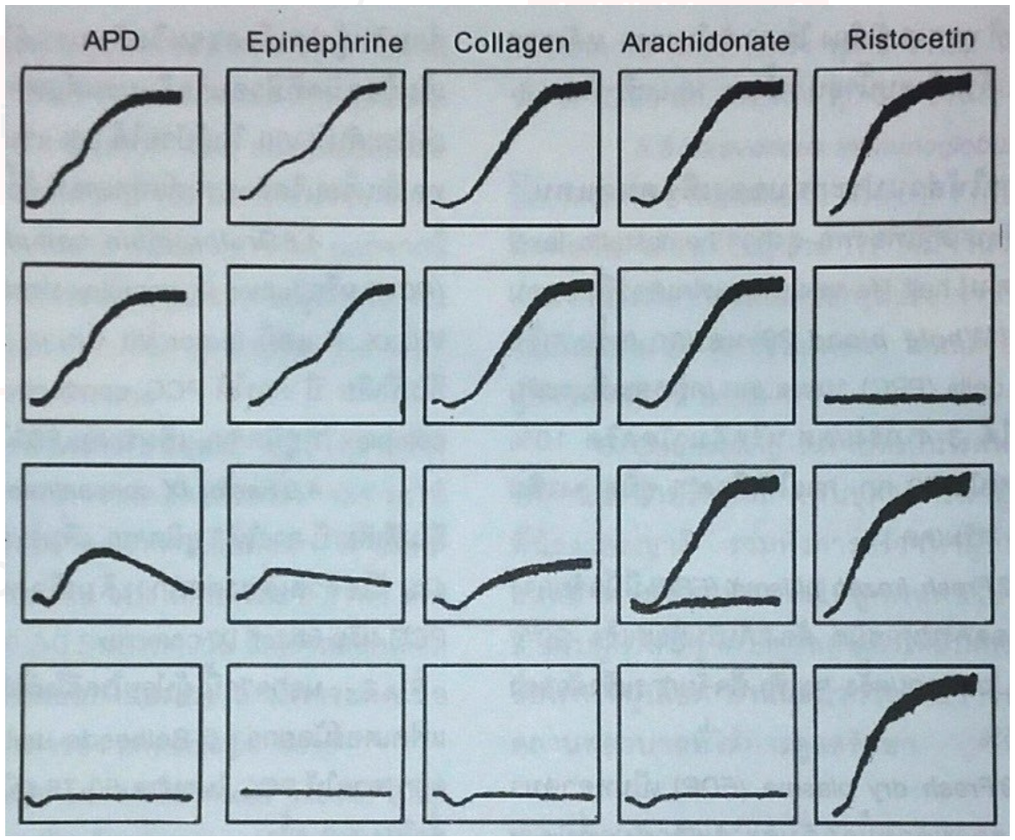
Von Willebrand factor study

Normal

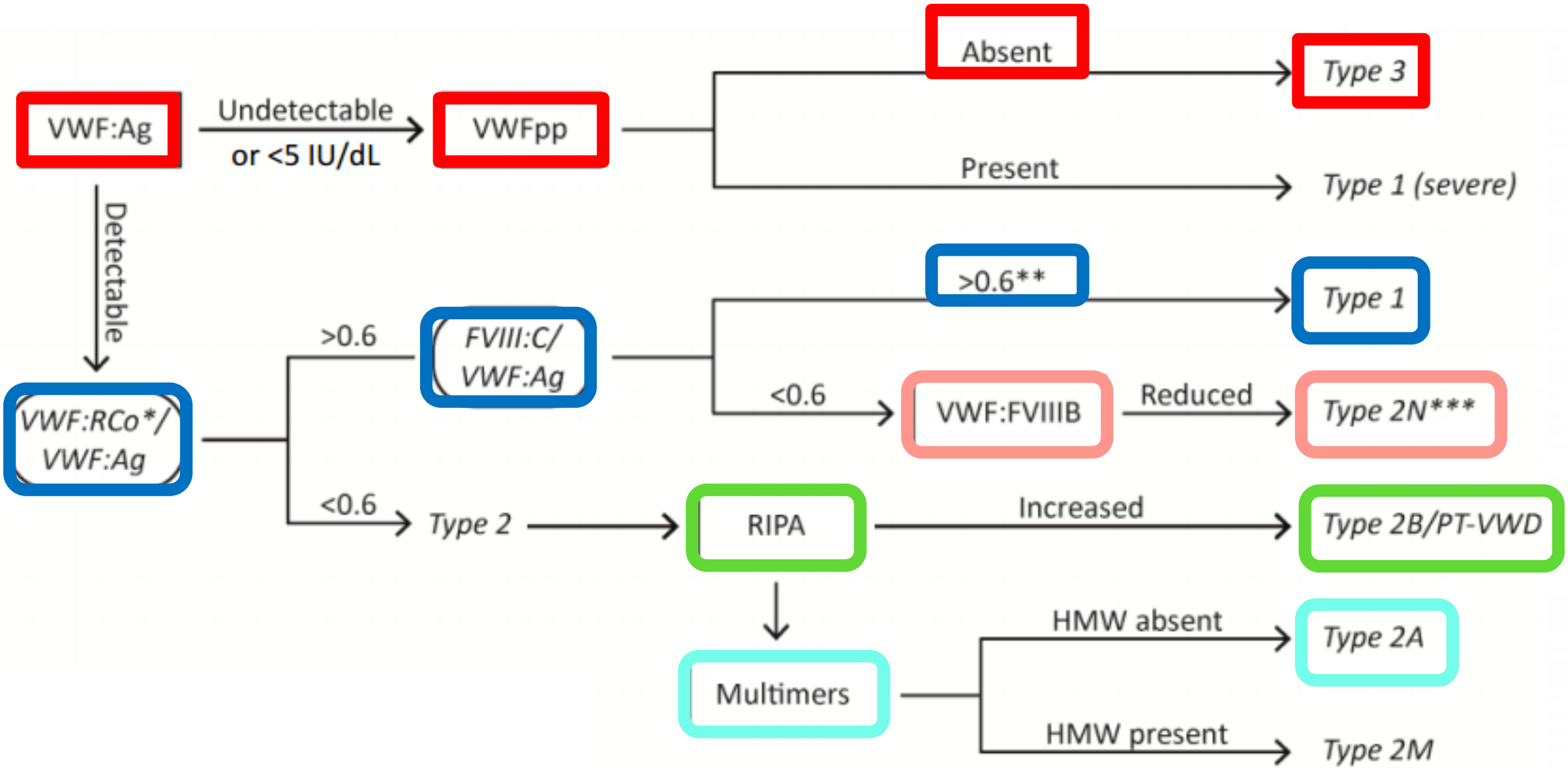
BSS, vWD

SPD

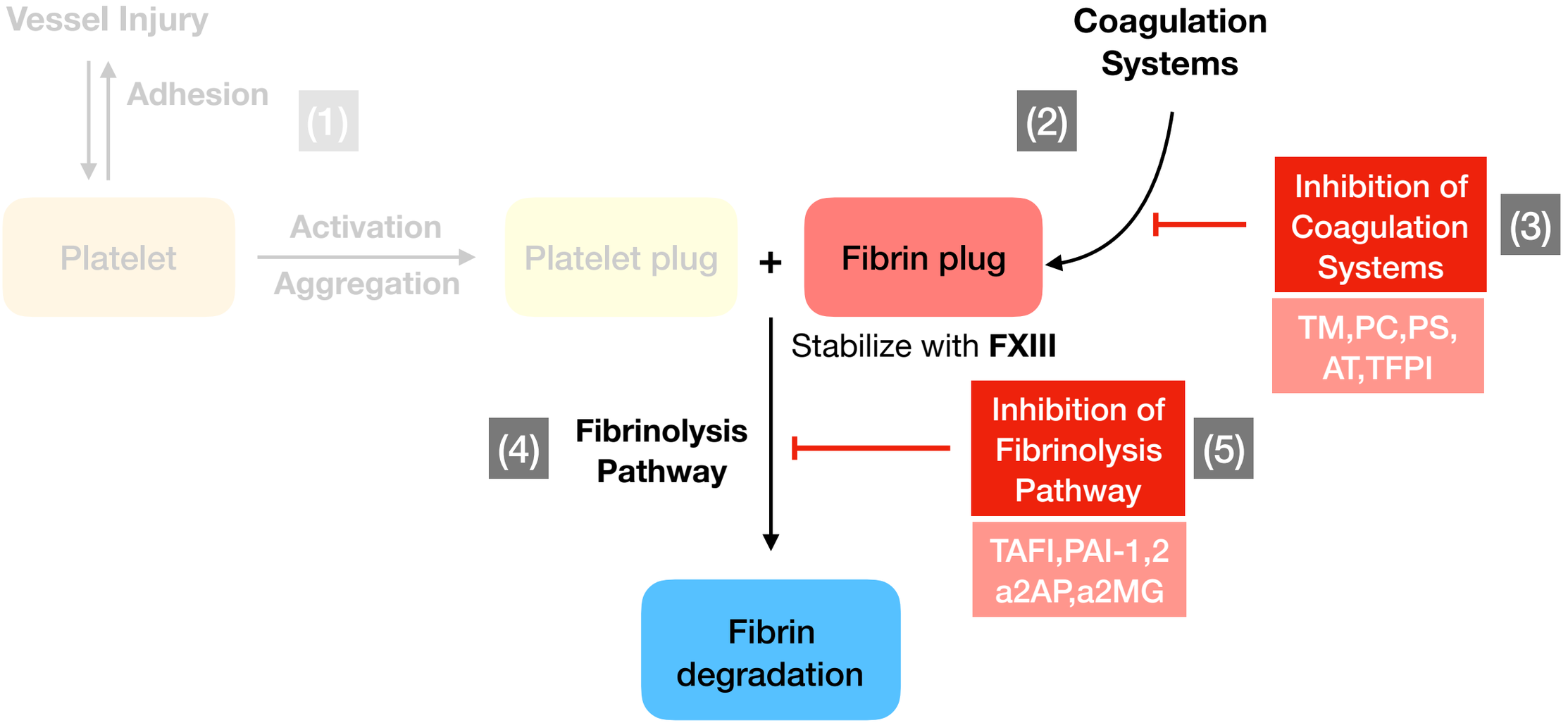
GT



von Willebrand Disease



Lab in Hemostasis



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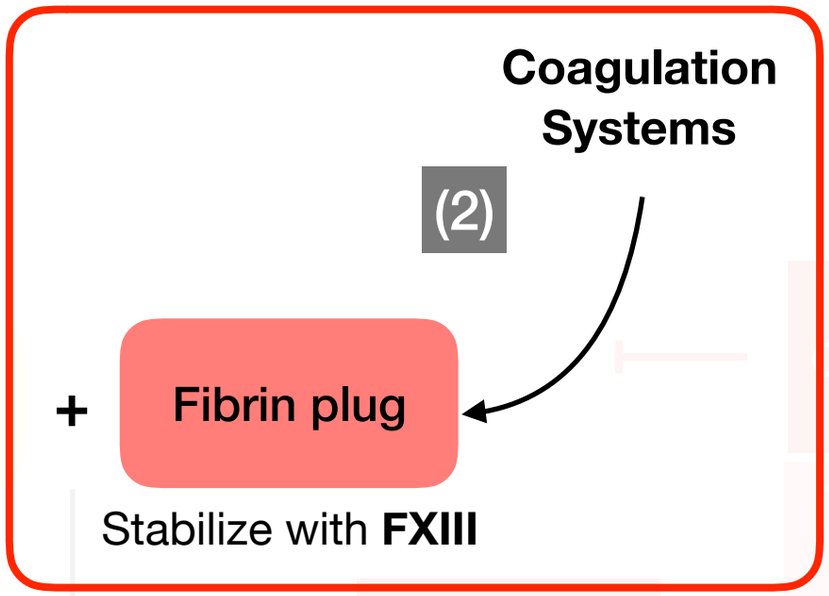
Lab in Hemostasis

activated Partial Thromboplastin time

Normal 25-35 sec
Detect : Intrinsic & Common pathway
Agent : **Kaolin** (ตัวกระตุ้น) + **PL** + **Plt-poor** plasma
ระวัง!!!
- **ส่งช้า 5,8 สลาย**
- **Hct เยอะ > 55, plasma น้อย factor ก็น้อย**

Mixing test

Detect : Factor def/ Inh/ APS
Agent : **Normal plasma**



Prothrombin Time

Normal 10-12 sec
Detect : Extrinsic & Common pathway
Agent : **TF** (ตัวกระตุ้น) + **PL** + **Plt-poor** plasma
ระวัง!!!
- **ส่งให้เร็ว, Hct > 55**

Clotting factor activity assays

Thrombin time

Normal 12-14 sec
Detect : Fibrinogen
Agent : **Thrombin** (ตัวกระตุ้น)
ระวัง!!!
- **Heparin contamination**

Reptilase time

Normal 15-20 sec
Detect : Fibrinogen
Agent : **Reptilase** (ตัวกระตุ้น)
ที่ไม่มีผลต่อ Antithrombin (AT)

