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- This speaker has nothing to disclose.

Laboratory Diagnosis of Von Willebrand Disease

Delu Zhou MD PhD
Pathology Resident
University of Utah

Objectives

After attending this seminar, the attendee are expected to be able to:

- Explain common symptoms and genetic causes of von Willebrand disease.
- Describe the common laboratory assays used to diagnose von Willebrand disease.
- List common subtypes of von Willebrand disease.

Topics

1. Introduction of von Willebrand factor and von Willebrand disease

2. Common diagnostic tests for von Willebrand disease and interpretation

3. Case studies

Topics

1. *Introduction of von Willebrand factor and von Willebrand disease*

2. Common diagnostic tests for von Willebrand disease and interpretation

3. Case studies

Von Willebrand factor (vWF)

vWF is synthesized in endothelial cells as a monomer that is subsequently made into multimers that are secreted.

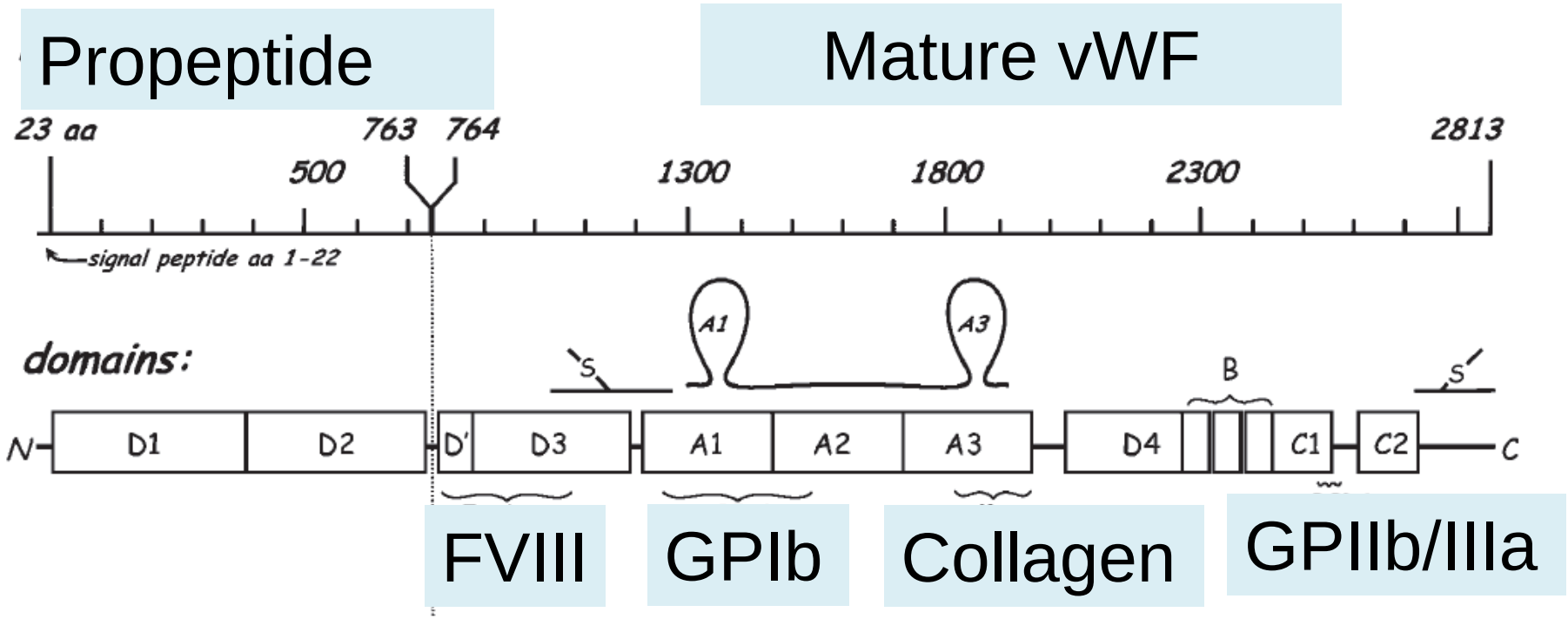
vWF is a critical protein in blood clotting

Von Willebrand factor (vWF)

The majority of vWF is circulating in the blood plasma.

A pool of vWF is also stored in the endothelial cells and megakaryocytes, the precursors of platelets.

Von Willebrand factor (vWF)



