

Laboratory testing for von Willebrand Disorder

*21st Australian Conference on Haemophilia,
VWD and rare Bleeding disorders*

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Topics

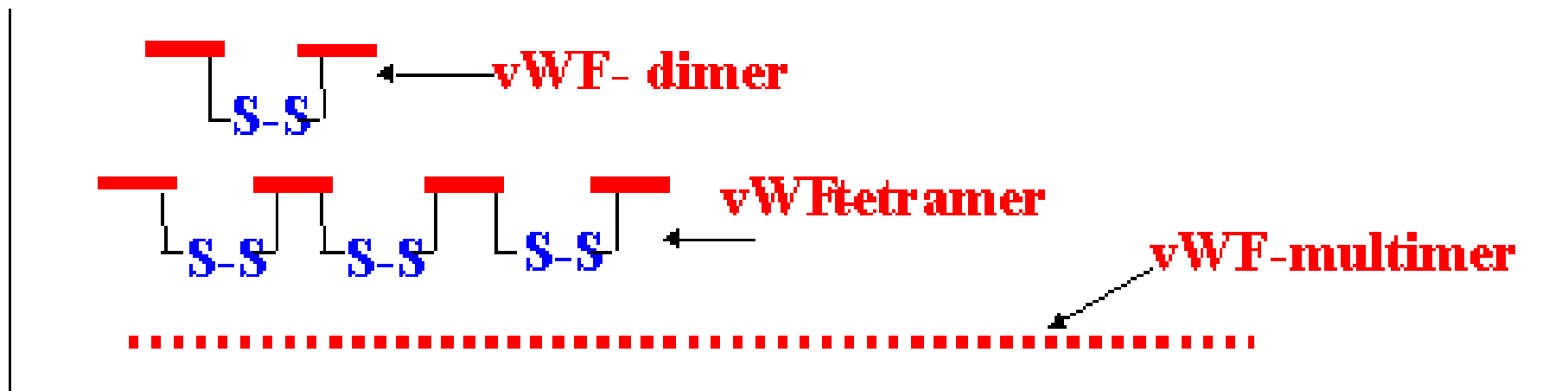
- VWD
- Structure & Function of von Willebrand Factor
- Laboratory tests
- Result patterns in VWD sub-types
 - Causes of result variability
 - External QAP

Von Willebrand Disorder (VWD):

- Most common inherited bleeding disorder.
- Arises from deficiencies or defects in von Willebrand factor (VWF).
- VWF has two primary functions/roles:
 - 'carries' FVIII, and protects/stabilises FVIII:C function.
 - permits adhesion of platelets to sites of vascular damage.
- VWF appears in plasma as a multimeric protein (low to high molecular weight or 'small' to 'large' size; higher MW = greatest adhesive function).

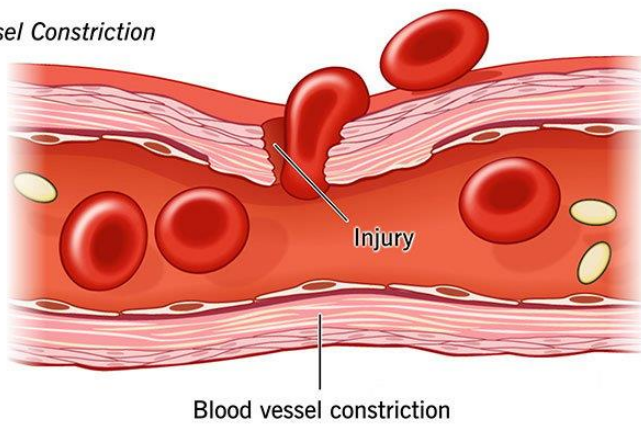
VWF

- synthesised in endothelial cells, megakaryocytes
- occurs as a dimer of 2 x 250kD subunits.
- these form tetramers and multimers.
- the highest molecular weight multimers have the most haemostatic function.

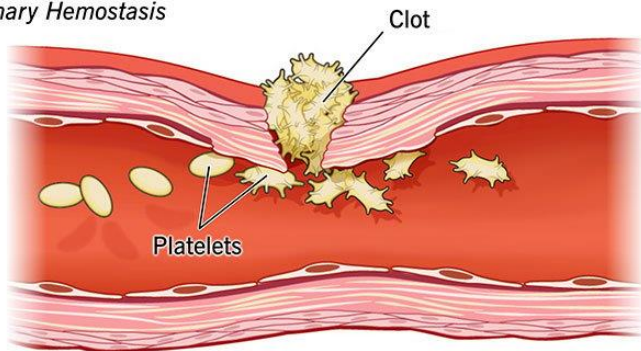


Hemostasis

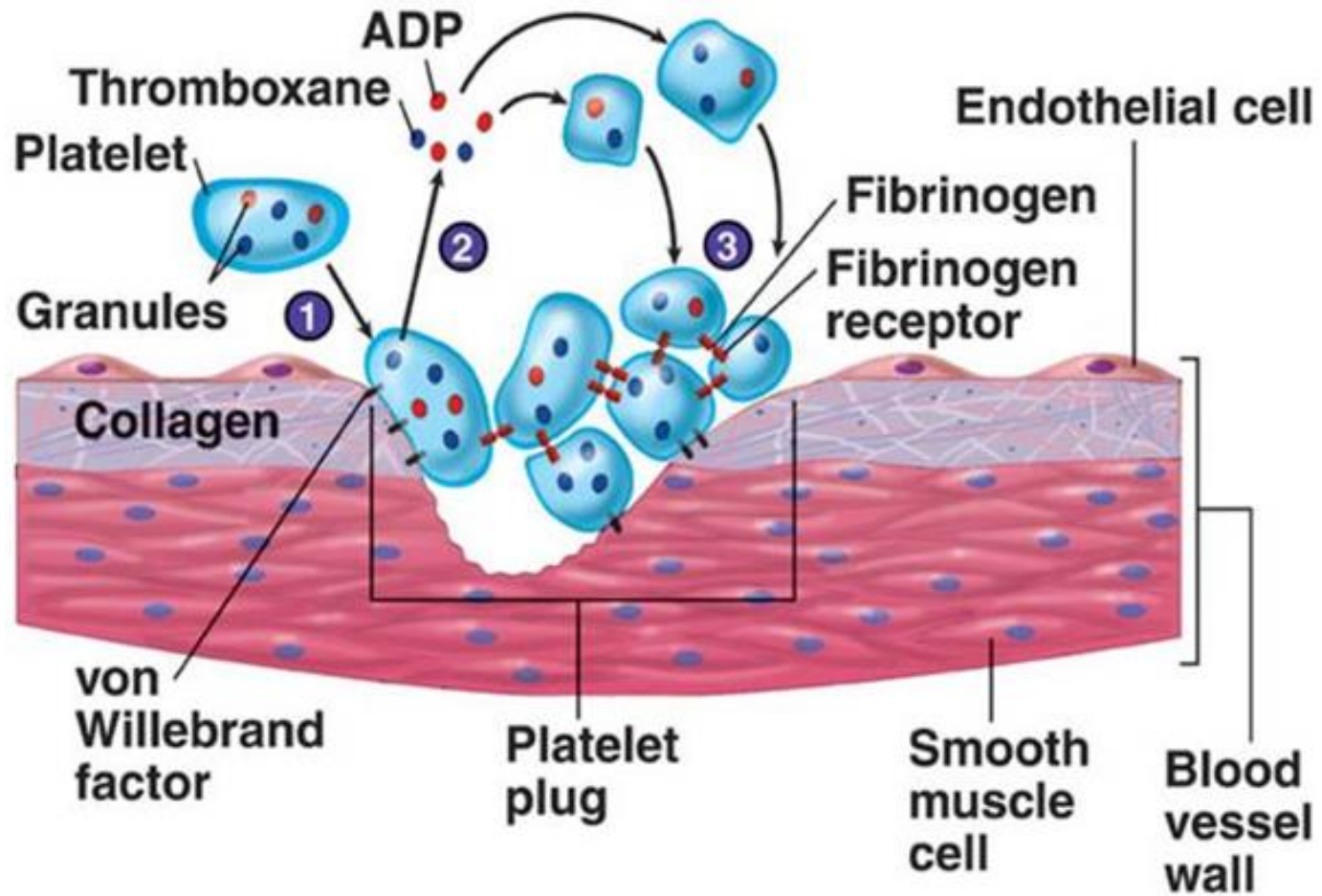
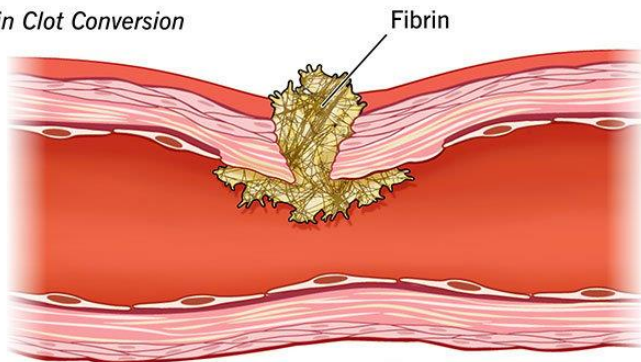
A) Vessel Constriction