Fibrinogen

Lab in Hemostasis

Euglobulin lysis time

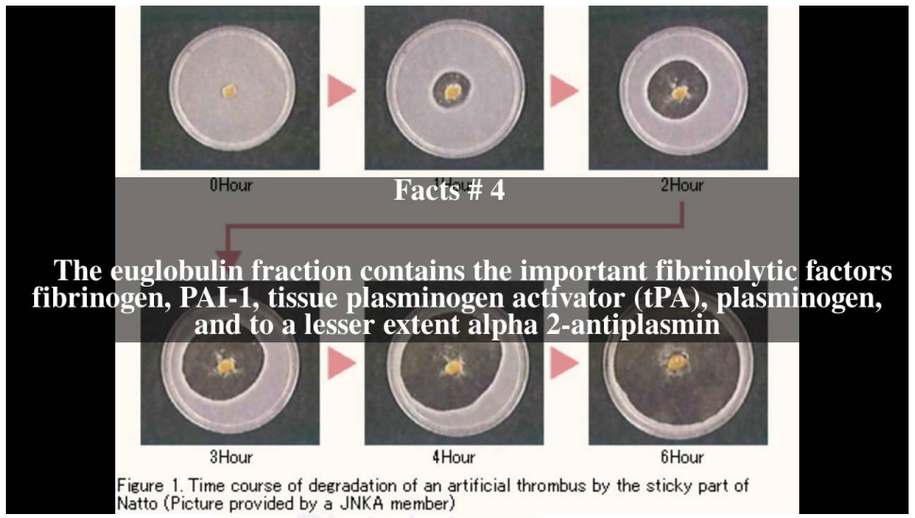
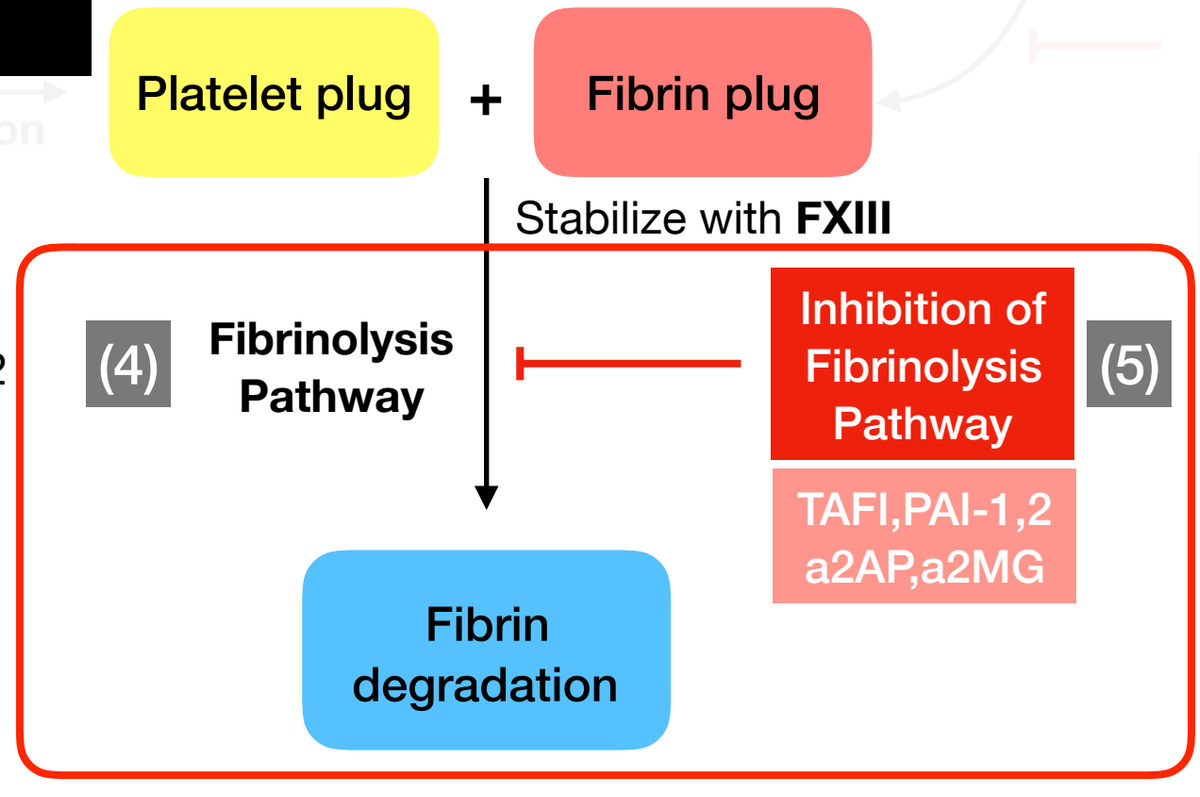