FVIII: Factor VIII

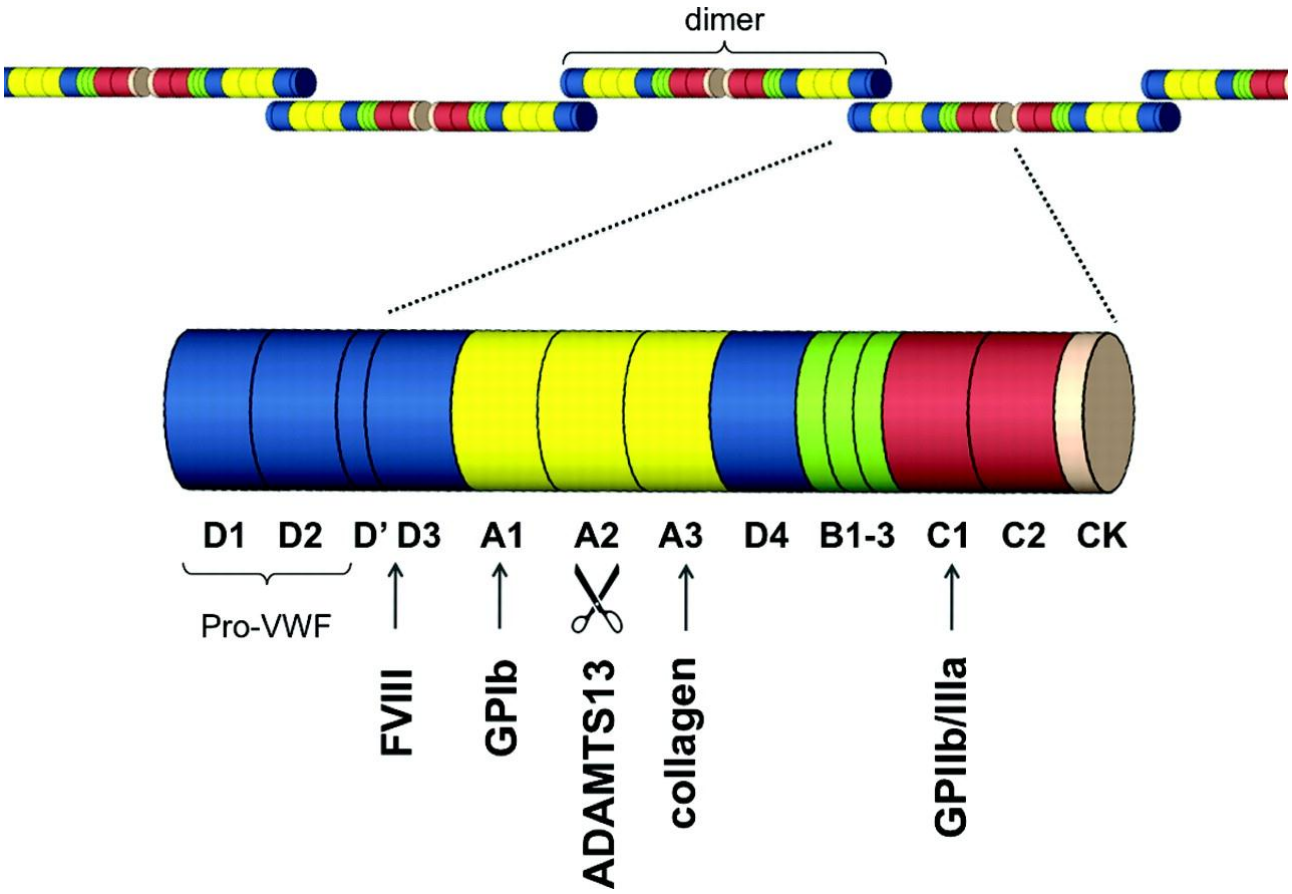
GPIb: platelet glycoprotein Ib

GPIIb/IIIa: platelet glycoprotein IIb/IIIa

vWD Guidelines, NHLBI

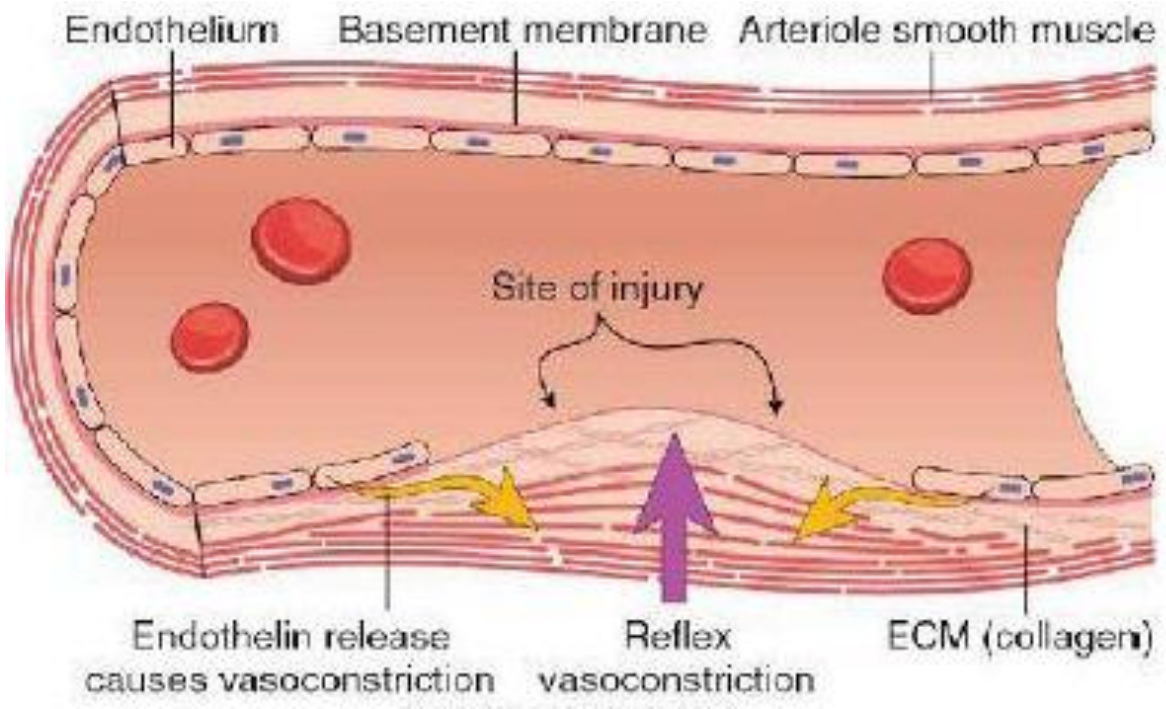
Von Willebrand factor (vWF)

vWF is a multimeric protein composed of dimeric building blocks.



