Automated Chemiluminescence Assay for von Willebrand Factor Antigen and Ristocetin Cofactor

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Introduction

Von Willebrand factor (VWF) is a multimeric plasma glycoprotein that acts as a mediator of platelet adhesion to subendothelial collagen after vascular injury and as a carrier for coagulation factor VIII in plasma. Quantitative or qualitative defects in VWF can produce different types of von Willebrand disease characterised by significant bleeding disorders. The typical screening panel used for vWD diagnosis and typing consists of the measurement of VWF antigen levels (VWF:Ag), the assessment of VWF’s ability to bind platelets in the presence of Ristocetin (VWF:RCo), and the measurement of FVIII activity. Von Willebrand Collagen Binding determination is used as a supplemental assay in VWD typing, and is a measure of VWF interaction with collagen.

The VWF:RCo is one of the most important tests for VWD diagnosis as it enables discrimination between qualitative (type 2) and quantitative defects (VWD type 1 or 3) when used in combination with the VWF:Ag assay. It is a widely used assay to estimate the functional properties of VWF, because it mimics platelet interaction with the GPIb/IX complex.

The aim of this study was to compare the ACL AcuStar chemiluminescence immunoassay for VWF:Ag and VWF:RCo with standard testing using the STAGO STA-R Evolution employing a latex immunoassay assay for VWF:Ag and a platelet agglutination based VWF:RCo assay.

All samples were tested by an ELISA based, solid phase collagen capture, collagen binding assay; Asserachrom CBA from STAGO.

In this study we tested a cohort of 51 samples with a range of both normal and von Willebrand disease patients. Citrated plasmas were analysed on each platform and were all tested on the same day. There were nine type 2M, four type 2B and two type 2A included in the cohort.
Methods

Blood was drawn by clean venepuncture with citrate anticoagulant at a ratio of 1:9 (v/v, sodium citrate/blood, final citrate concentration 0.109M). Platelet poor plasma (PPP) was prepared by centrifugation at 2500 g for 15 minutes at 15°C then frozen in aliquots and stored at -80 °C until analysis.

Patient citrate samples from IMVS were sent to Alfred Haematology for testing on the AcuStar analyser. All samples were tested on the same day for all analytes using HemosIL AcuStar VWF:Ag, HemosIL AcuStar VWF:Ag, platelet agglutination based VWF:RCo assay on a STAGO STA-R, STAGO VWF:Ag Latex immunoassay and with the Asserachrom VWF:CBA. Statistical analysis was performed using MedCalc® (version 13.2.2.0).

An automated chemiluminescent immunoassay, HemosIL AcuStar von Willebrand Factor Ristocetin Cofactor Activity has been developed for the quantitative determination of VWF Ristocetin Cofactor activity in human citrated plasma. This method is a two step immunoassay to quantify VWF:RCo activity in human citrated plasma using magnetic particles as a solid phase and a chemiluminescent detection phase. In the first step, the sample is mixed with the ristocetin containing assay buffer and magnetic particles coated with a recombinant fragment of glycoprotein receptor of VWF (rGPIbα) by means of a specific monoclonal antibody which orientates the GP1bα fragment to allow interaction with the VWF of the patient sample in the presence of ristocetin.

The VWF present in the sample binds to the magnetic particles proportionally to its ristocetin cofactor activity. After magnetic separation and washing, an anti-VWF monoclonal antibody labelled with isoluminol is added and incubated in the second step. After a new magnetic separation and washing, two triggers are added and the resulting chemiluminescent reaction is measured as relative light units by the ACL AcuStar optical system. The light units are directly proportional to the VWF:RCo activity concentration in the sample.
Results

Fig 1. Correlation of VWF:Ag by AcuStar & STA-R. Correlation Coefficient 0.9934, N=51.

Fig 2. Correlation of VWF:RCo by AcuStar and STA-R. Correlation Coefficient 0.9547, N=51.

Fig 3. Bland Altman Plot of RCo Assays. 95% limits of agreement -21.1 to 13.5

Fig 4. Correlation of STA-R VWF:Ag & CBA. Correlation Coefficient 0.8936, N=51.

Fig 5. Correlation of AcuStar VWF:Ag & CBA. Correlation Coefficient 0.9070, N=51.

Fig 6. Correlation AcuStar RiCo and CBA. Correlation Coefficient 0.9586, N=51.
Results and Conclusions

The Ristocetin Cofactor assay evaluated the quality of the VWF in the sample, because it reproduces in vitro the first VWF interactions with the platelet receptor and remains the standard method for determination VWF activity.

There was good correlation between VWF:Ag assay with a correlation coefficient (r value) of 0.9934 (figure 1, 95% confidence interval of 0.9885 to 0.9963) and 0.9547 (figure 2, 95% confidence interval of 0.9217 to 0.9740) for the VWF:RCo. Bland Altman analysis (figure 3) illustrated slightly lower values with the VWF:RCo AcuStar (bias -3.8) compared with the STAGO STA-R. This is reflected in the different reference ranges; the reference range for the AcuStar Ristocetin assay is 45.6 to 176.3 IU/dL compared to 50 to 150 IU/dL for the platelet agglutination method. The VWF:Ag AcuStar illustrated a slightly lower values (bias -6.4, data not shown) compared to the STA-R.

There was good agreement between the AcuStar VWF:RCo assay and the agglutination method and the for the different VWF:Ag methods. The correlation coefficient between the AcuStar VWF:Ag and collagen binding was 0.9070 and for the STA-R latex immunassay for the VWF:Ag was 0.8936 (figures 4 and 5 respectively ). The correlation coefficient for the AcuStar VWF:RCo and collagen binding was 0.9586 (figure 6).

Importantly, all of the type 2 patients were identified as such by the AcuStar method, with the ratio of VWF:RCo to VWF:Ag <0.5 for all.

In this study the HemosIL AcuStar VWF:RCo was compared with our traditional platelet agglutination method using a commercial source of fixed platelets from Siemens on a STAGO STA-R both on normal control samples and patient samples. The reference ranges for the assays were different, with the AcuStar having a manufacturer's target with a lower limit of 45.6 IU/dL compared to 50 IU/dL for the agglutination method.

The AcuStar VWF:Ag and VWF:RCo compared well with our conventional testing systems. There was greater discordance with the VWF:RCo testing compared to the VWF:Ag and this may be due to a variation differences in technology. The AcuStar delivers results in 24 minutes and provides up to 32 tests per hour. It is fully automated, easy to use and has a broad dynamic range.

The negative bias is consistent with the different reference ranges and suggests that the reference range for the AcuStar method would need to be confirmed.