Comparison of the Siemens vWF:RCo and the Instrumentation Laboratory HaemosIL von Willebrand factor activity assays for the diagnosis of von Willebrand disease

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Abstract
von Willebrand disease is a bleeding disorder caused either by a quantitative or a qualitative abnormality in the plasma protein, von Willebrand factor. The diagnosis and classification of von Willebrand disease relies upon a panel of screening and confirmatory laboratory tests. In this study the Siemens von Willebrand factor ristocetin cofactor assay was used to test 59 patient samples submitted to Waikato Hospital Laboratory. The samples were also tested using the Instrumentation Laboratory HaemosIL von Willebrand Factor activity assay which is a potential replacement for the Siemens assay. The two assays showed good correlation of results below the 80% level. The HaemosIL assay showed better intra-batch precision, however, inter-batch precision revealed little difference between the two assays. The Siemens assay was more time consuming to perform, however, reagent costs were lower than the HaemosIL assay. This study demonstrated that the HaemosIL assay could be useful in the laboratory investigation of von Willebrand disease. Whether it is a suitable replacement for the von Willebrand factor ristocetin cofactor assay remains unanswered. The lack of sufficient samples with low von Willebrand factor and the unavailability of samples from von Willebrand types and subtypes did not allow for a complete evaluation of the performance of the HaemosIL assay in this study.

Keywords: von Willebrand disease, von Willebrand factor, activity assay

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Introduction
von Willebrand disease (vWD) is a congenital bleeding disorder with an incidence of approximately 1.3% of the human population (1). The disorder varies in its clinical presentation from asymptomatic to severe bleeding. vWD is caused by a mutation in the vWF gene or gene promoter located on chromosome 12. This can result in either a quantitative or qualitative defect in the plasma protein von Willebrand factor (vWF). vWD is subdivided into 3 types and 4 subtypes based on the type of vWF abnormality (2). Type 1 vWD is a partial quantitative deficiency in vWF; Type 2 (subtypes A, B, N and M) is a qualitative defect and Type 3 an extreme quantitative defect where vWF is absent (1).

vWF has an important role in primary haemostasis promoting platelet adhesion to sub endothelial structures such as collagen and platelet to platelet interaction following vessel injury. Both phases are important in the proper formation of the primary platelet plug. vWF also plays a role in the secondary phase of haemostasis preventing the degradation of Factor VIII in the circulation and its rapid inactivation during the clotting process (3).

Patients are diagnosed with vWD following both clinical and laboratory evaluation. The patient may present with a history of excessive bleeding or easy bruising and there may be some related family history. Laboratory tests used most commonly to evaluate fibrin formation, do not directly evaluate plasma vWF. Laboratory investigation is usually comprised of the prothrombin time (PT), activated partial thromboplastin time (APTT) test, the platelet count, the bleeding time test (now mostly historical) and the platelet function assay (PFA) developed for the PFA-100 analyser (4).

A normal PT, normal/abnormal APTT and a prolonged closure time for both the collagen/ADP and the collagen/epinephrine cartridges in the PFA-100 assay, will lead to further laboratory investigations. Confirmatory tests for vWD disorders are the vWF:Ristocetin cofactor assay (vWF:RCo) assay, the vWF antigen assay, the factor VIII coagulation activity assay, and the collagen binding assay (5). The vWF:RCo assay measures plasma vWF by testing the ability of vWF to induce platelet agglutination in the presence of the antibiotic ristocetin (3). The HaemosIL vWF activity assay was manufactured to provide an alternative method to assess in vivo platelet/vWF factor interaction and is a potential alternative to the vWF:RCo (6). The HaemosIL assay directly measures plasma vWF through its ability to bind a latex bound monoclonal antibody (7).

At the Waikato Hospital laboratory the Siemens vWF:RCo activity assay is currently used for the investigation of vWD. In this study the HaemosIL vWF activity assay and the Siemens vWF:RCo assay were used to quantify vWF in plasma. Samples used in the study were those that had been submitted to the Waikato Hospital laboratory for vWD investigation. The two assays were compared in terms of cost, ease of use, precision and correlation, to determine whether the HaemosIL assay could replace the vWF:RCo assay for vWD investigations.

Materials and methods
Siemens vWF:RCo assay
The vWF:RCo is the classic functional assay used for the investigation of disorders associated with vWF. At Waikato Hospital laboratory the Siemens vWF:RCo assay is run on the Behring Coagulation
Timer (BCT) analyser. In the assay plasma vWF binds to the platelet gp1b receptor on lyophilised reagent platelets and agglutinates the platelets in the presence of ristocetin. Agglutinated platelets decrease reagent turbidity which is measured at OD405 nm with reduced light transmittance proportional to plasma VWF (5). vWF levels are obtained using a pre-programmed standard curve and presented as vWF %. Samples with a vWF result below 50% were retested against a calibration curve established for low values.