De Meyer S F et al. Stroke 2012;43:599-606

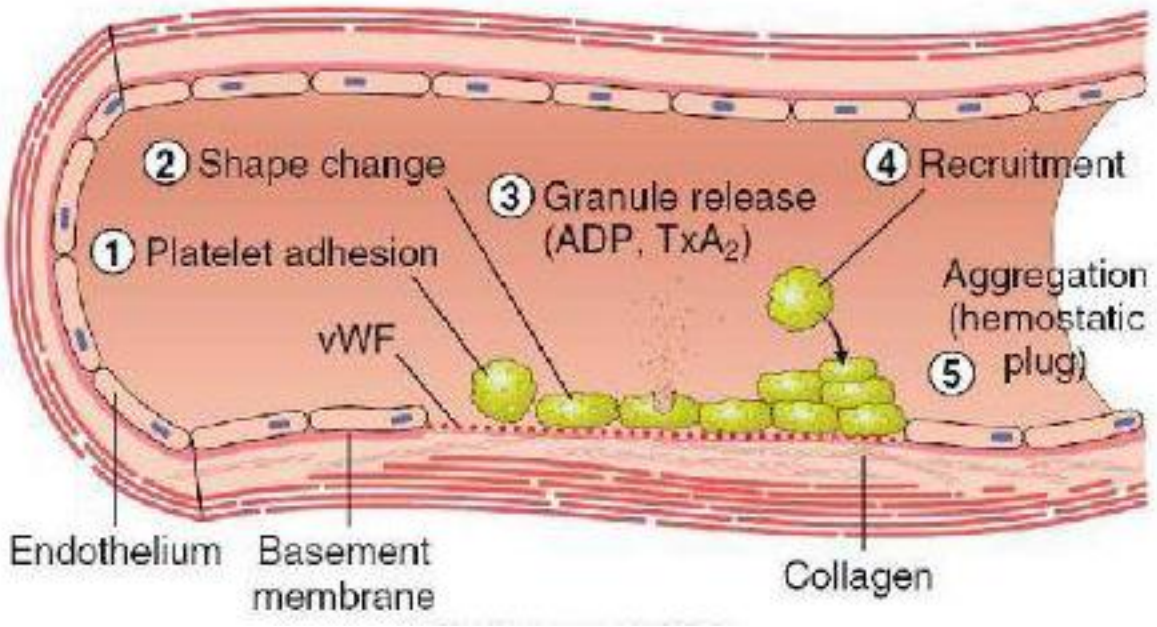
Body Reactions to Bleeding



1. Constriction of blood vessels

Robbins and Cotran Pathologic Basis of Disease 8th edition

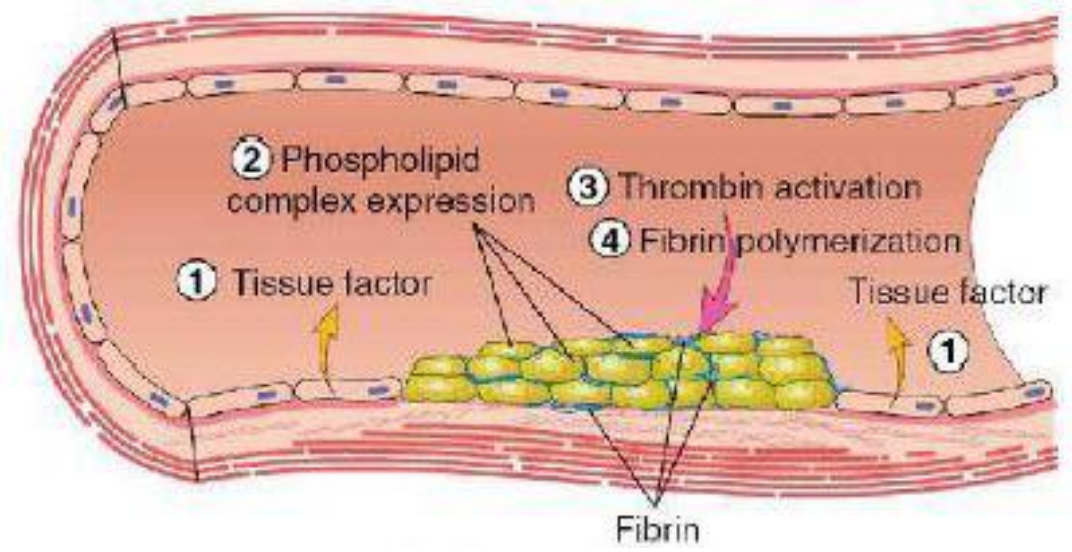
Body Reactions to Bleeding



2. Adhesion of platelets

Robbins and Cotran Pathologic Basis of Disease 8th edition

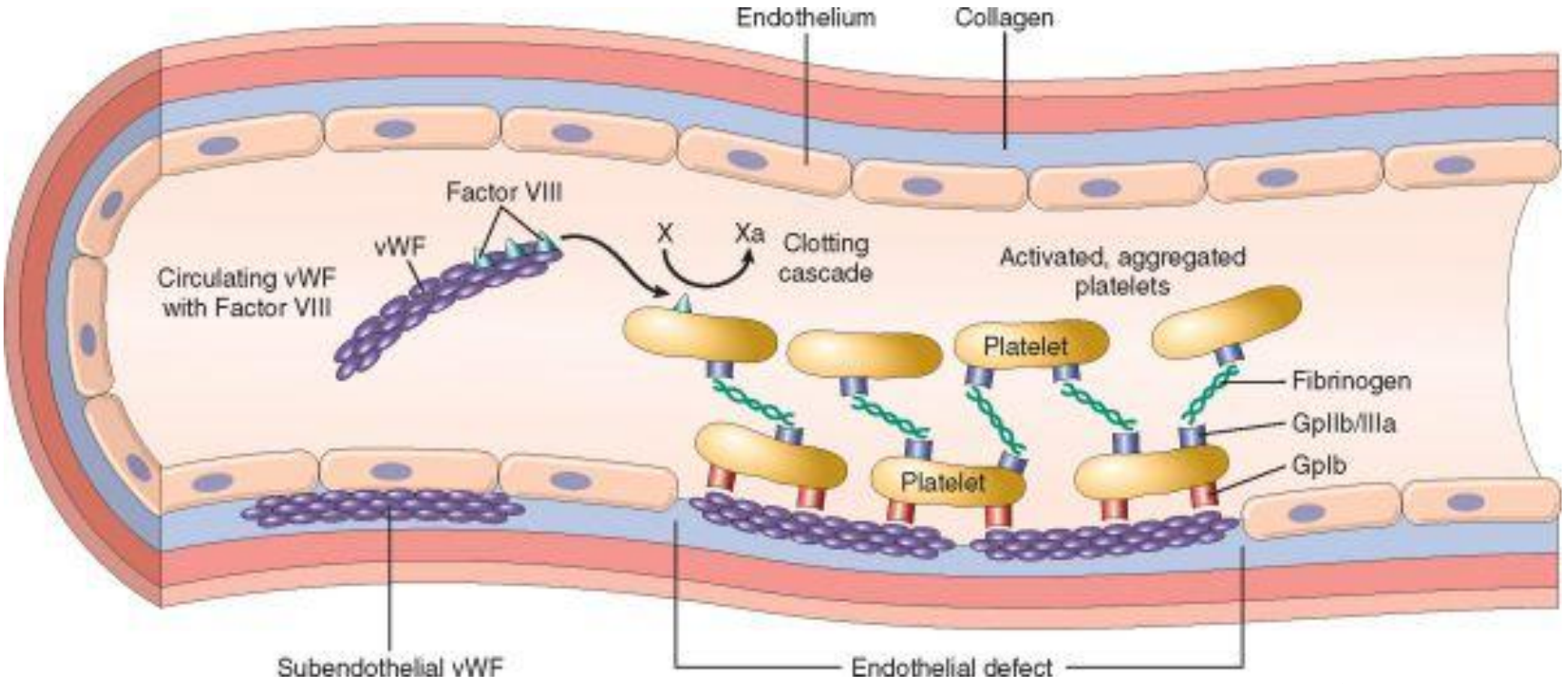
Body Reactions to Bleeding



3. Formation of fibrin reinforces platelets

Robbins and Cotran Pathologic Basis of Disease 8th edition

vWF Plays Two Major Roles



vWF tethers the platelet to exposed collagen

vWF serves as a carrier protein for factor VIII

Kumar: Robbins Basic Pathology, 9th ed.

Von Willebrand disease (vWD)

Von Willebrand disease (vWD) was first described in 1926 by a Finnish physician named Dr. Erik von Willebrand.

Quantitative deficiency of vWF or to functional deficiencies of vWF

Autosomal inheritance pattern / Males and females are affected equally

Von Willebrand disease (vWD)

Dr. Erik von Willebrand

The first manuscript describing a haemorrhagic disorder in people who were living on the Aland islands off the coast of Finland.

Von Willebrand disease (vWD)

His first case was a little girl, who was five years old when first examined.

She was one of 12 siblings, all but two of whom had had bleeding symptoms.

Her parents had severe nose bleedings.

The girl herself had had several severe episodes of bleeding from the nose and lips, and following tooth extractions. At the age of 13, she bled to death during her fourth menstrual period.

The history of von Willebrand disease. Haemophilia. Volume 5 (s2), p7–11, May 1999

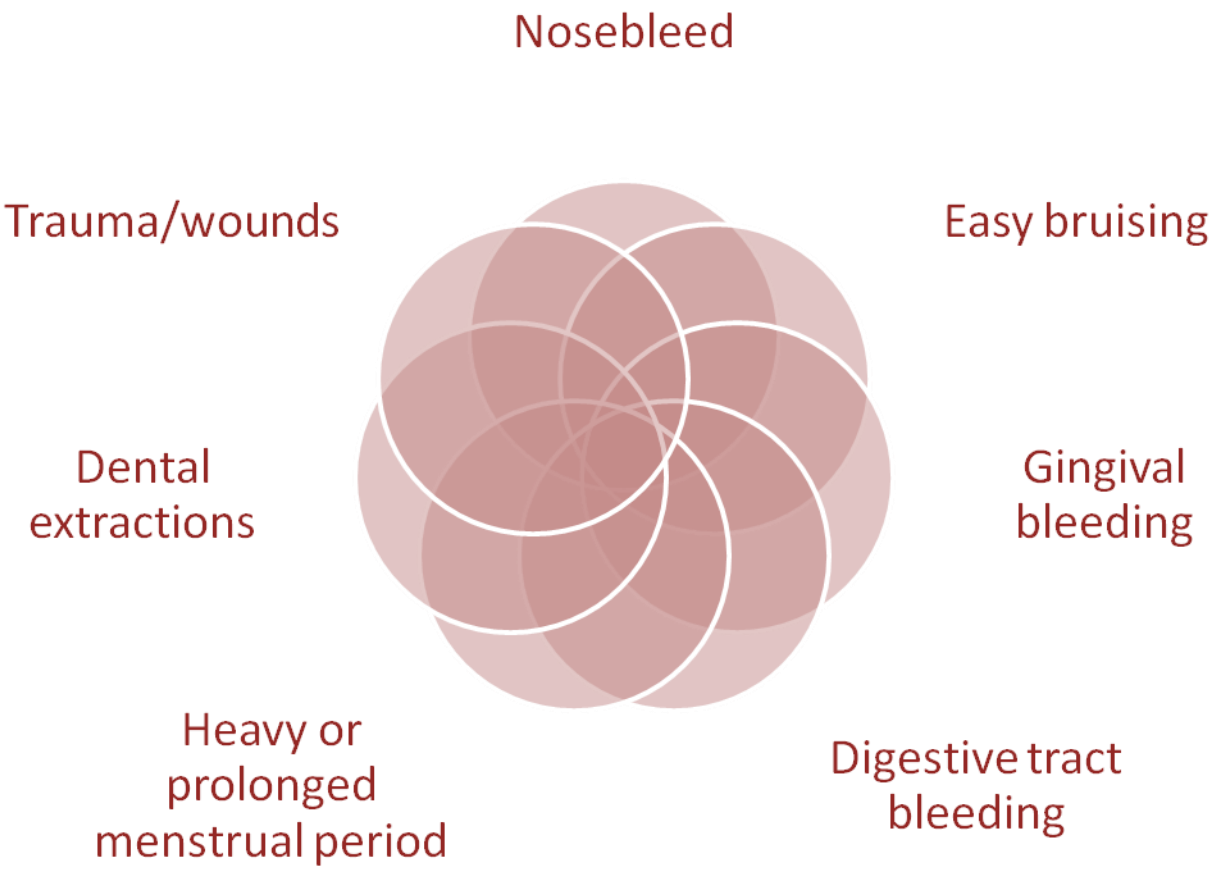
Von Willebrand disease (vWD)

