Diagnosis of von Willebrand Disease: comparison between conventional versus automated assays

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INTRODUCTION

One of the biggest challenges in diagnosing von Willebrand disease (VWD) is that the traditionally used Ristocetin Cofactor (VWF RCo) assay presents high variability, low sensitivity, and can be influenced by genetic variants. In addition, the test is highly laborious. The new ISTH 2021 guidelines for VWD diagnosis recommend the use of more recent assays.[1] The fully automated INNOVANCE VWF Ac assay (VWF Ac) has emerged as an automated method for measuring VWF activity.

1. James PD, et al. Blood Adv. 2021;5(1):280–300.

AIM

To compare the analytical performance of the INNOVANCE VWF Ac assay to the semi-automated Helena VWF RCo method:

- Evaluate the analytical performance of the commercial INNOVANCE VWF Ac assay based on the binding of VWF to recombinant GPIb (parameter = VWF:Ac), in comparison with the VWF RCo method (parameter = VWF:RCo)
- Assess the relationship between the INNOVANCE VWF Ac and VWF RCo assays in relation to von Willebrand Factor antigen (VWF:Ag) using the INNOVANCE VWF Ag assay
- Compare the cutoff points of 0.6 and 0.7 for the VWF:Ac/VWF:Ag ratio using both the INNOVANCE VWF Ac assay and VWF RCo method.

METHOD

A total of 124 plasma samples were analyzed from 95 patients with previously diagnosed VWD receiving treatment at Fundação Hemominas, Brazil, and from 20 presumed healthy blood donor controls with no indications of blood clotting disorders. Laboratory analyses were conducted to determine von Willebrand factor activity levels using VWF:RCo aggregometry (VWF RCo method) and VWF:Ac turbidimetry (INNOVANCE VWF Ac assay), as well as VWF:Ag turbidimetry (INNOVANCE VWF Ag assay), with subsequent evaluation of correlation between the methods. The study was approved by the local Ethics Committee (CAAE: 64775822.8.0000.5118). Free and informed consent was not required for anonymous samples from outpatients, in accordance with Resolution 466/2012, however informed consent was obtained from the controls.

CONCLUSIONS

We conclude that the INNOVANCE VWF Ac assay demonstrates superior analytical performance compared to the traditional VWF RCo assay for the laboratory evaluation of von Willebrand disease. Analysis of VWF:Ac/VWF:Ag ratio cutoffs showed that using the 0.7 cutoff determined with the INNOVANCE VWF Ac assay and the 0.6 cutoff using the VWF RCo method resulted in the lowest disagreement rate (4%), while using a cutoff of 0.7 for both increased discordance (8.8%). Given that clinical guidelines recommend a cutoff of 0.7, the INNOVANCE VWF Ac assay aligns better with current standards. These analytical advantages support its use for consistent and reliable VWF activity measurement in routine clinical practice.

RESULTS

Table 1 shows that the INNOVANCE VWF Ac assay yielded a higher median functional activity (71.15% for all samples) compared to the traditional VWF:RCo method (64.00% for all samples) This difference was statistically significant ($p < 0.0001$), which shows that the automated method tends to provide consistently higher values. Furthermore, the ratio between functional activity and VWF:Ag level was closer to 1 when using the INNOVANCE VWF Ac assay (0.96) than when using the VWF RCo method (0.86), which may indicate a better alignment with the actual VFW:Ag concentration, assuming that Type 2 VWD is rare in our cohort.

Table 1. Determination of VWF:Ac using the semi-automated VWF RCo assay, the authomated INNOVANCE VWF Ac Assay and VWF:Ag using the INNOVANCE VWF Ag assay, FVIII, and the VWF:RAc / VWF:Ag ratios using theVWF RCo and INNOVANCE VWF Ac assays for 124 patient samples. Results are reported as median and 1st and 3rd quartiles (Q1 ; Q3).

		Median % activity (Q1 ; Q3)		
Parameter	**Assay**	**Patient samples (n = 104)**	**Control samples (n = 104)**	**All samples (n = 124)**
VWF:Ac	VWF RCo assay	59.00 (34.75 ; 85.50)	96.00 (77.00 ; 102.50)	64.00 (40.25 ; 94.00)
	INNOVANCE VWF Ac assay	62.05 (39.30 ; 96.38)	110.95 (95.00 ; 119.25)	71.15 (43.85 ; 105.15)
VWF:Ag	INNOVANCE VWF Ag assay	66.50 (38.50 ; 95.00)	118.50 (95.50 ; 128.00)	74.00 (46.75 ; 108.00)
Factor VIII	INNOVANCE Factor VIII	76.60 (56.00 ; 111.30)	NA[a]	76.60 (56.00 ; 111.30)
VWF:Ac / VWF:Ag	VWF RCo assay INNOVANCE VWF Ag assay	NA[b]	NA[b]	0.86 (0.80 ; 1.00)
	INNOVANCE VWF Ac assay INNOVANCE VWF Ag assay	NA[b]	NA[b]	0.96 (0.90 ; 1.10)

[a] Factor VIII testing was not performed for the control samples. FVIII analysis was only available for analysis in 102 of 104 patient samples.

[B] Ratio results were only calculated for all samples.

Table 2 shows strong correlation and agreement between the VWF RCo method and the INNOVANCE VWF AC assay, reinforcing the analytical equivalence between them.

Table 2. Correlation and Intraclass Correlation Coefficient between the VWF RCo assay and INNOVANCE VWF Ac assay results (n = 124 samples)

Variables	Spearman correlation	Intraclass Correlation Coefficient (ICC)
VWF RCo assay x INNOVANCE VWF Ac assay	0.944*	0.953 (0,900; 0,974)**

*p-value <= 0.05 of Spearman's correlation test; **p-value <= 0.05 of ICC test

Regarding the analysis of the cutoff points for the VWF:Ac/VWF:Ag ratios, the combination of using a cutoff of 0.6 for the VWF Rco assay and a cutoff of 0.7 for the INNOVANCE VWF Ac assay showed the lowest disagreement rate (five samples; 4%), with the same result being found when using 0.6 for both methods. On the other hand, the adoption of a 0.7 cutoff for both ratios resulted in 11 disagreements (8.8%, Table 3). Discordance refers to disagreement between the two assays with respect to the VWF RCo assay method results.

Table 3. Cutoff points for VWF:Ac/VWF:Ag ratios determined using and VWF RCo and INNOVANCE VWF Ac assays

Cutoff point used	Concordant individuals	Discordant individuals
0.6 for both methods	119 (96.0%)	5 (4.0%)
0.6 using VWF RCo assay X 0.7 using INNOVANCE VWF Ac assay	119 (96.0%)	5 (4.0%)
0.7 for both methods	113 (91.2%)	11 (8.8%)

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