Figure 1. Time course of degradation of an artificial thrombus by the sticky part of Natto (Picture provided by a JNKA member)

Abnormal ถ้าเริ่มละลายก่อน 2 hr/
หรือ หมดก่อน 4 hr

Detect : H**yperfibrinolysis**

How : plasma เอา anti-plasmin ออก แล้วทำให้เป็น **Euglobulin clot** โดยเติม CaCl2 แล้วจับเวลาจนละลายหมด



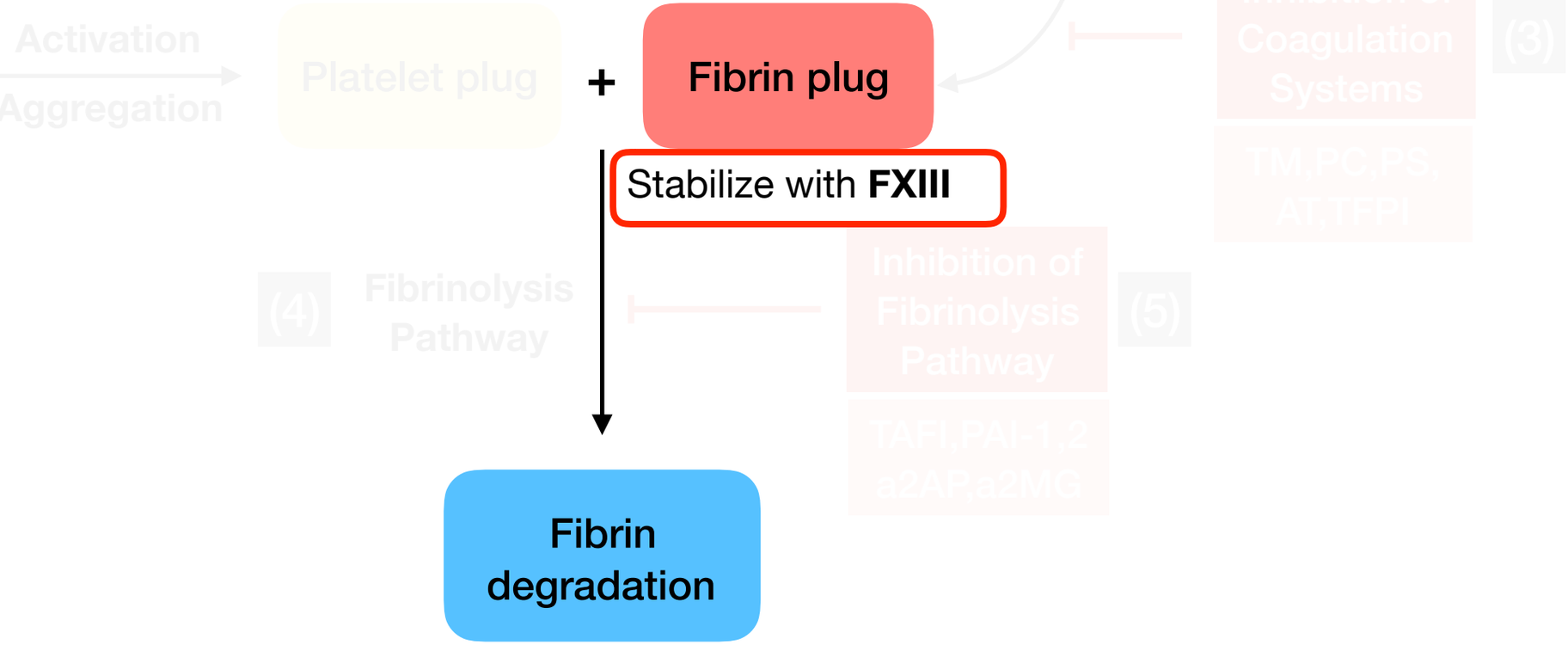
Lab in Hemostasis

Urea clot lysis assay /Urea solubility time

Detect : severe FXIII deficiency
How : Clot จะถูก degrade เมื่อ incubate 37 ด้วย 5M urea ถ้าไม่มี FXIII
ระวัง!!!
- **detect ได้แค่ severe FXIII def < 1%**