Most frequent inherited bleeding disorder

Estimated prevalence of 1% in general population

Clinically significant vWD: 100 persons per million population

Clinical Manifestations



vWD Classification

Type 1: **Partial quantitative** deficiency of vWF
Mild-moderate disease
70% of cases

Type 2: **Qualitative** deficiency of vWF
Mild to moderate disease
25% of cases

Type 3: Total or near **total quantitative** deficiency of vWF
Severe disease
5% of cases

vWD Classification

Type 1: **Partial quantitative** deficiency of vWF
Mild-moderate disease
70%

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Mild to moderate disease
25%

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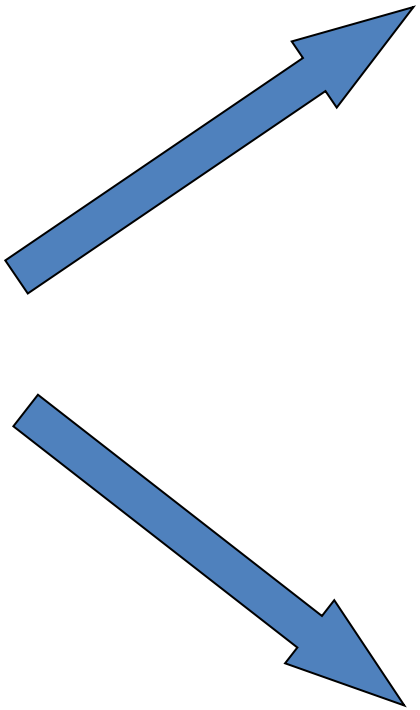
5%

vWD Classification



Type 2A ↓vWF-dependent platelet adhesion with selective deficiency of high molecular weight vWF multimers

Type 2B Increased vWF affinity for platelet GPIb; ± ↓platelet numbers

Type 2M ↓vWF-dependent platelet adhesion without selective deficiency of high molecular weight vWF multimers

Type 2N Markedly decreased vWF binding affinity for FVIII

Topics

1. *Introduction of von Willebrand factor and von Willebrand disease*

2. *Common diagnostic tests for von Willebrand disease and interpretation*

3. Case studies

Initial vWD testing

- von Willebrand Factor Antigen
- von Willebrand Factor Activity (Ristocetin Cofactor)
- Factor VIII Activity

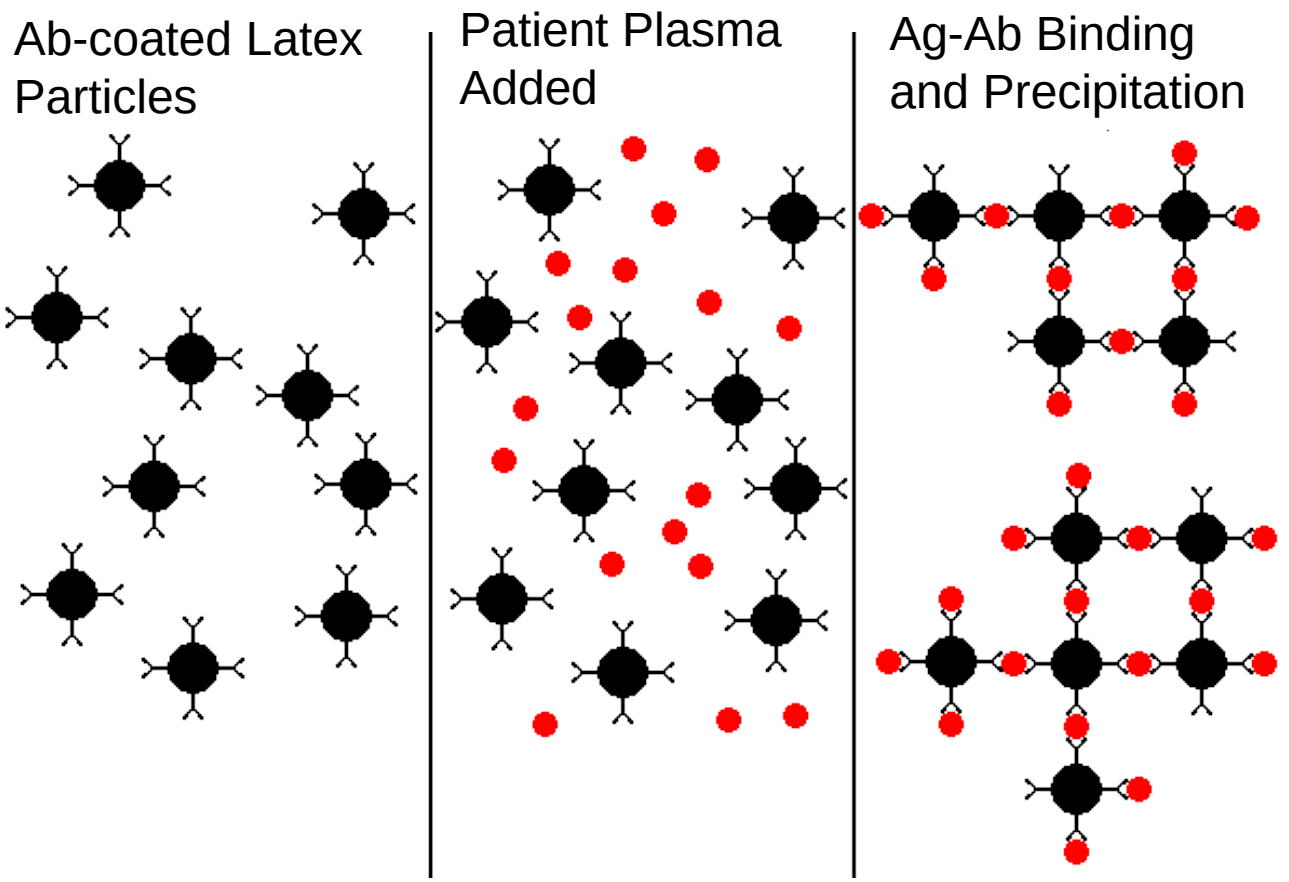
von Willebrand Factor Antigen

vWF:Ag

Immunological assay that measures the concentration of the vWF protein in plasma.

