Evaluation of the Sebia Hydragel 5 von Willebrand factor (VWF) assay compared to an in-house agarose gel electrophoresis method for VWF multimer analysis

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INTRODUCTION

VWF multimer analysis is essential to characterise the subtype of VWF abnormalities, along with assays such as VWF activity/antigen, factor VIII, factor VIII binding and genetic testing.
Multimer analysis is currently laborious, non-standardised and interpretation of gel results subjective.

OBJECTIVES

The aim of this study was to determine whether the Sebia Hydragel semi-automated system will improve our interpretation of results and reduce hands-on staff time, as our in-house method takes 4 days to produce results and are interpreted visually.
Normal and patient samples were analysed in parallel with both the in-house method and the Sebia Hydragel 5 VWF multimer assay. VWF:Ag and VWF:Act assays were carried out using Siemens reagents.

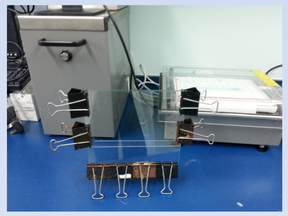
METHODS

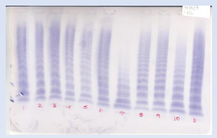
In-house multimer method :

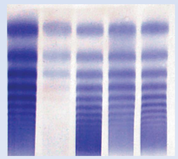
- 1.6 % SDS Agarose gel electrophoresis
- Visualisation using alkaline phosphatase -conjugated antibody
- Low resolution gel used for identification of types
- High resolution gels used to further categorise type 2A
- Total hands on time: 190 mins over 4 days

Sebia Hydragel 5 VWF multimers with Hydrasys 2 Scan instrument :

- Electrophoresis in 2.0% SDS agarose gel
- Direct immunofixation and visualisation with peroxidase-labelled antibody
- Followed by densitometry
- Total hands-on and assay steps = 6 hours and 40 mins

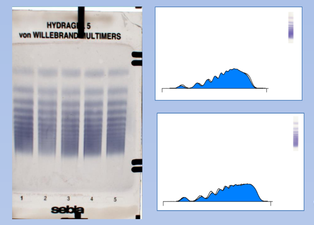






RESULTS

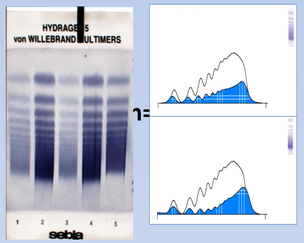
Normal Donors : n=23



Lane 1
VWF:Ag 109 IU/dl
VWF:Act 70 IU/dl

Lane 3
VWF:Ag 93 IU/dl
VWF:Act 61 IU/dl

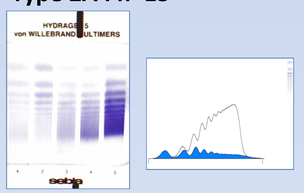
Type 1 : n=16



Lane 1
VWF:Ag 45 IU/dl
VWF:Act 36 IU/dl

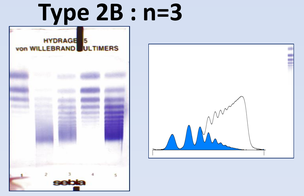
Lane 3
VWF:Ag 38 IU/dl
VWF:Act 35 IU/dl

Type 2A : n=15



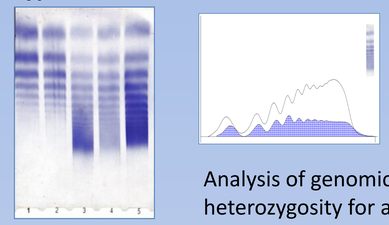
Lane 2
VWF:Ag 23 IU/dl
VWF:Act <3 IU/dl

Type 2B : n=3



Lane 4
VWF:Ag 45 IU/dl
VWF:Act 16 IU/dl

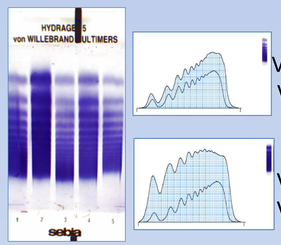
Type 2M : n= 10



Lane 4
VWF:Ag 36 IU/dl
VWF:Act 7 IU/dl

Analysis of genomic DNA shows heterozygosity for a c.3845T>C P.Leu1282 Pro missense mutation consistent with a diagnosis of Type 2M

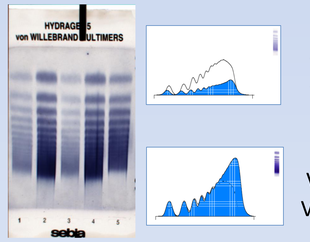
Thrombotic thrombocytopenic purpura (TTP) : n=4



Lane 1
VWF:Ag 224 IU/dl
VWF:Act 220 IU/dl AD13 39%

Lane 2
VWF:Ag 399 IU/dl
VWF:Act 277 IU/dl AD13 26%

Type 1 : Pre and Post DDAVP : n=2



Lane 1
VWF:Ag 45 IU/dl
VWF:Act 36 IU/dl

Lane 2
VWF:Ag 195 IU/dl
VWF:Act 180 IU/dl

Type 2N : n=2 and Acquired VWS : n=4 also completed (data not shown)

SUMMARY

The Hydragel 5 von Willebrand Multimers and Hydrasys 2 Scan instrument :-

- Ready to use gel and reagents are provided
- Results are obtained within the working day
- Clear reproducible bands (no triplets) are obtainable
- Densitometry improves interpretation of gels
- Excellent screening gel

The Hydragel 5 von Willebrand multimer technique correlates with the in-house technique for :

- Normal samples
- VWD Type 1
- VWD Type 2A
- VWD Type 2B
- Acquired VWS
- TTP

For 6 /10 of the VWD Type 2M a slight decrease of HMWM has been found in the Hydragel system in contrast to the in-house method where all HMWM appear normal.

CONCLUSIONS

Our in-house 4 day labour-intensive multimer method is prone to interpretation difficulties due to the lack of densitometry.
The hydragel sysytem which included all phases of analysis : sample application, migration, incubation, staining/destaining, drying and densitometry on one instrument saved on staff time and allowed for easy interpretation of results.

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