B) Primary Hemostasis



C) Fibrin Clot Conversion

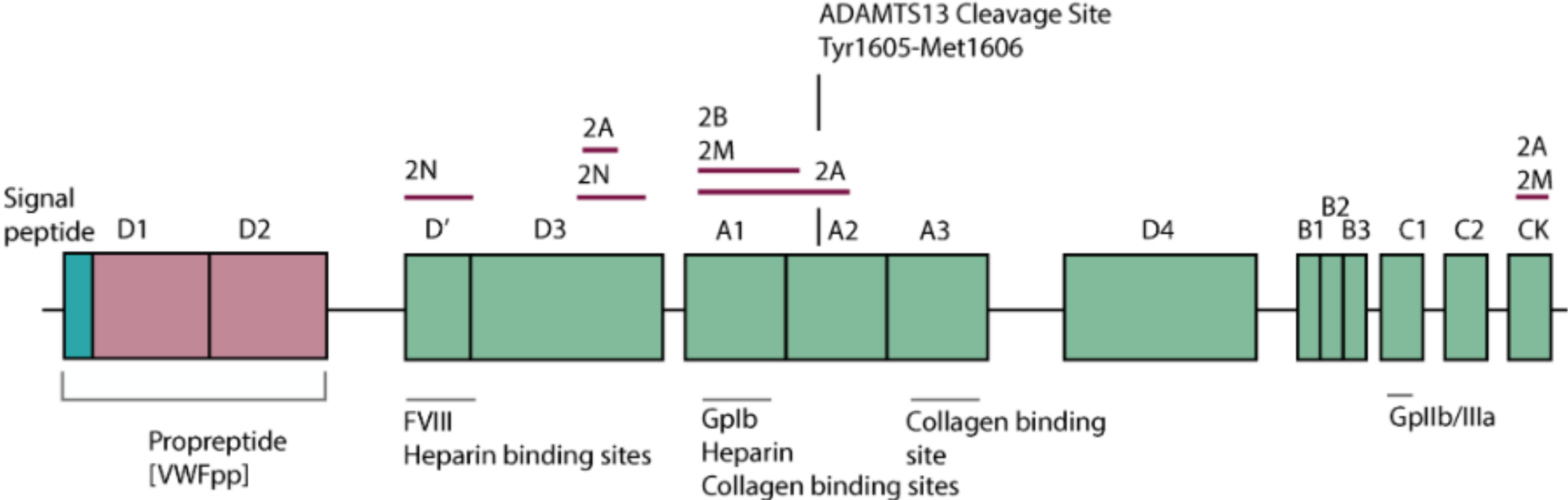


VWF structure

Different regions have different functions:

- binding to collagen
- binding to FVIII
- binding to platelets through Gp1b

These relate to laboratory assays available



Laboratory tests for VWD

Plasma-based:

FVIII:C

VWF:Ag

VWF:Rco

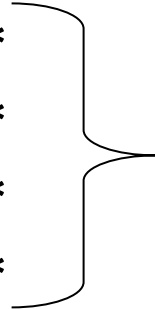
VWF:CB

VWF:Acty

VWF multimers

FVIII binding activity

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Basic screen

Whole blood:

PFA100 – useful quick screening test

- Platelet –rich plasma aggregometry with ristocetin

Von Willebrand Factor Antigen [VWF:Ag]

Measure of the total amount of VWF in plasma. All sizes are counted

Current Methods:

ELISA

-capture of VWF in microtitre wells coated with anti-VWF

Liatest

- latex micro-particles coated with anti-VWF.

-adjacent particles become linked by VWF bridges

Chemiluminescent immunoassays - Acustar

What are the variables affecting measured VWF levels ?

Pre-analytical variables

- Levels can increase with stress, physical exertion, inflammatory illnesses.....
- Quality of sample: temperature control of blue tube:
 - Adhesion of VWF to red cells if kept on ice; must be at RT
 - Loss of FVIII if temperature is too high, or a delay in plasma separation
 - Clean venipuncture, early mixing with anti-coagulant
 - Plasma must be frozen at below -20°C within 2 hours of collection