Methodology: Microlatex Particle-Mediated Immunoassay

Principle of Latex Immunoassay



Turbidity ↑

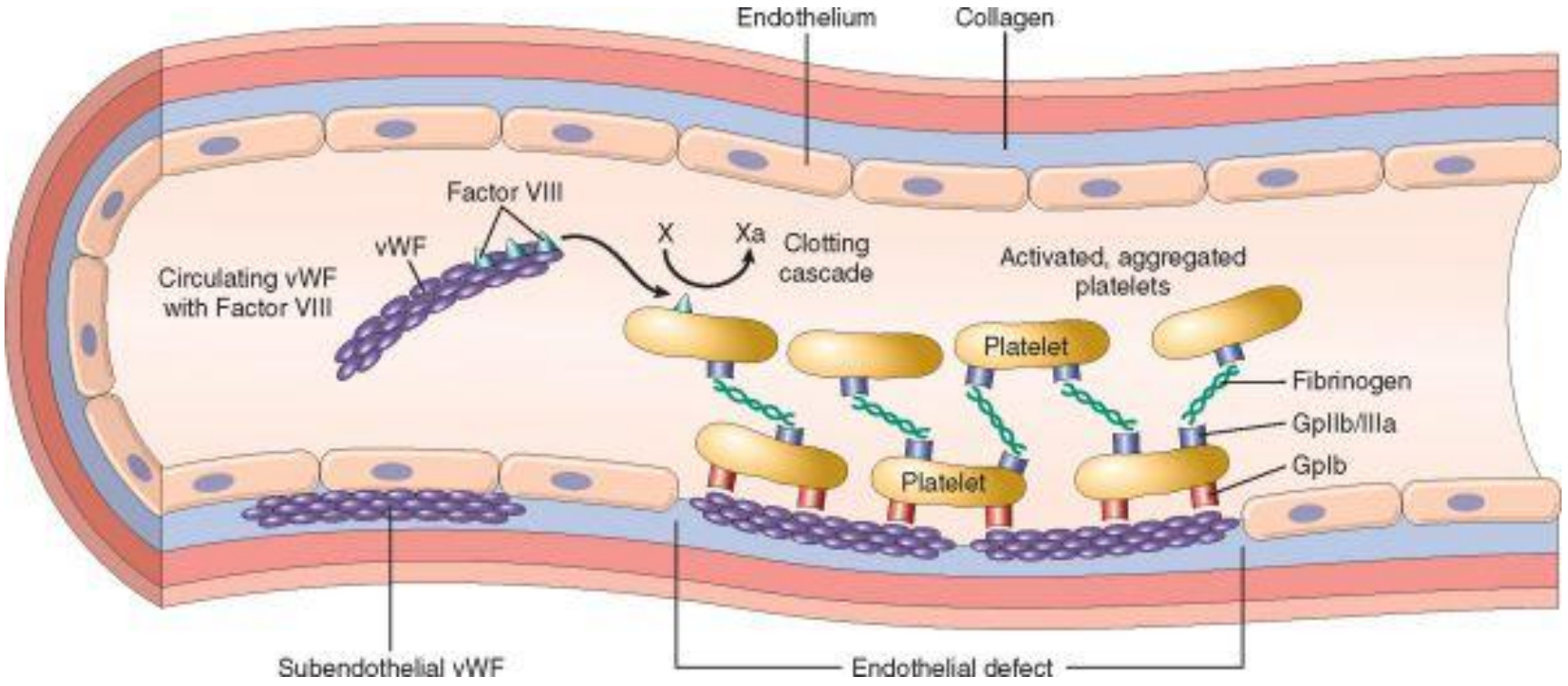
Absorbance ↑

http://tiger.kobiljak.msu.edu/WebSites/Web_Path/webpath/microbio/microbe/serology.htm

von Willebrand Factor Activity (Ristocetin Cofactor)

- vWF:RCo
- Measures the ability of a patient's plasma to agglutinate platelets in the presence of the antibiotic **Ristocetin**.
- Methodology: Platelet Agglutination

von Willebrand Factor Activity (Ristocetin Cofactor)

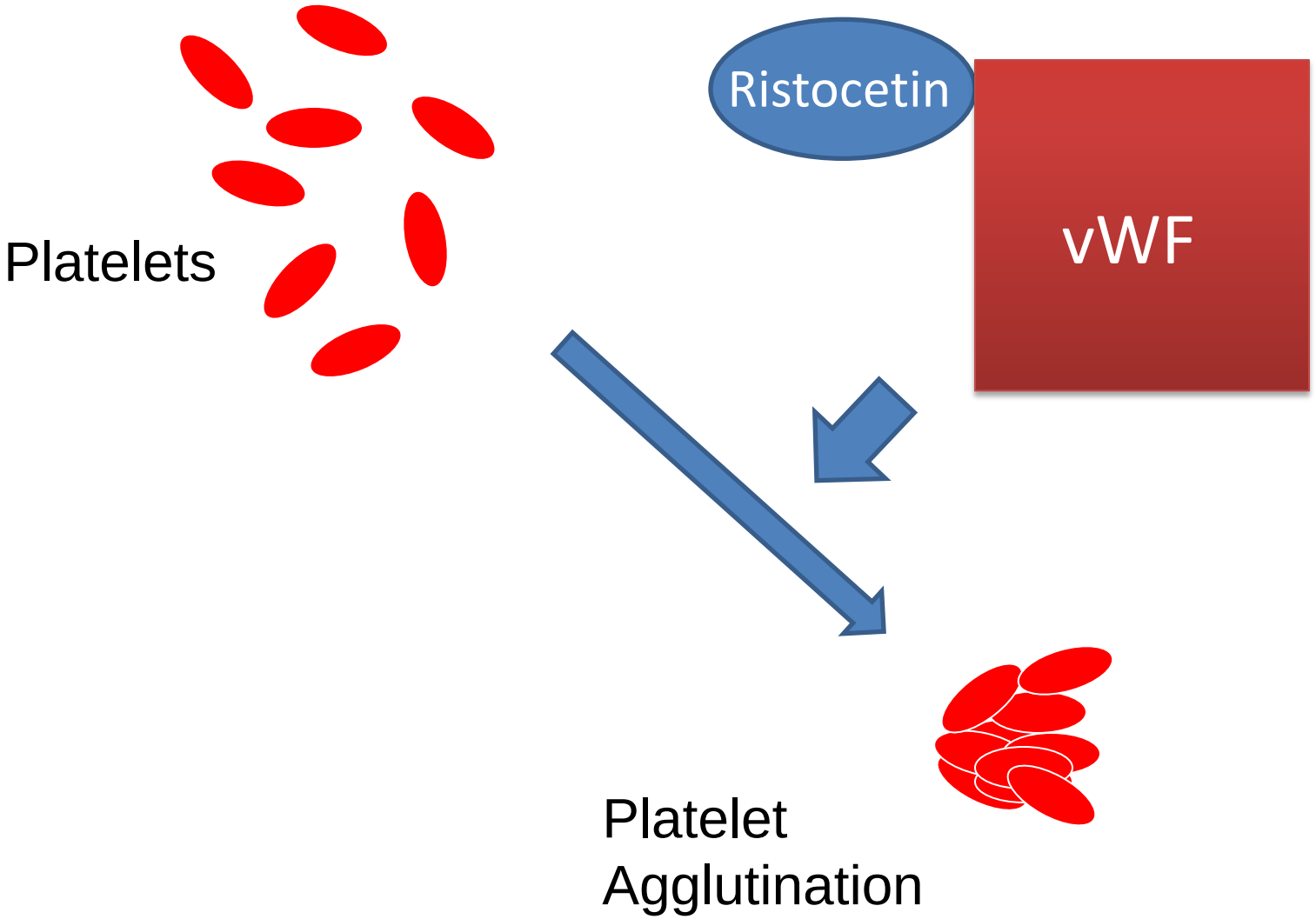


Kumar: Robbins Basic Pathology, 9th ed.

von Willebrand Factor Activity (Ristocetin Cofactor)

- Ristocetin is an antibiotic
- Side effect: activates vWF and induces platelet agglutination and cause thrombocytopaenia
- Removed from the market