HaemosIL vWF activity assay
The HaemosIL (Instrumentation Laboratories) assay was performed on a Sysmex CA7000 coagulation analyser. In the HaemosIL assay plasma vWF is determined by turbidometric assay after it reacts with a reagent monoclonal anti-vWF (gp1bα platelet binding site) bound to latex particles (7). Decreased turbidity of the reagent is measured at OD405 with vWF levels determined from a pre-programmed standard curve.

Samples
Blood samples from 59 patients sent to the laboratory for vWD testing over a 10 week period were included in the study. Whole blood collected into 3.2% tri-sodium citrate was centrifuged at 2000g for 10 minutes as soon as possible after arrival in the laboratory. Plasma separated into capped plastic tubes was stored frozen at -20°C. Samples were batch tested weekly following thawing of the plasma for 5 minutes at 37°C. Blood samples were collected into 3.2% tri-sodium citrate from twenty random blood donors to establish a normal range. Plasma samples were separated and stored as for patient samples. Both patient and donor samples were later batch tested.

Instrument calibration and quality control
Performance of the Siemens and IL assays were controlled using Siemens Control plasma N and P and IL ST1 and ST2 controls. Control samples were run for each assay on the two analysers prior to patient testing: control plasma N had a vWF:RCo level of 100% (8) and control plasma P a level of 30% (9). The IL ST1 control plasma had a value for the VWF Activity Assay of 55% and the ST2 20%. Valid results for control plasmas required values within ± 2 standard deviations from the target mean before sample testing could proceed. Calibration curves were derived for the vWF:RCo assay using the Siemens standard normal plasma and for the HaemosIL assay the IL calibrator plasma. Each curve was prepared as part of the calibration cycle for each assay on both analysers.

Statistical analysis
The results of the vWF testing were evaluated by Deming regression analysis and Spearman’s ranking. In addition, results data was compared by Bland Altman plot. These provided correlation and mean difference values for both assays. Both procedures utilised Microsoft Excel and Analyse-it software (10). Intra-batch precision of the two assays was assessed from the coefficient of variation (CV) calculated from 10 replicate tests on a single plasma sample. Inter-batch precision was assessed using CV values obtained from the testing of the control plasmas N and P with batches of test plasmas. The same method was used to establish the precision of the HaemosIL assay using IL ST1 and ST2 plasmas.

Results
Testing of the 59 patient samples using the Siemens and the IL assays provided a range of results from 30.4 - 259.30%. Deming regression was used to compare the correlation of the two methods. With the ratio of variances set at 1 this provided a slope of 0.925 (0.705 to 1.145 - 95% confidence interval). Results showed good correlation between the two methods with $r = 0.901$ (Figure 1).

The results were also compared using a Bland–Altman plot to demonstrate the overall difference between the two methods. A mean difference of -6.68% (Figure 2) showed a close relationship between the results. At lower levels near the cut-of points the results were more accurate. The Spearman non-parametric test of correlation provided an $r$ value of 0.92 and a $p$-value of <0.0001. His confirmed the close similarity of results demonstrated by the Bland Altman plot.

Precision studies
Intra batch precision for the two assays was established by retesting a patient sample with low vWF and a healthy donor plasma with a normal level of vWF. Sample testing was repeated 10 times for each sample in the same run. Mean values were used to establish the coefficient of variation. Precision for the IL assay was 5.3% and 1.3% for low and normal levels of VWF respectively (Table 1). The CVs for the Siemens assay for the same samples were 8.2% and 8.6% (Table 1) respectively. Inter batch precision was determined from the results of the control plasmas P and N (Siemens assay) and the ST1 and ST2 controls (IL assay) run with each batch of patient samples. The CVs for the Siemens assay were higher than those for the HaemosIL assay, a finding reported by others (7, 11). The CVs for the HaemosIL assay were a little higher than expected, however, numbers were low (n=8) which may have overly skewed the results (Table 2).
Table 1. Intra-batch precision

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<tr>
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<th>Siemens assay</th>
<th>IL assay</th>
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<tbody>
<tr>
<td></td>
<td>Low vWF</td>
<td>Normal vWF</td>
</tr>
<tr>
<td>Mean</td>
<td>47.7</td>
<td>118.0</td>
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<tr>
<td>SD</td>
<td>3.9</td>
<td>10.1</td>
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<tr>
<td>CV</td>
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<td>10.1%</td>
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Table 2. Inter-batch precision

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<tr>
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<th>Siemens assay</th>
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<tbody>
<tr>
<td></td>
<td>Control P</td>
<td>Control N</td>
</tr>
<tr>
<td>Test number</td>
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</tr>
<tr>
<td>Mean</td>
<td>26.4 (30%)</td>
<td>97.7 (100%)</td>
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<tr>
<td>CV</td>
<td>10.3%</td>
<td>9.4%</td>
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Reference range

The reference range (Cl 95% ± 2SD) for the IL assay was calculated from the testing of the plasma from 20 random (not selected for ABO group) healthy blood donors (Table 3). The results provided a reference range of 44.9 - 145.8%. This was comparable to the reference range of 50-150% for the Siemens assay currently in use at Waikato Hospital.