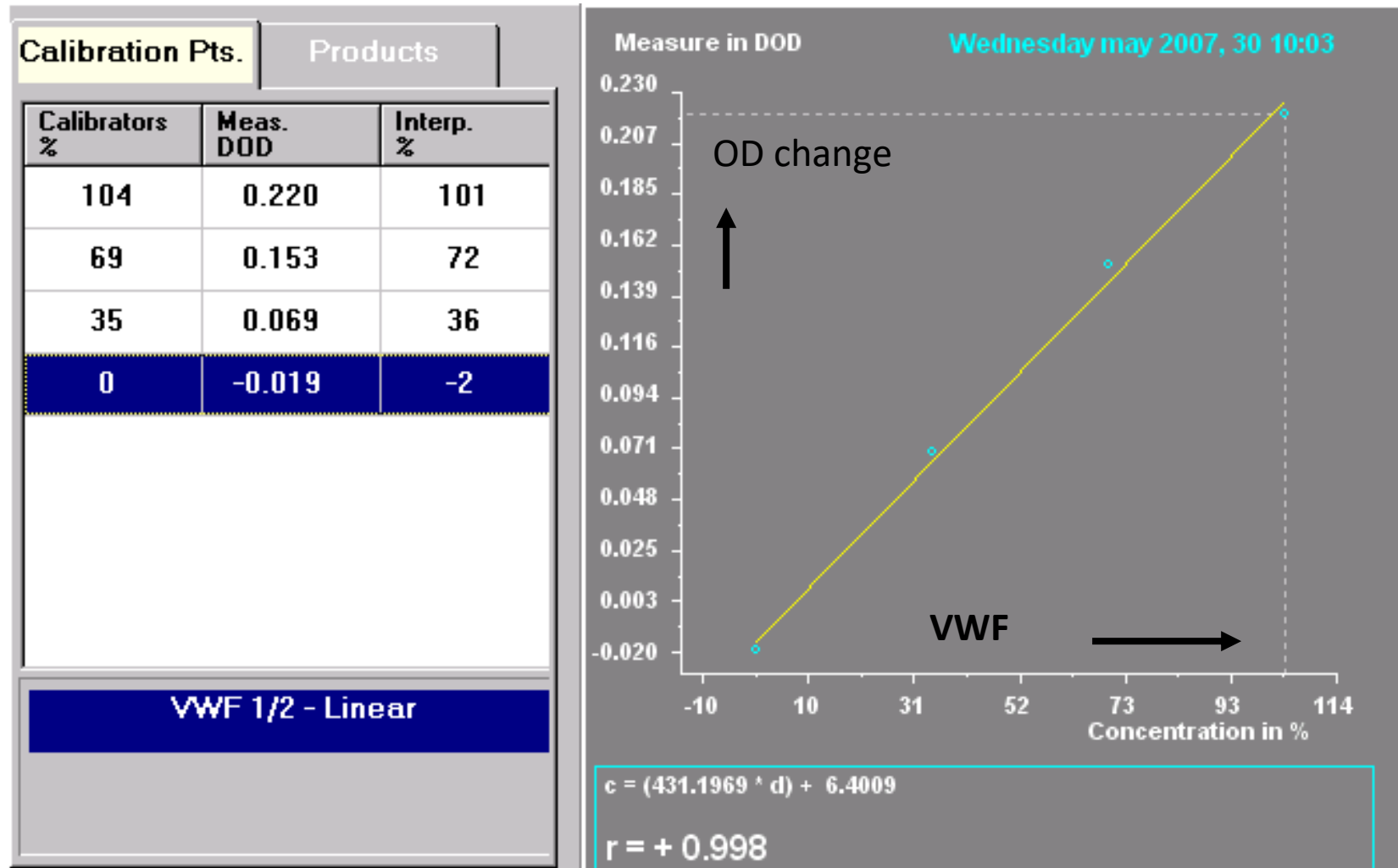
Analytical variable

- Adequate VWF test panel. Minimum of F8, VWF Antigen and one functional assay, but preferably two if levels not high.
- Assays validated for sensitivity to detecting low levels
- Quality control

Post-analytical variables

Interpretation of results, comments and suggestions for follow-up

VWF:Ag Latest calibration line on STAR-evolution



The greater the amount of latex particle agglutination the larger the change in light transmission

VWF:RC Automated assay.

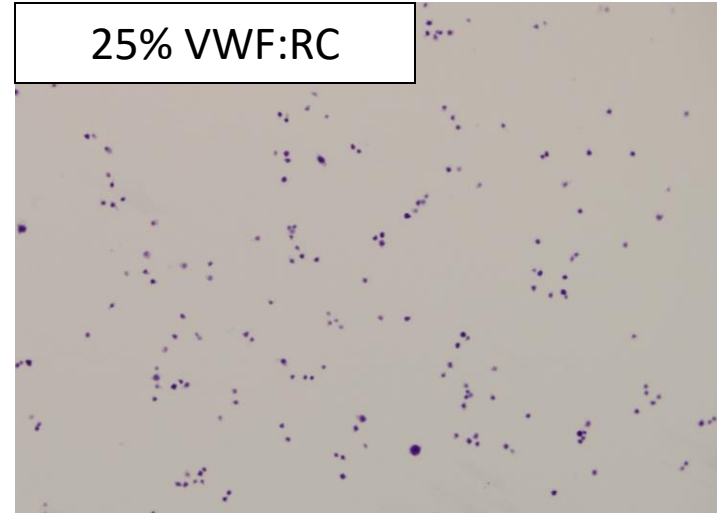
The degree of platelet clumping varies with plasma VWF level.

Test plasma is mixed with a suspension of platelets in the presence of ristocetin, causing spontaneous platelet clumping