Principles of von Willebrand Factor Activity Assay



Principles of von Willebrand Factor Activity Assay

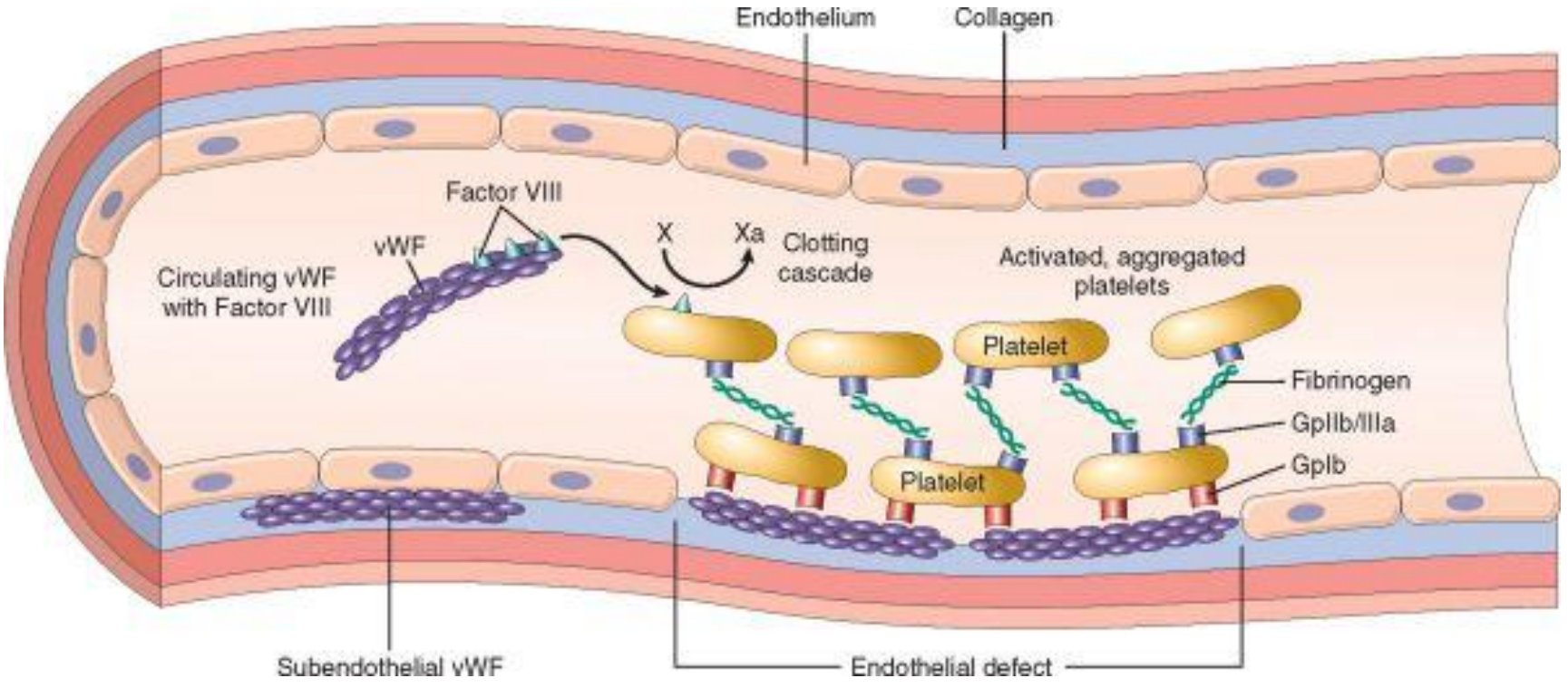
In quantitative vWF deficiency (types 1 and 3), it parallels the vWF antigen

In qualitative vWF deficiency resulting in decreased affinity for platelets (types 2A and 2M), vWF RCo is more severely affected compared to vWF antigen.

Principles of von Willebrand Factor Activity Assay

However this assay is not a true 'functional' assay but rather the interaction of vWF with the Gp1b platelet receptor in the presence of ristocetin.

Factor VIII Activity



Measures the activity of factor VIII

Functional clot-based assay

Result Interpretation

Type 1: **Partial quantitative** deficiency of vWF

Condition	vWF:RCo (%)	vWF:Ag (%)	FVIII (%)	Ratio of vWF:RCo /vWF:Ag
Normal	51-215	52-214	56-191	>0.5-0.7
Type 1	<30	<30	↓ or Normal	>0.5-0.7

Result Interpretation

Type 3: **Total quantitative** deficiency of vWF

Condition	vWF:RCo (%)	vWF:Ag (%)	FVIII (%)	Ratio of vWF:RCo /vWF:Ag
Normal	51-215	52-214	56-191	>0.5-0.7
Type 3	<10	<10	↓ ↓ ↓	N/A

Result Interpretation

Type 2A: ↓vWF-dependent platelet adhesion with selective deficiency of high molecular weight vWF multimers

Condition	vWF:RCo (%)	vWF:Ag (%)	FVIII (%)	Ratio of vWF:RCo /vWF:Ag
Normal	51-215	52-214	56-191	>0.5-0.7
Type 2A	<30	30-200	↓ or Normal	<0.5-0.7

Result Interpretation

Type 2B: Increased vWF affinity for platelet GPIb; ± ↓platelet numbers

Condition	vWF:RCo (%)	vWF:Ag (%)	FVIII (%)	Ratio of vWF:RCo /vWF:Ag
Normal	51-215	52-214	56-191	>0.5-0.7
Type 2B	<30	30-200	↓ or Normal	Usually <0.5-0.7

Ristocetin-Induced Platelet Aggregation (RIPA)

Type 2B: Increased platelet aggregation at low dose of ristocetin

Result Interpretation

Type 2M: ↓vWF-dependent platelet adhesion without selective deficiency of high molecular weight vWF multimers

Condition	vWF:RCo (%)	vWF:Ag (%)	FVIII (%)	Ratio of vWF:RCo /vWF:Ag
Normal	51-215	52-214	56-191	>0.5-0.7
Type 2M	<30	30-200	↓ or Normal	<0.5-0.7

Result Interpretation

Type 2N: Markedly decreased vWF binding affinity for FVIII

Condition	vWF:RCo (%)	vWF:Ag (%)	FVIII (%)	Ratio of vWF:RCo /vWF:Ag
Normal	51-215	52-214	56-191	>0.5-0.7
Type 2N	30-200	30-200	↓ ↓	>0.5-0.7

