

INTEREST OF THE NEW RAPID TEST "HYDRAGEL 5 VON WILLEBRAND MULTIMERS" FOR THE ANALYSIS OF VON WILLEBRAND MULTIMERS

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AIMS

- von Willebrand disease (vWD) is one of the most common inherited bleeding disorders. It is traditionally classified into type 1 and type 3, which corresponds to quantitative deficiencies of vWF, while types 2 are characterized by qualitative defects.
- Diagnosis of vWD requires measurement by both an immunological (vWF:Ag) and a functional test to check its activity (vWR:Act).
- A ratio vWR:Rco/vWF:Act below 0.5 suggests a type 2 vWD, but this ratio can be in a gray zone (0.5 – 0.75)
- The gold standard to identify qualitative vWD is the analysis of multimers distributions, in discontinuous SDS agarose gel followed by a western blotting step. Only a few specialized laboratories perform this analysis due to the complexity of the method itself and to its very slow turnaround time (2-3 days).
- We present here the use of a ready-to-use SDS agarose gel (Hydragel 5 von Willebrand Multimers*) for multimer analysis, which is rapid (1 day) and easy to perform (no western blot). * For Research Use Only

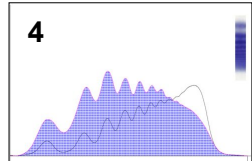
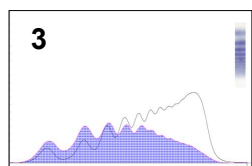
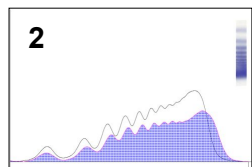
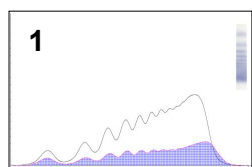
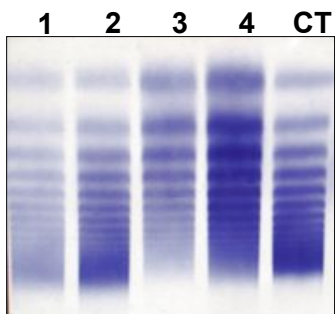
PATIENTS & METHODS

- Plasmas from 20 healthy controls and 42 patients with an haemorrhagic diathesis were tested. Among them, a parallel decrease of vWF:Ag and vWF:Act suggested a type 1 vWD, whereas in 5 patients, a decreased ratio vWF:Act/vWF:Ag suggested a type 2 vWD. We also analysed samples of patients with clinical situations associated with an acquired vWD (5 cases with Extra Cellular Membrane Oxygenation),
- Plasma were loaded and separated in continuous SDS agarose gel system (no stacking and running gel) within 110 mn.
- Multimers were probed in gel by immunofixation using horse-radish peroxidase (HRP) conjugated to a rabbit anti-vWF (90 min).
- Visualisation of multimers was achieved by colorimetry using commercially available TTF1/TTF2 reagents.
- Curves were produced using the manufacturer's gel scanner and interpretation software
- Tests were done on the Hydrasys 2 Scan (Sebia, Lisses, France)



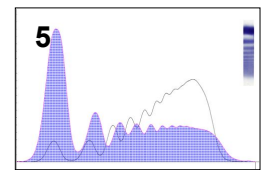
RESULTS

Here are presented some typical gels obtained, and the scanning of the corresponding gels



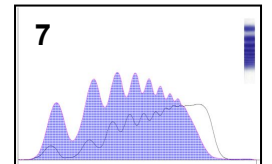
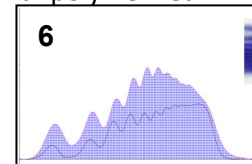
• Scanning of the gel clearly shows the distribution of the multimers. Scan of the plasma of the patients are colored in blue, the black line shows the distribution of multimers in a control (CT) plasma

- Line 1: classical type 1 vWD
- Line 2: same patient than in line 1, after surgery (Day 2). The increase of multimers is due to the inflammatory state of the patient
- Line 3: absence of multimers of high molecular weight (HMW) in a patient with a type 2 vWD.
- Line 4: same patient than in line 3, but during pregnancy. A partial correction of the deficiency in HMW multimers is observed



- Line 5: Analysis of vWF multimers of a woman with a bleeding tendency and a prolonged BT (PFA > 300 sec). vWF:Ag = 228 %, vWF:Act 68%

The analysis of multimers indicates an excess of unpolymerized vWF.



Variations of vWF multimers in a patient before lung transplantation (6) and at the end of a 15 hour surgery (7) with an extra cellular membrane oxygenation device. A clear decrease of HMW multimers is evidenced.

	1	2	3	4	CT
vWF:Ag (%)	37	95	132	197	102
vWR:Act (%)	37	79	77	118	101
Ratio Co/Ag	1	0.82	0.58	0.6	1
BT (PFA-100)	ND	ND	> 220	130	ND
PLT (x10 ⁹ /L)	307	274	165	97	ND

BT: Bleeding Time; PLT: Platelet count;
ND: not determined

CONCLUSION

The "Hydragel 5 von Willebrand Multimers" method for vWF multimer analysis is :

- simple to carry out,
- produces results within 1 day,
- performed on commercially available instruments (Hydrasys 2 and GelScan or Hydrasys 2 Scan)

It provides clear pattern of vWF multimer distribution and is particularly valuable in emergency situations when a molecular abnormality of vWD is suspected (discordance between vWF:Act and vWF:Ag).