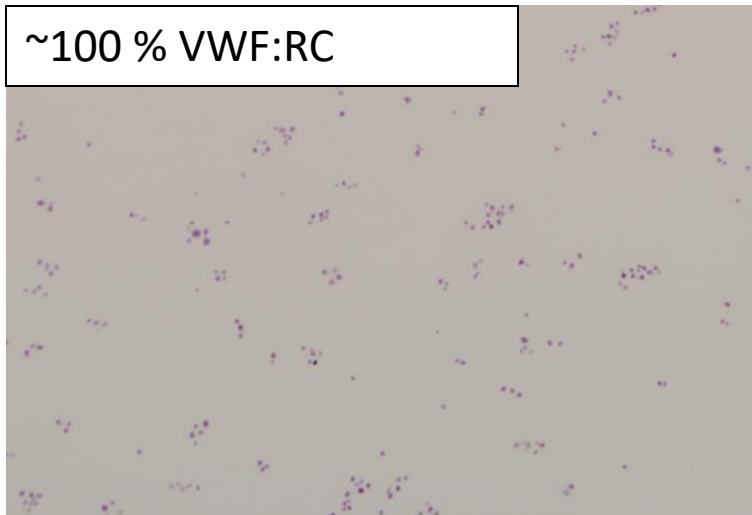
Plt/ristoc reagent only



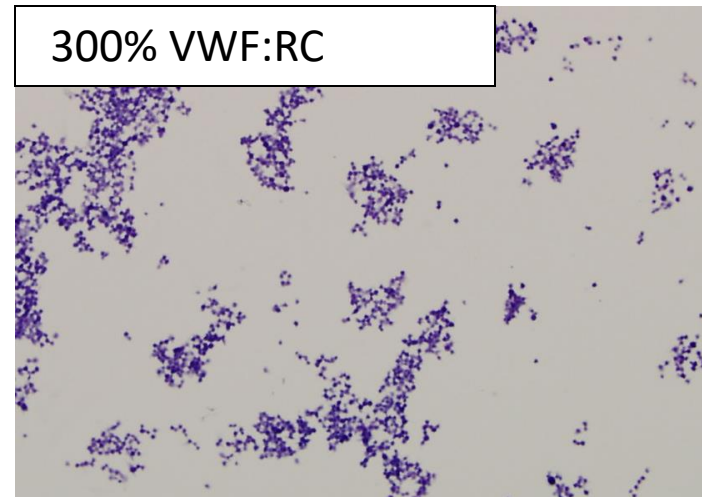
25% VWF:RC



~100 % VWF:RC

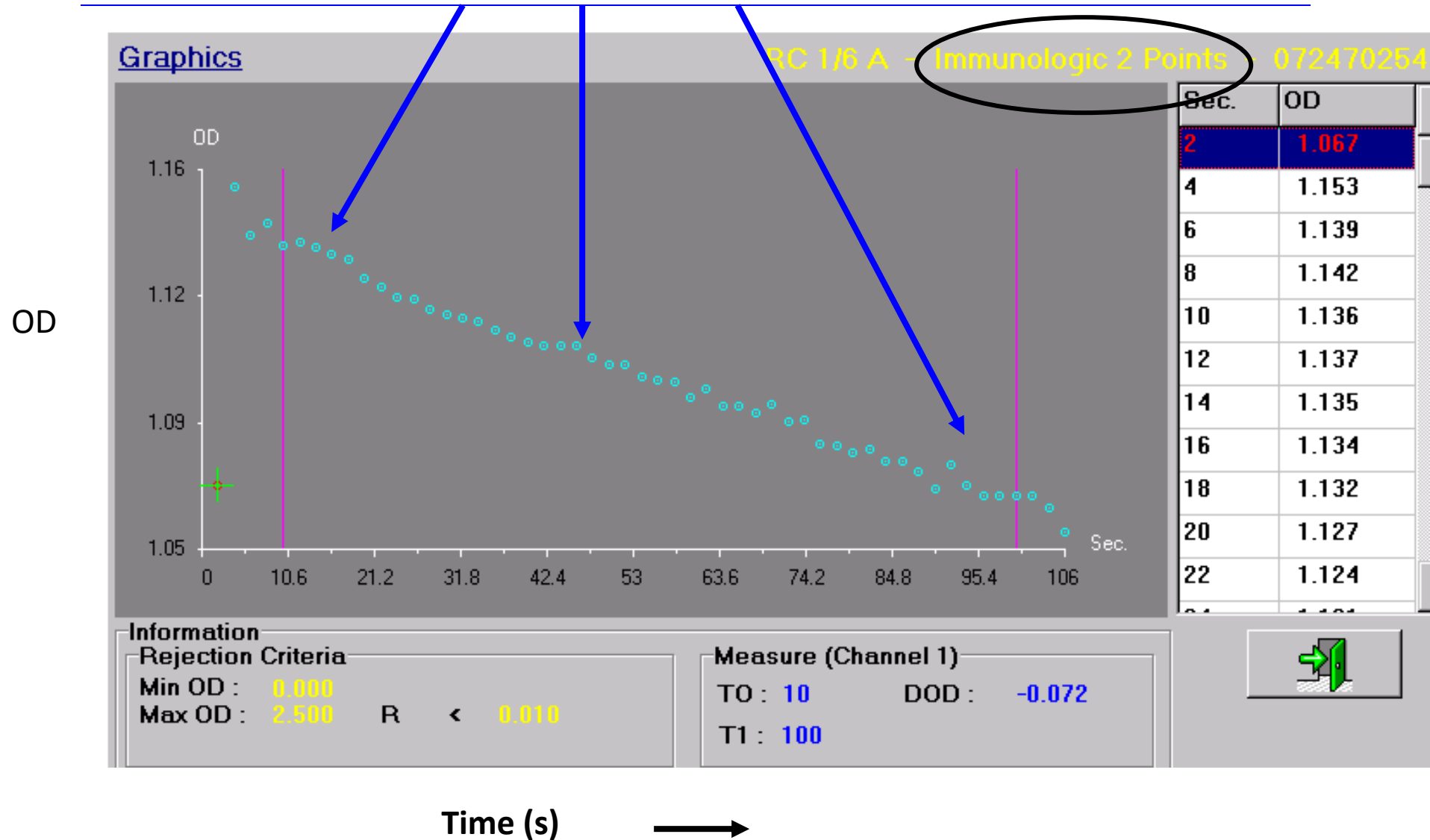


300% VWF:RC



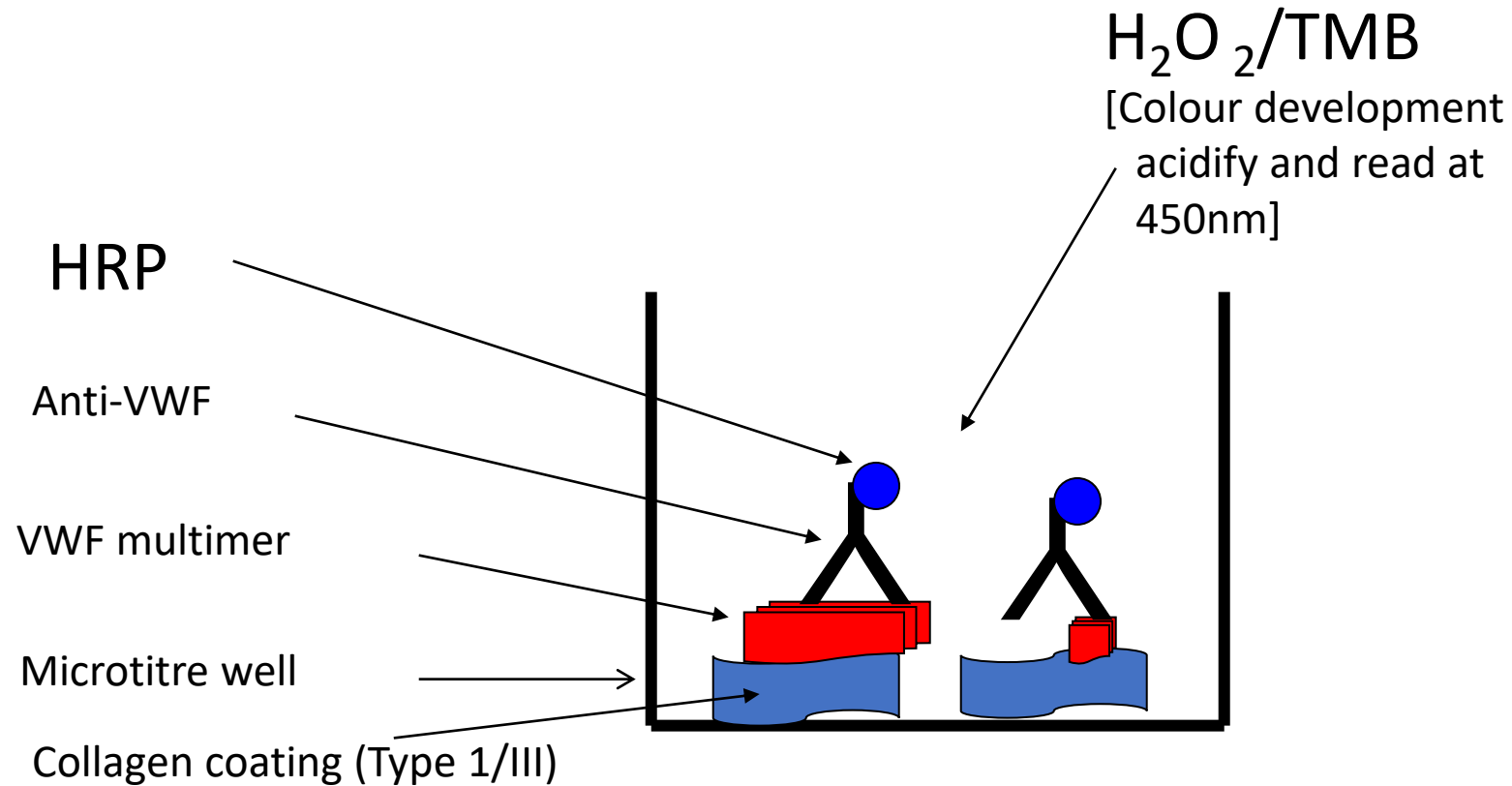
VWF:RC Real time plot of absorbance change from 10 to 100 secs.