vWF:FVIII binding (vWF:FVIIIB) assay

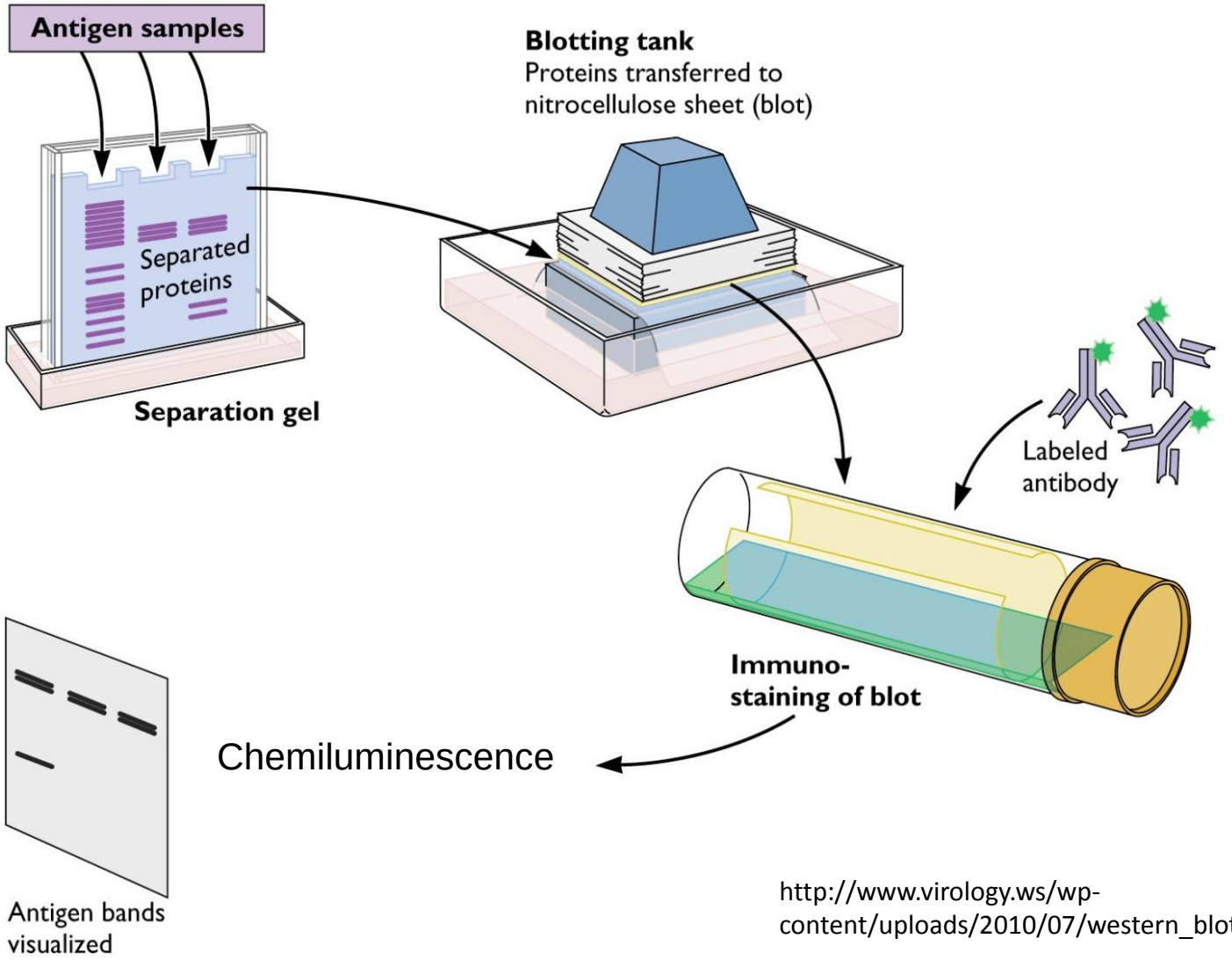
von Willebrand Factor Multimers

vWF monomer is about 250 kD

vWF is released from endothelial cells to the plasma as a *multimers* ranging from 500-20,000 *kD*

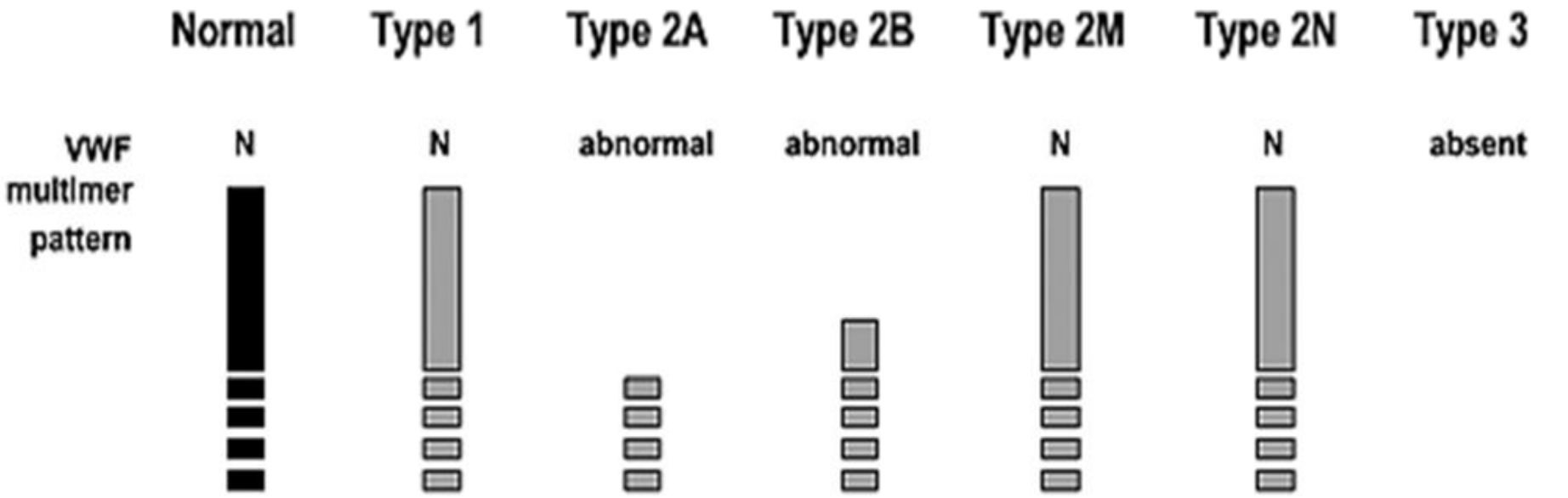
Analysis of vWF multimeric forms by this procedure is predominantly designed to evaluate type II vWD

Principle of Gel Electrophoresis and Western Blot



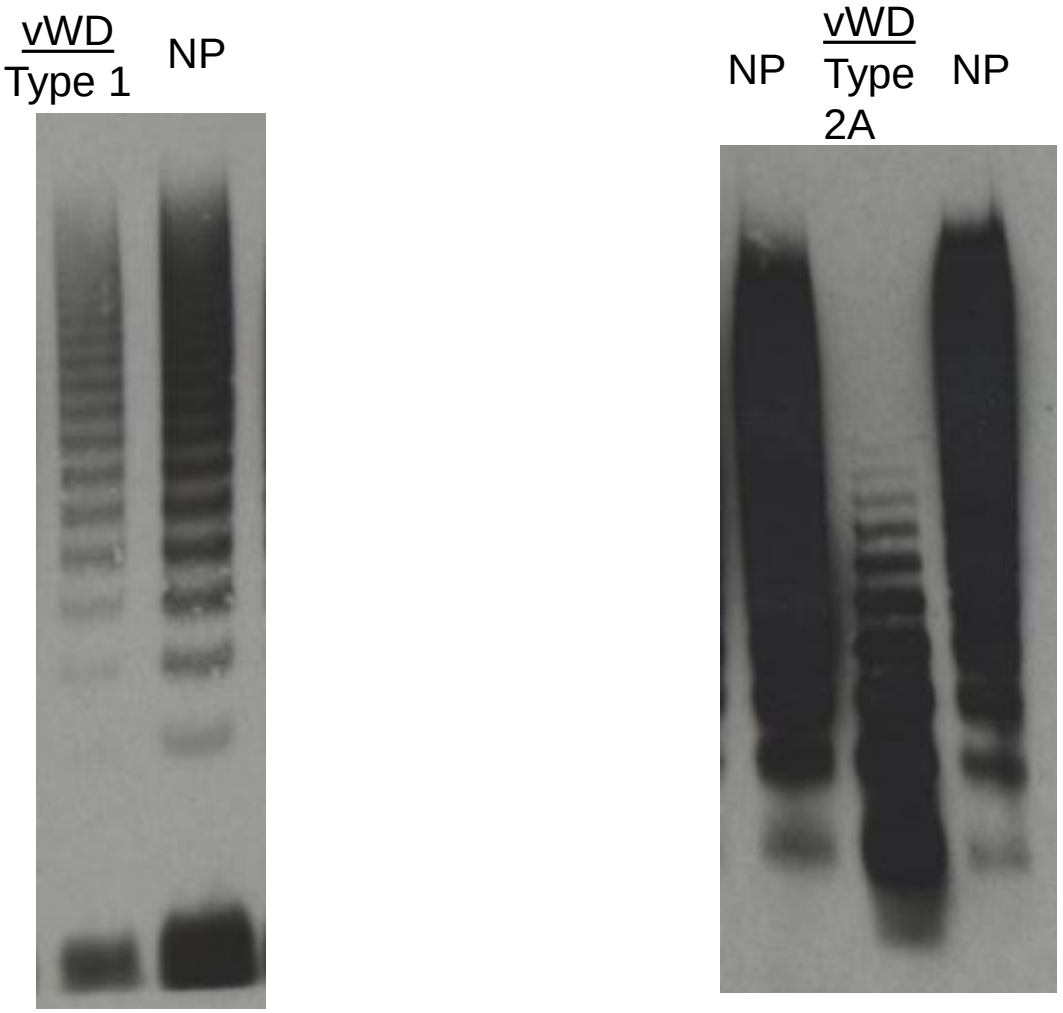
http://www.virology.ws/wp-content/uploads/2010/07/western_blot.jpg

