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Full Length Article

Evaluation of a von Willebrand factor three test panel and chemiluminescent-based assay system for identification of, and therapy monitoring in, von Willebrand disease

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ABSTRACT

von Willebrand disease (VWD) is reportedly the most common bleeding disorder and arises from deficiency and/or defects of von Willebrand factor (VWF). Laboratory diagnosis and typing of VWD has important management implications and requires a wide range of tests, including VWF antigen (VWF:Ag) and various activities, involving differential identification of qualitative vs quantitative VWF defects. We have assessed a new hemostasis instrument, the chemiluminescent assay based ACL AcuStar™, and an associated HemosIL AcuStar three test panel comprising VWF:Ag, VWF ristocetin cofactor (VWF:RCo) and VWF collagen binding (VWF:CB) (Instrumentation Laboratory, Bedford, Ma. USA) for ability to identify VWD, to help provisionally type VWD, and for potential use in therapy monitoring. This test system was compared to previously evaluated and validated test systems including VWF:RCo on CS-5100 and BCS analyzers, the new Siemens INNOVANCE assay (VWF Ac) on CS-5100, and VWF:Ag and VWF:CB assays performed by automated ELISA. We employed a large total sample test set (n = 535) comprising plasma and platelet-lysate samples from individuals with and without VWD, some on treatment, normal plasmas, and normal and pathological controls. We also evaluated desmopressin (DDAVP) responsiveness, plus differential sensitivity to reduction in high molecular weight (HMW) VWF. The chemiluminescent test panel (VWF:Ag, VWF:RCo, VWF:CB) showed good comparability to similar assays performed by alternate methods, and broadly similar data for identification of VWD, provisional VWD type identification, DDAVP and VWD therapy, and HMW VWF sensitivity, although some notable differences were evident. The chemiluminescent system showed best low level VWF sensitivity, and lowest inter-assay variability, compared to all other systems. In conclusion, we have validated theACL AcuStar and the chemiluminescent HemosIL AcuStar VWF test panel for use in VWD diagnostics, and have identified some favorable characteristics that may improve the future diagnosis of VWD.
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1. Introduction

von Willebrand disease (VWD) is reportedly the most common congenital bleeding disorder and arises from deficiency and/or defects of von Willebrand factor (VWF), an adhesive plasma protein essential for effective primary haemostasis [1,2]. Clinical identification, diagnosis and typing of VWD are supported by laboratory testing, but this remains imperfect for many reasons. First, VWD is extremely heterogeneous, as evidenced by the fact that VWF performs many adhesive functions that enables effective capture and localization of platelets and factor VIII (FVIII) to damaged vasculature, to facilitate both primary and secondary hemostasis and arrest bleeding. VWF accomplishes this role because it can bind to platelets (via several receptors, but most notable glycoprotein Ib (GPIb)), sub-endothelial matrix components (most notably collagen), and FVIII (thereby preserving its function) [3]. Therefore, defects may occur anywhere within *VWF*, leading to a wide variety of clinical and laboratory phenotypes. Second, the laboratory tests used to aid identification, diagnosis and typing of VWD are very heterogeneous in methodology and diagnostic efficacy. These tests are also imperfect, reflecting different procedures of varied sensitivity to VWF level and activity, as well as other limitations including poor reproducibility (or high assay variability), poor sensitivity to low levels of VWF, and variable sensitivity to high molecular weight (HMW) forms of VWF [4–8].

Abbreviations: CLA, Chemiluminescent assay; ELISA, enzyme linked immunosorbent assay; FVIII:C, FVIII coagulant; GPIb, glycoprotein Ib; HMW, high molecular weight (VWF); LIA, latex-particle immuno-assay; VWD, von Willebrand disease; VWF, von Willebrand factor; VWF Ac, VWF INNOVANCE activity (assay); VWF:Ag, VWF antigen; VWF:CB, VWF collagen binding (assay); VWF:RCo, VWF ristocetin cofactor (assay).