Optical density decreases progressively with platelet aggregation



Principle of collagen binding assay by ELISA

Enzyme-linked Immunosorbent assay



ORIGINAL ARTICLE

A comparative evaluation of a new fully automated assay for von Willebrand factor collagen binding activity to an established method

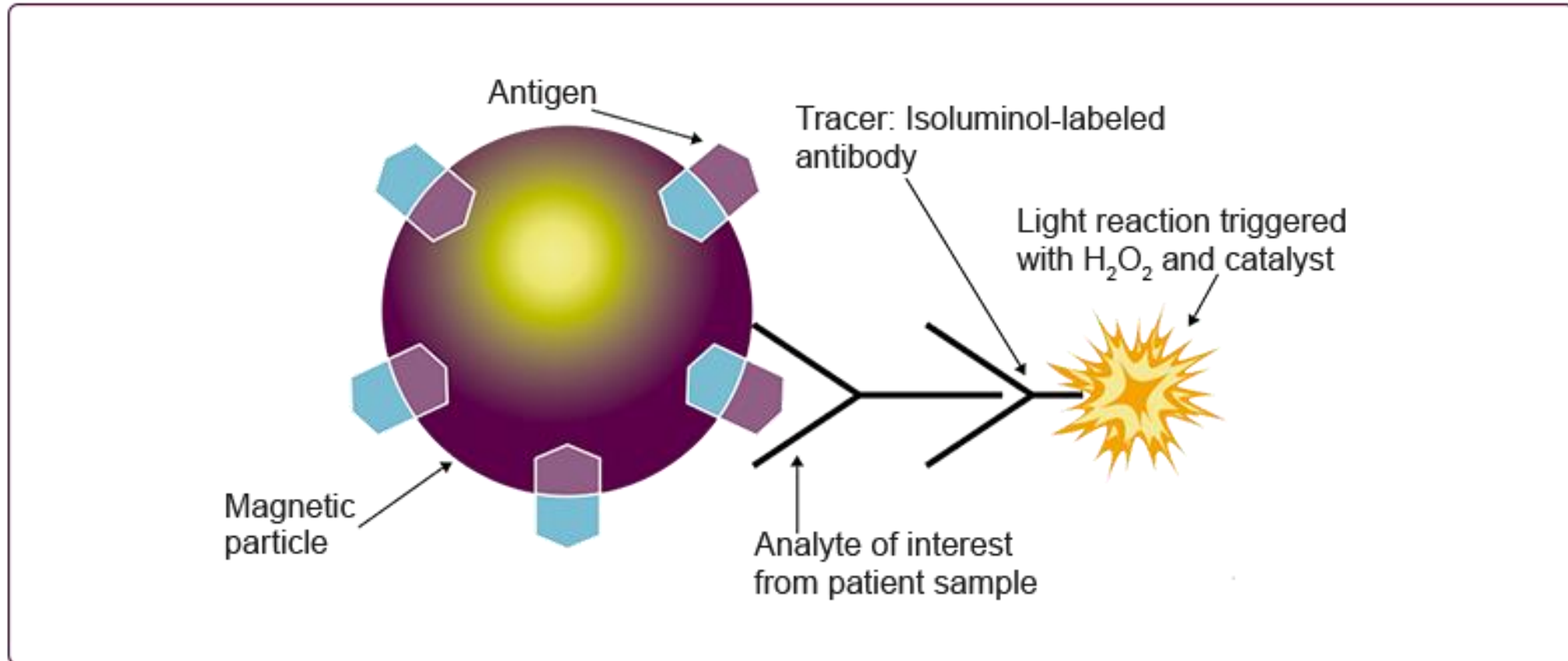
F. Stufano, L. Baronciani, D. Mane-Padros, G. Cozzi, S. Faraudo, F. Peyvandi ✉



Conclusion

The new assay is rapid and simple to use, with its ready-to-use reagent cartridges. This VWF:CB assay, in addition to the measurement of VWF:Ag and VWF:RCO made on the same platform, gives additional information for the diagnosis of VWD in both nonspecialized and reference laboratories.

Test Principle - Acustar



Advantages:

Random access

All three assays run simultaneously on one system

Large measuring range 0.5% to >400%

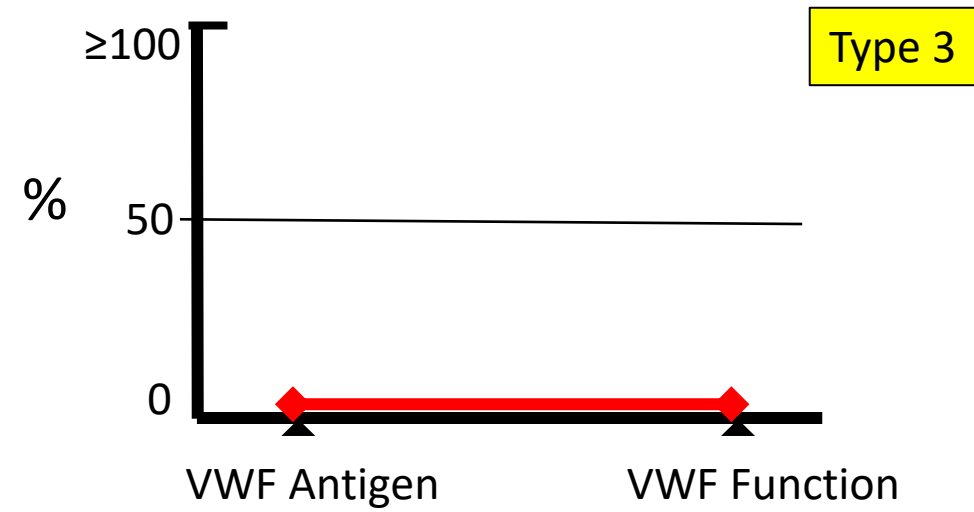
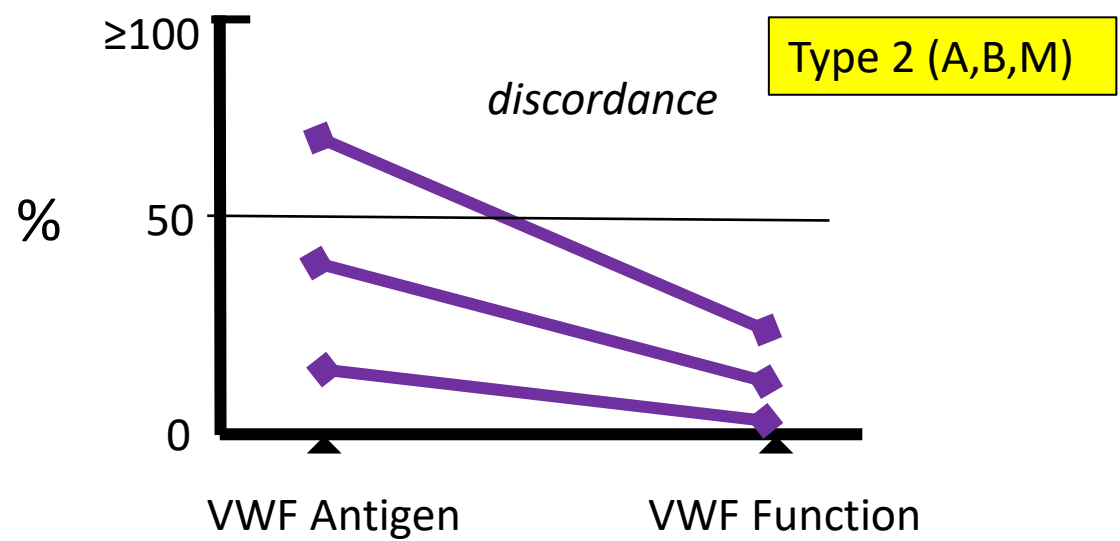
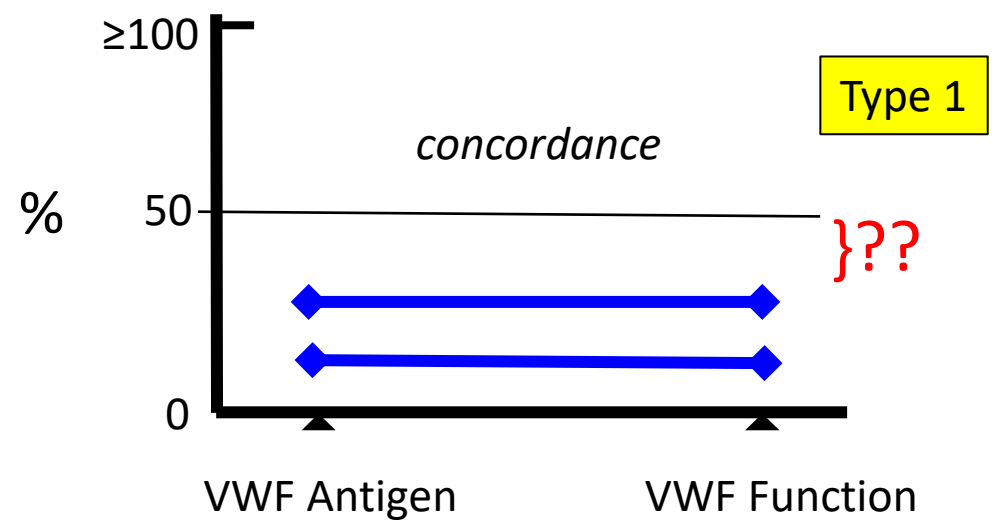
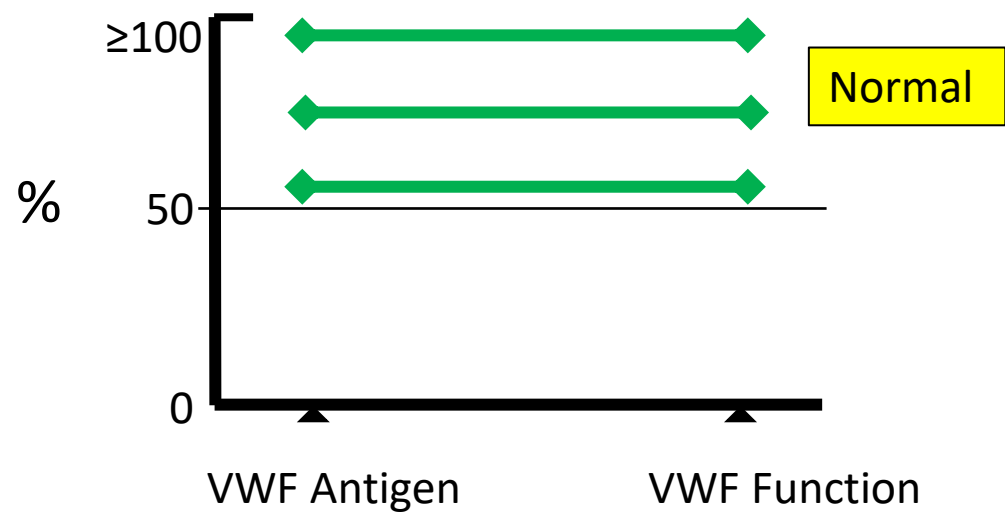
Von Willebrand Disorder (VWD): Primary Classification:

Type 1: Partial quantitative deficiency (reduced levels of functionally normal VWF).

Type 2: Qualitative defect (absolute levels of VWF low or normal, but VWF 'function' diminished).

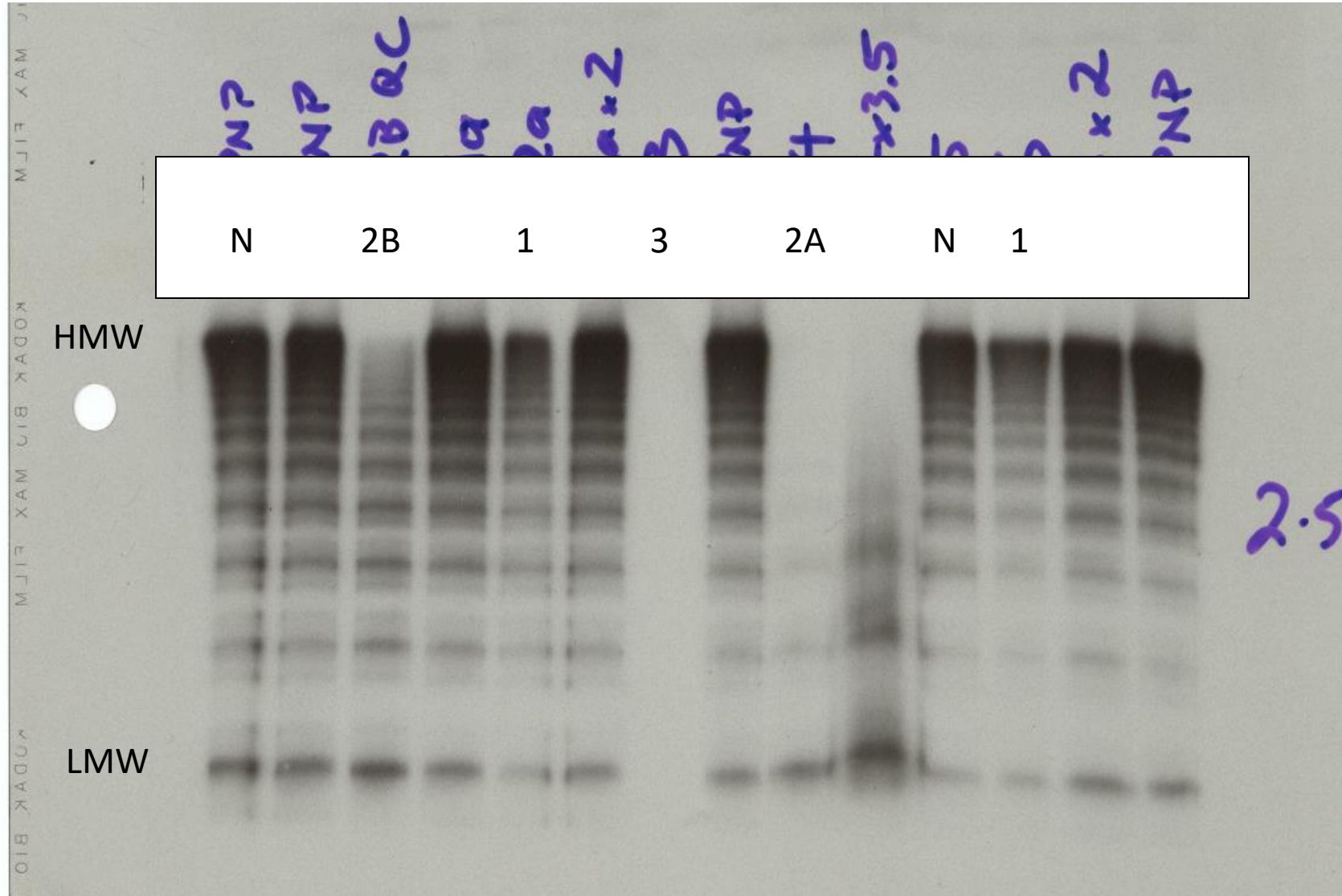
Type 3: Total quantitative deficiency (VWF 'absent').