Principle of Gel Electrophoresis and Western Blot

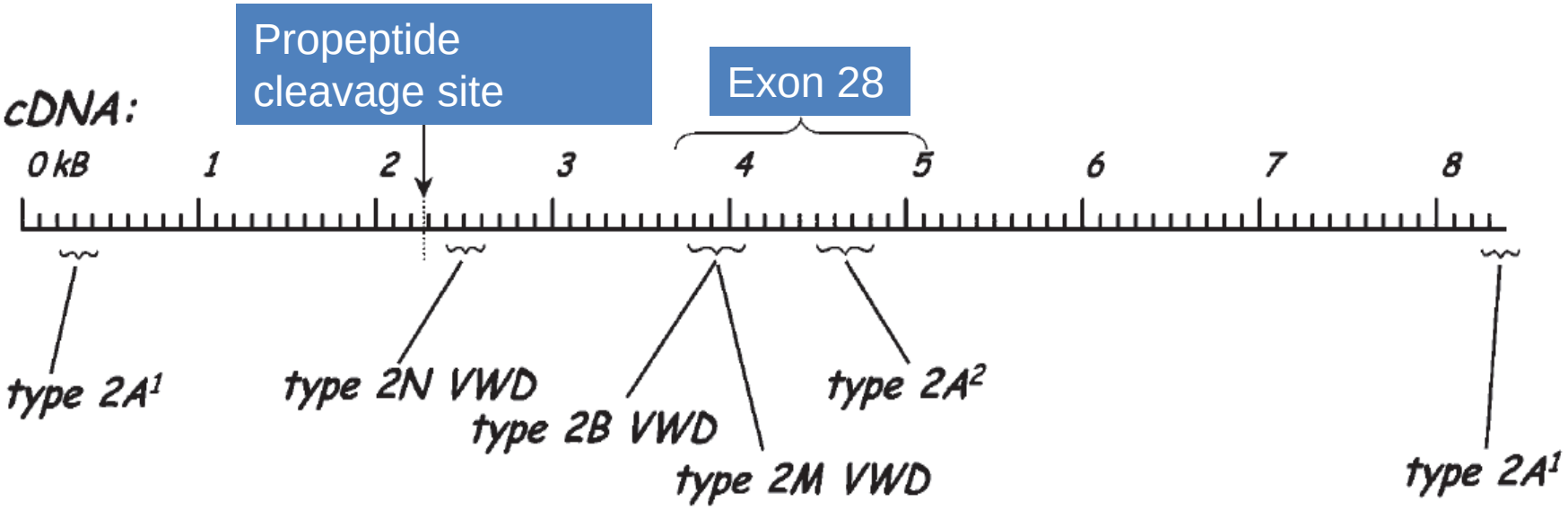


http://www.nhlbi.nih.gov/guidelines/vwd/3_diagnosisandevaluation.htm

Principle of Gel Electrophoresis and Western Blot



Genetic testing



vWD Guidelines, NHLBI

Type-specific sequencing tests

Genetic testing

von Willebrand Disease, Type 2A (*VWF*) Sequencing Exon 28 with Reflex to 9 Exons 2005480 Method: Polymerase Chain Reaction/Sequencing	
von Willebrand Disease, Type 2B (*VWF*) Sequencing 2005486 Method: Polymerase Chain Reaction/Sequencing	
von Willebrand Disease, Type 2M (*VWF*) Sequencing 2005490 Method: Polymerase Chain Reaction/Sequencing	
von Willebrand Disease, Type 2N (*VWF*) Sequencing 2005494 Method: Polymerase Chain Reaction/Sequencing	

Topics

1. *Introduction of von Willebrand factor and von Willebrand disease*

2. *Common diagnostic tests for von Willebrand disease and interpretation*

3. *Case studies*

Case 1

A one-year-old girl was referred to our hospital for prolonged oral bleeding following a mouth wound. Physical examination revealed many bruises. There was no documented familial history of hemorrhage.

Case 1

Lab Results:

Platelet count: 346 (229-465 K/ul)

aPTT (Partial Thromboplastin Time): 68 s (24-35 s)

vWF:Ag	<10% (0-6 years: 52-214%)
vWF:RCo	<10% (0-6 years: 51-215%)
Factor VIII	7% (0-6 years: 56-191%)

Case 1

What is the most likely diagnosis:

A. vWD type 1

B. vWD type 2A

C. vWD type 2M

D. vWD type 3

Case 1

What is the most likely diagnosis:

A. vWD type 1

B. vWD type 2A

C. vWD type 2M

D. vWD type 3

Case 1

- vWD Type 3
- Recessive disorder
- vWF protein is virtually undetectable
- Absence of vWF causes a secondary deficiency of FVIII and a subsequent severe combined defect in blood clotting and platelet adhesion

Case 2

A 6-year-old boy with frequent nosebleeds.

Physical examination revealed occasional ecchymosis (subcutaneous hemorrhage / purple discoloration of the skin).

Case 2

Lab Results:

Platelet count: 360 K/ul (229-465 K/ul)

aPTT (Partial Thromboplastin Time): 30 s (24-35 s)

vWF:Ag	21% (0-6 years: 52-214%)
vWF:RCo	20% (0-6 years: 51-215%)
Factor VIII	60% (0-6 years: 56-191%)

Case 2

Ctrl Patient

Case 2

What is the most likely diagnosis:

A.vWD type 1

B.vWD type 2A

C.vWD type 2B

D.vWD type 3

Case 2

What is the most likely diagnosis:

A.vWD type 1

B.vWD type 2A

C.vWD type 2B

D.vWD type 3

Case 2

- vWD Type 1
- Mild to moderate disease
- Mild quantitative deficiency of vWF
- vWF is functionally normal
- Usually autosomal dominant

Case 3

A 5-year-old boy recent had gingival bleeding and nosebleed.

Lab Results:

Platelet count: 289 K/ul (229-465 K/ul)

aPTT (Partial Thromboplastin Time): 85 s (24-35 s)

vWF:Ag	156% (0-6 years: 52-214%)
vWF:RCo	135% (0-6 years: 51-215%)
Factor VIII	8% (0-6 years: 56-191%)

Genetic testing ruled out Hemophilia A (FVIII deficiency)

Case 3

What is the most likely diagnosis:

A.vWD type 1

B.vWD type 2A

C.vWD type 2N

D.vWD type 3

Case 3

What is the most likely diagnosis:

A.vWD type 1

B.vWD type 2A

C.vWD type 2N

D.vWD type 3

Case 3

vWD Type 2N

Markedly decreased affinity of vWF for FVIII

Results in markedly reduced FVIII level.

vWF:FVIII binding (vWF:FVIIIB) assay

Summary

von Willebrand factor (vWF)

- Large multimeric protein
- Two major functions:

1. Tethers the platelets to exposed collagen during injuries
2. Serves as a carrier protein for Factor VIII

Summary

von Willebrand disease (vWD)

- Most frequent inherited bleeding disorder
- Autosomal inheritance pattern
- Quantitative: Types 1 and 3
- Qualitative: Types 2A, 2B, 2M and 2N

Summary

Laboratory tests for vWD

Initial vWD testing

- vWF:Ag
- vWF:RCo
- FVIII activity

Further testing

- vWF multimer analysis
- Ristocetin-induced platelet aggregation (RIPA)
- vWF:FVIII binding (vWF:FVIIIB) assay
- Genetic testing

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References

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