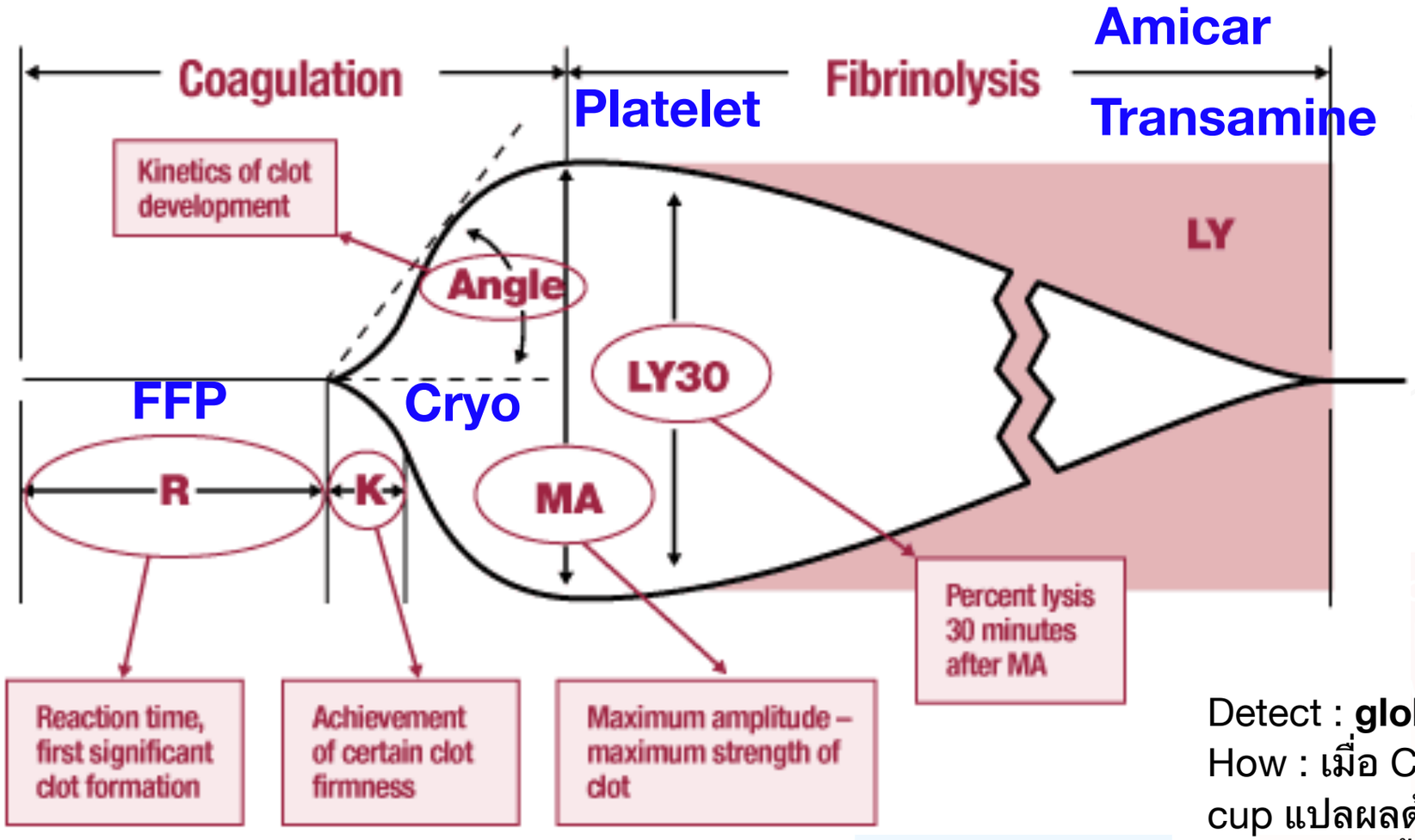
FXIII activity, antigen FXIII A and B subunit sequencing

Detect : confirm dx of FXIII deficiency



Lab in Hemostasis

Thromboelastography (TEG)



Detect : **global hemostatic test**

How : เมื่อ Clot จะเกิด torque ระหว่าง pin กับ cup แปลผลด้วย electronic เกิด graph

- บอกได้ตั้งแต่เริ่ม clot จนถึง fibrinolysis

Take home message

- Understanding lab in hematology based on the basic physiology
- RBC disorder : Membrane defect vs Enzymatic defect vs Hemoglobinopathy
- Primary hemostatic disorder : Platelet and vWF
- Secondary hemostatic disorder : Coagulopathy and Fibrin pathway