VWF patterns in normal and VWD plasma

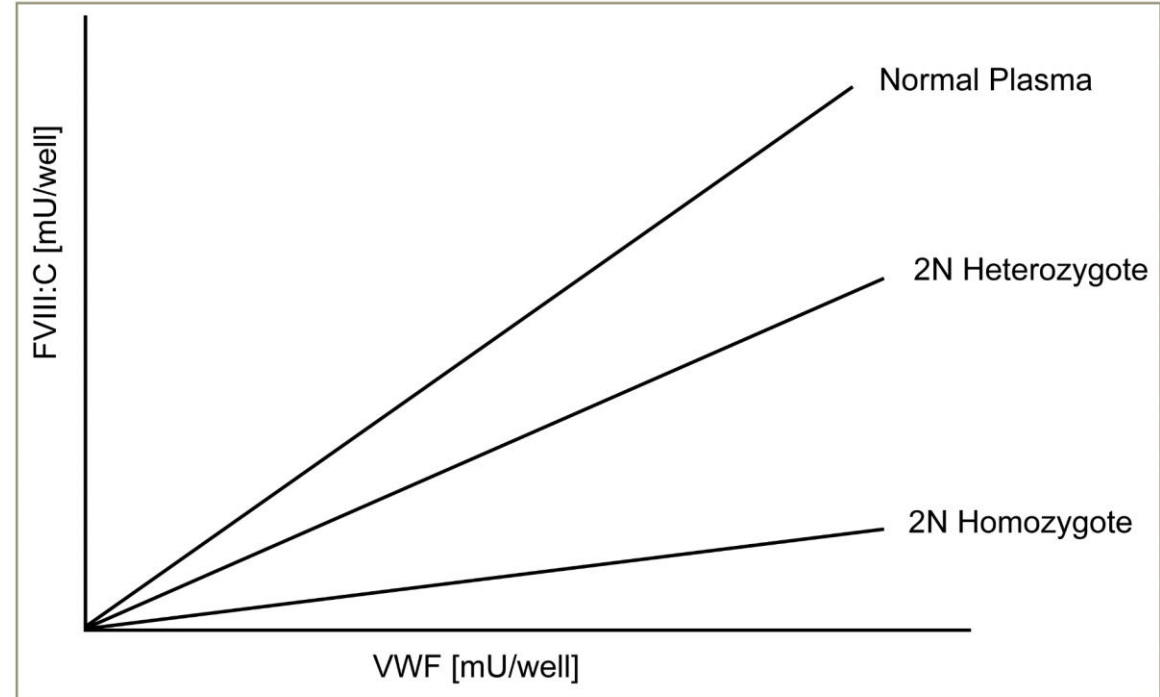
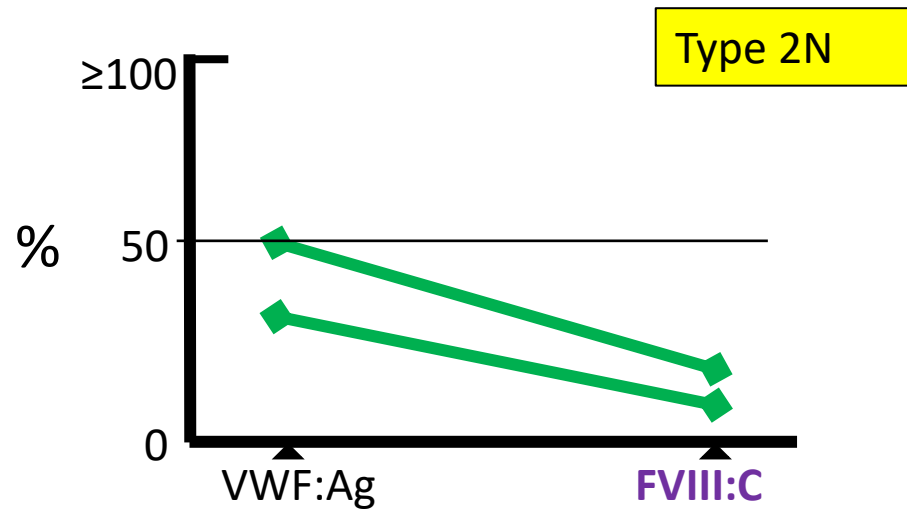


Example multimer patterns

Direction of protein migration when current is applied



VWF patterns in normal and VWD plasma



Individuals with 2N VWD will show defective binding of FVIII i.e. a reduced VWF:FVIIIIB.

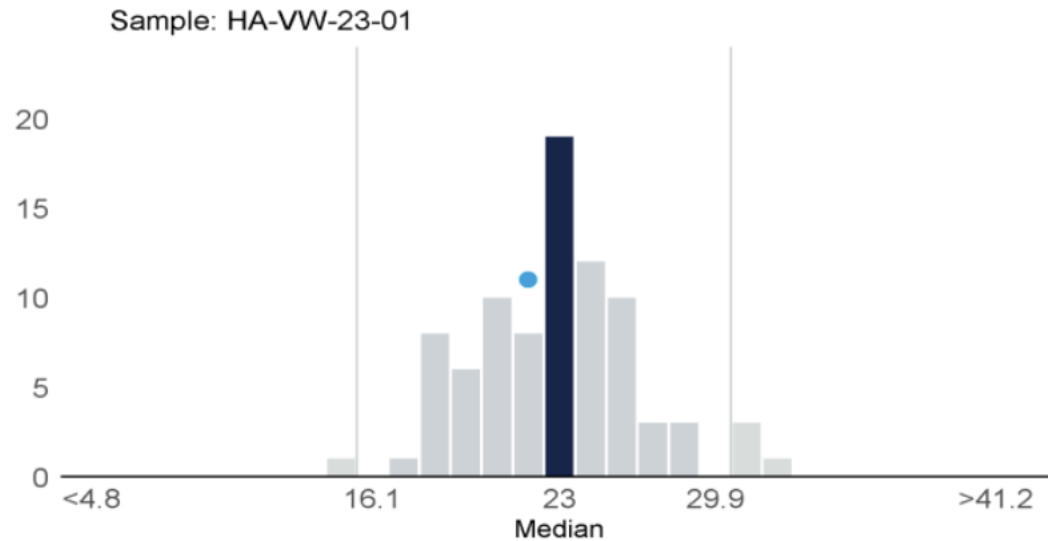
External Quality Assurance

-eg RCPA, ECAT, UKNEQAS

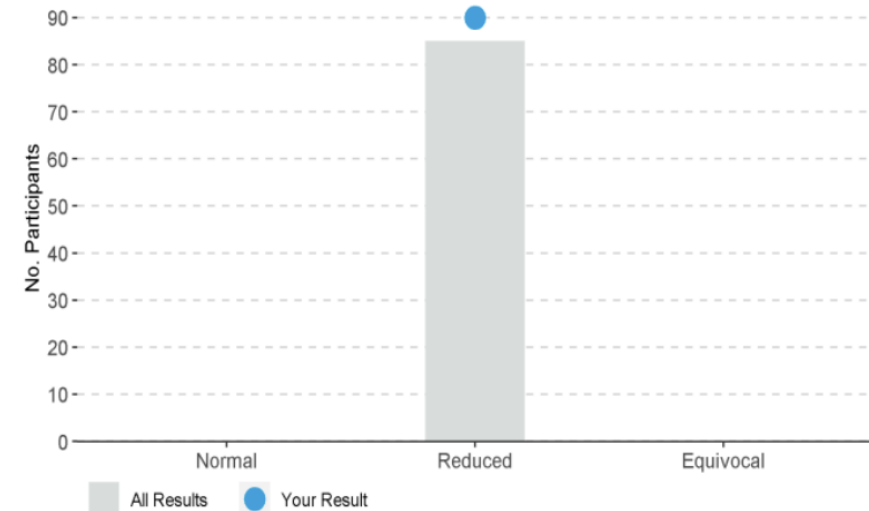
- mandatory for diagnostic laboratories to participate [NATA]
- periodic testing of distributed test plasma sample
- comparison of results with peer group results