Table 3  HaemosIL vWF activity assay reference range

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<table>
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<tbody>
<tr>
<td>Mean</td>
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<tr>
<td>± 2 Standard Deviations</td>
<td>50.4</td>
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<tr>
<td>Reference Range</td>
<td>44.9 - 145.8%</td>
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Discussion

The results of the 59 patient samples tested using the HaemosIL and Siemens assays showed good correlation r = 0.901 (Figure 1) and a small mean difference of -6.68% between the two methods. The similarity of the results was closest in the lower range of normal, with results above 80% demonstrating a greater difference (Figure 2). While not ideal, the performance of the HaemosIL assay was still considered acceptable as differences in the upper range present less of an interpretative issue compared to those close to the 50% cutoff. The five outliers (Figure 2) were each above 80% with 3 of the 5 above 180%. The reasons for this level of disparity cannot be satisfactorily explained, however, the two assays measure plasma vWF differently and this may have been a contributing factor. Removal of these from the Bland Altman calculation did not alter the mean difference of values overly and so were retained for completeness.

Studies to demonstrate intra-batch precision showed the HaemosIL assay to be more precise than the Siemens assay. This has been reported by others. Poorer performance of the Siemens assay in inter-batch precision compared to the HaemosIL assay has also been reported (7,11). This was the finding in this study although difference between the two was not marked. Of note was the higher than expected CV values for inter-batch precision testing. A possible explanation for this was the relative inexperience of the operator and the fact that only a low number of results were available for the calculation.

The Siemens vWF:RCo assay took longer to perform as the BCT analyser together with the loss of laboratory bench space to store the machine, makes retaining the Siemens assay increasingly more difficult to justify. At Waikato Hospital the Sysmex CA7000 is used for the Factor VIII, vWF:AG and vWF activity assays for the investigation of vWD. The use of this machine and the HaemosIL assay, as a replacement for the vWF:RCo assay, has obvious attraction. In addition, the elimination of extra sample handling and storage and the risk of transcription error in results reporting required for the Siemens assay, further favour the centralising of vWF testing on to a single platform.

Reagent costs for the Siemens assay are less per test than those of the HaemosIL assay. The Siemens vWF:RCo reagent kit contains five vials each with 4mL of reagent. Reagent costs per test were estimated to be approximately NZ$3.16. Once reconstituted the Siemens reagent had a 48hr shelf-life at 4°C. The HaemosIL assay reagent kit contained 2 vials of 4.5mL of reagent with the cost per test estimated to be NZ$9.10. Once reconstituted the HaemosIL reagent was stable for 1 month at 4°C. Reagent stability is an advantage with the HaemosIL assay enabling vWF activity to be assayed in urgent cases. This is currently not offered because of excessive reagent wastage with the Siemens assay. Preparation of reagents for both assays was simple, however, unlike the Siemens reagents which were ready for use immediately after reconstitution (Siemens package insert), the HaemosIL reagents had to be left for 30 minutes following reconstitution before use. This delay could result in a significant disruption if the analyser unexpectedly ran out of reagent while testing a batch of samples.

Conclusion

The findings of this study showed that the HaemosIL assay has the potential to become an alternative to the Siemens vWF:RCo assay or another laboratory test useful in the diagnosis of vWD. While both methods measure plasma vWF, each does so employing a different approach. Whether the two assays measure the same thing and whether both are able to similarly predict in vivo platelet adhesion in response to vessel injury remains the question. This probably mitigates against the replacement of the HaemosIL assay in place of the classic vWF:RCo assay.

The reference range calculated for the HaemosIL assay was very similar to that in use for the Siemens vWF:RCo assay at Waikato Hospital. While this was derived from a fairly small number of samples, this supports the other results of this study indicating similar performance characteristics of the two assays. Correlation studies showed good overall correlation of the results especially around the important 50% cutoff figure. Results were less well correlated with values greater than 80%. Precision studies also showed the HaemosIL assay to have improved intra and inter batch precision over the Siemens assay.

This study was limited by the finding of only three samples with a vWF:RCo level of less than 50%. Overall seven samples produced results that were ≥10% of the 50% cutoff, the range in which the potential exists for patient diagnosis to be affected. A change to the HaemosIL assay in place of the Siemens vWF:RCo assay may be possible sometime in the future. Before this can happen more work to evaluate the performance of the HaemosIL assay against samples close to the 50% cutoff is required. In addition, evaluation of assay performance against samples from known vWD types and subtypes would also be required. Because of this it is not likely that many laboratories will replace the traditional Siemens vWF:RCo with the HaemosIL vWF activity assay. Instead it may find a place as another useful assay in the often technically challenging evaluation of vWD.

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Author contributions

RP conducted the study and substantially drafted the main article, HS supervised the laboratory work and critically reviewed the main article, and CK substantively drafted the final article for critical content. The authors declare no conflicts of interest.

References


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