Evaluation of a new commercial method for von Willebrand Factor multimeric analysis

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Introduction

von Willebrand factor (VWF) is a large glycoprotein with a multimeric structure. Molecular weights of VWF multimers in plasma normally range from approximately 800 to 20,000 kDa. Qualitative multimer analysis evaluates the size distribution of VWF multimers, including small, intermediate, large, and very large forms. Qualitative evaluation of plasma VWF multimeric distribution is useful for subclassification of von Willebrand disease (VWD) as certain inherited (type 2) and acquired subtypes are characterized by defects in multimeric distribution. Multimer analysis has historically been a manual, labor-intensive, laboratory-developed test requiring significant time and technical expertise. The first commercial method for multimeric analysis has recently been developed and utilizes an "all-in-one" analyzer for gel electrophoresis, staining, and densitometry, thereby streamlining the complicated, multi-step method of the laboratory-developed test.

Objectives

The current study was undertaken to evaluate the performance characteristics of the new commercial Sebia Hydragel 5 VWF Multimers method on the Sebia HYDRASYS 2 SCAN instrument.

Methods

Accuracy was assessed by testing plasma specimens from 24 patients being evaluated for VWD with both the laboratory-developed multimer method (electrophoresis and western blot with chemiluminescent detection) and the Sebia method. Multimeric patterns on both methods were reviewed independently by 3 medical directors and the consensus pattern (defined as 2/3 agreement) was used as the final multimer assignment. Plasma specimens from 38 normal donors were also evaluated on the Sebia method. A subset of 9 specimens with VWF antigen concentrations significantly lower- or higher- than normal were re-tested on the Sebia method at both the standard specimen pre-dilution (1:6) and an optimized dilution based on protein concentration (1:4, 1:10, or 1:20). Precision was determined by evaluating pattern reproducibility of normal and abnormal controls included on each Sebia gel in the evaluation studies to confirm protein migration and reactivity of VWF antisera and included data from 27 gels. Sensitivity was determined by diluting normal plasma with VWF-deficient plasma to achieve VWF protein concentrations of 50, 25, 10, 5, and 1 percent of normal, followed by multimeric analysis.

Conclusion

- The Sebia Hydragel 5 VWF Multimers methods on the HYDRASYS 2 SCAN instrument demonstrates comparable performance characteristics to our current laboratory-developed method with the advantage of electrophoresis gels and densitometry scans to aid interpretation.

Results

- **Accuracy**
  - Concordant multimer interpretations were obtained in 19 of 24 comparisons including 12 with normal and 7 with various abnormal multimeric patterns (see examples, **Figure 1**).
    - The 5 specimens with discordant interpretations all involved slight differences in interpretation of the degree of multimeric abnormality or discrimination between normal and a subtly abnormal pattern.
    - No trends toward one method were observed in the discordance comparisons and none were considered clinically significant.
  - All 38 normal donor specimens demonstrated normal multimer patterns.
  - All 9 specimens re-tested using optimized specimen pre-dilution demonstrated the same pattern observed at the standard 1:6 dilution.

Figure 1, panel 1 (Sebia)

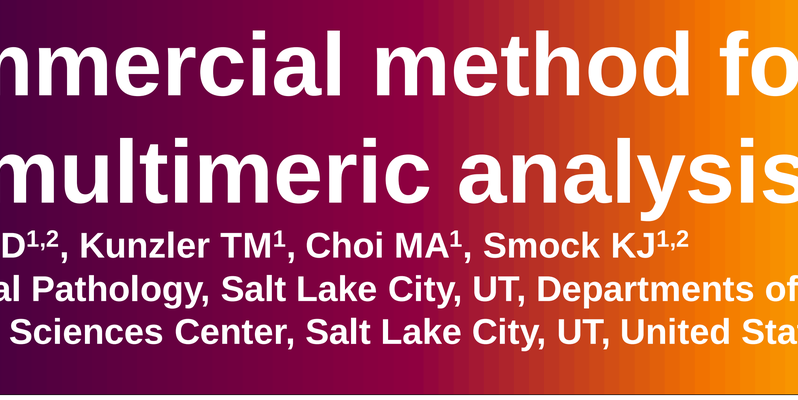

Figure 1, panel 2 (laboratory-developed)

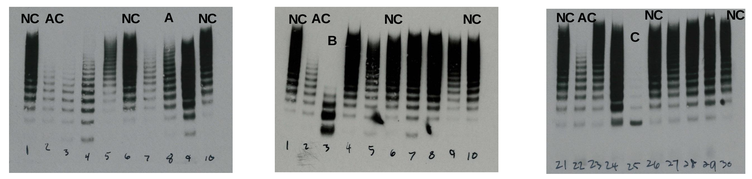

Figure 1: Example comparisons between the Sebia (panel 1) and the laboratory-developed (panel 2) VWF multimer methods in normal and abnormal controls and 3 specimens with abnormal multimeric patterns.

The Sebia method provides both electrophoresis gels and densitometry scans. The scans display the normal control multimeric distribution (transparent) superimposed against each test specimen's multimeric distribution (shaded).

- **NC: normal control demonstrating a normal multimeric pattern.**
- **AC: abnormal control demonstrating missing high-molecular-weight (HMW) multimers.**
- **A: comparison specimen demonstrating a slightly abnormal multimeric pattern with absence of only the highest-molecular-weight multimers.**
- **B: comparison specimen demonstrating missing high- and intermediate – molecular-weight (HMW IMW) multimers.**
- **C: comparison specimen demonstrating missing high- and intermediate – molecular-weight (HMW IMW) multimers.**

- **Precision -** pattern reproducibility was observed in 100% of normal and abnormal controls run on 27 gels (see examples, **Figure 1)**

- **Sensitivity** - Adequate visualization of multimers was determined to require minimal VWF protein concentrations of approximately 5-10% of normal (**Figure 2**).

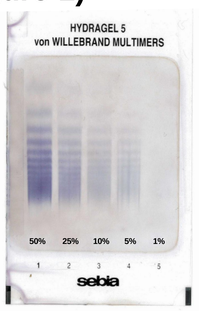

Figure 2: VWF sensitivity study demonstrating adequate visualization of multimers at minimal VWF protein concentrations of 5-10% of normal