Eg 2023 recent survey, with 85 participant laboratories

Result review - vWF Antigen (%)



Result review - vWF Antigen interpretation HA-VW-23-01



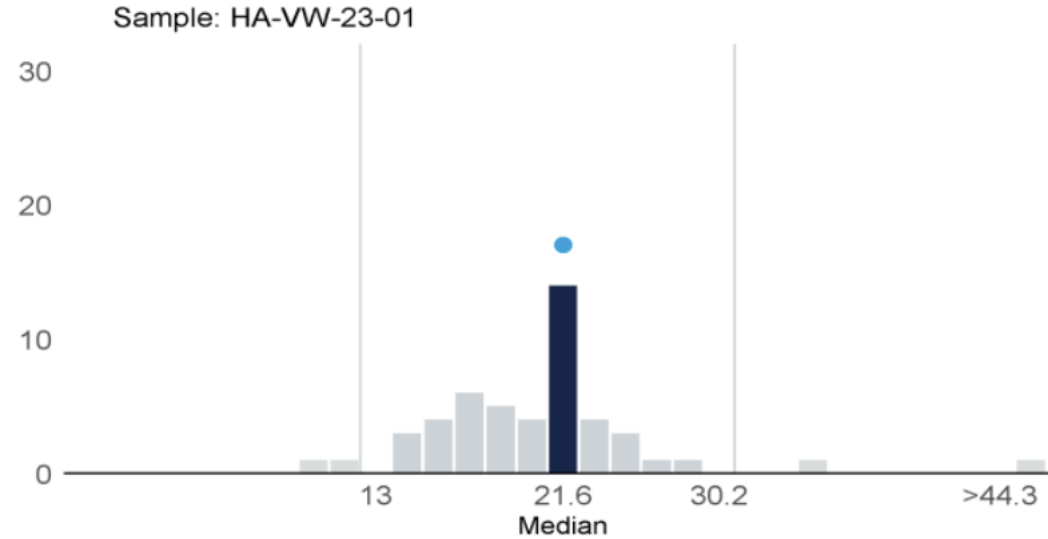
External Quality Assurance

Method Comparison - vWF Antigen interpretation

Reagent	Reduced
All Result	85
Instrumentation Laboratory AcuStar VWF Ag	11
Instrumentation Laboratory VWF: Activity Assay	2
Instrumentation Laboratory vWF-Antigen	18
Siemens VWF Antigen assay	29
Stago Liatest vWF:Ag	24
Technoclone VWF:Ag Elisa	1

External Quality Assurance

Result review - vWF Ristocetin Co-factor (%)

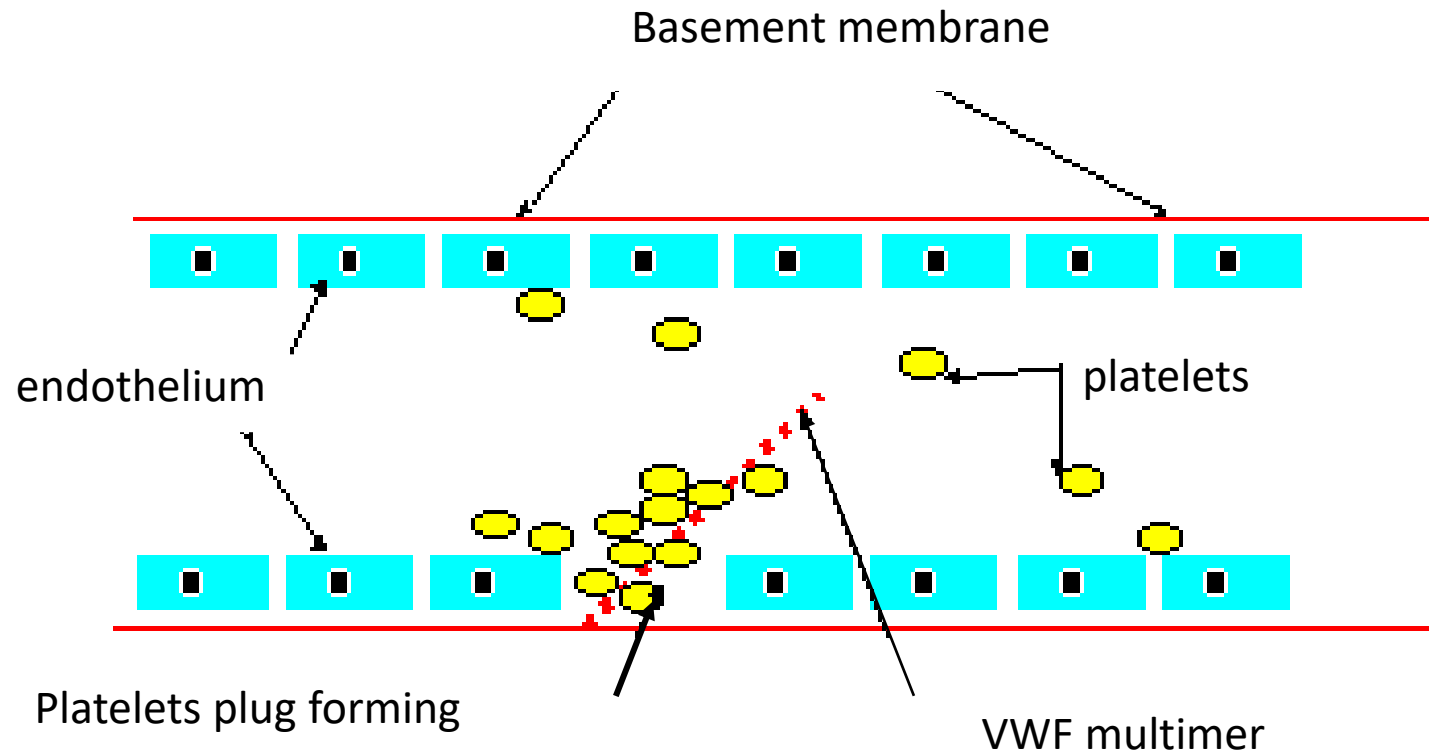


Method Comparison - vWF Ristocetin Co-factor (%)

Reagent	n Results	Median
ALL RESULTS	49	21.6
In-house	2	
Instrumentation Laboratory AcuStar VWF Rco	14	23.0
Instrumentation Laboratory VWF:RCo activity	14	22.0
Instrumentation Laboratory vWF-Antigen	1	
Siemens BC von Willebrand Reagent	4	17.0
Siemens VWF	8	16.5
Stago VWF:Rco	6	18.5

Questions?

VWF acts as a 'glue' to bind platelets to sub-endothelial collagen exposed after blood vessel damage.



Multimer Examples