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The current classification scheme from the International Society on Thrombosis and Haemostasis (ISTH) identifies six different types of VWD [9]. For cases reflecting a minor quantitative deficiency of VWF but without a formal diagnosis of VWD, the concept of 'low VWF' as a risk factor for bleeding has alternatively been proposed [10]. Quantitative VWD defects are identified within type 1 (representing a partial deficiency of VWF), and type 3 (representing 'complete' deficiency of VWF). In type 1 VWD, the VWF produced in lower amount is 'functionally normal'. Qualitative VWF defects are characterized within type 2 VWD, which actually represents a very heterogeneous group, comprising (i) 2A VWD (loss of HMW VWF), (ii) 2B VWD (enhanced functional binding of VWF that leads to loss of HMW VWF and typically mild thrombocytopenia), (iii) 2N VWD (loss of VWF-FVIII binding), and (iv) 2M VWF (VWF dysfunction not associated with loss of HMW VWF).

The proper identification of VWD and differentiation of VWD type is important for therapeutic management [6,11]. In normal laboratory practice, identification of VWD and differentiation of type can be determined by laboratory testing encompassing a broad panel of different validated tests [1,2,4–10]. The majority of laboratories perform FVIII coagulant (FVIII:C) and VWF antigen (VWF:Ag) [1,5], which respectively measure levels of FVIII activity and VWF protein, the latter most commonly using either ELISA (enzyme linked immunosorbent assay) or LIA (latex-immuno-assay) technologies. VWF 'activity' can be assessed by a wide variety of methodologies, including ristocetin cofactor (VWF:RCo), collagen binding (VWF:CB) and newer direct GPIb binding assays (e.g., Siemens INNOVANCE VWF Ac) [8,12–15]. Which assays, and how many of these laboratories use as a diagnostic test panel, and what methodology laboratories employ within each individual assay, strongly impacts the accuracy of VWD identification, diagnosis and typing [5,8]. VWF:RCo represents the most commonly performed activity based test [1,2,4–10], usually by platelet agglutination assay using aggregometry or automated methods with modern hemostasis instruments, and sometimes by LIA technology [8]. Direct GPIb binding assays provide similar values to VWF:RCo in published studies [8,12–15]. VWF:CB, although typically performed by fewer laboratories, is nonetheless an important activity assay, usually performed by ELISA [1,2, 4–10], and whose omission will compromise VWD identification, diagnosis and typing [5,8].

Moreover, laboratories and clinicians also need to deal with many pre-analytical variables affecting laboratory test results [16], as well as significant intra-patient variability in test results, including that VWF and FVIII are acute phase proteins that increase at times of stress and also during pregnancy [17]. These considerations further challenge diagnosis, since a normal test result does not always exclude VWD (might be acute phase or pregnancy increase in VWF, or type 2 VWD with normal VWF:Ag) and an abnormal test result does not always confirm VWD (could be one of many pre-analytical events).

In the current study, we have evaluated a fairly new instrument in the hemostasis laboratory armamentarium, the ACL AcuStar™, which employees chemiluminescent technology, for its ability to identify VWD, to help type VWD, and for potential use in therapy monitoring. We have used a three VWF test panel on this instrument, namely HemosIL AcuStar VWF:Ag, HemosIL AcuStar VWF:RCo and HemosIL AcuStar VWF:CB, and compared findings to previously evaluated and validated VWF test systems, employing a large total sample test set (n = 535) comprising a variety of patient, normal and control material. This is the first such study to include an evaluation of such a three test system on a single platform.

2. Methods

2.1. Assays and instrumentation

The tests evaluated in this study are summarized in Table 1.

Table 1
Summary of VWF test methods comparatively evaluated in this study.

VWF assay	Description
VWF:Ag	• Assessment of VWF protein level using an 'antigen' assay. • Historically performed by manual ELISA. • Reference assay performed in this study by automated ELISA using an ELISA workstation (Best 2000). • Comparator assay was an automated chemiluminescent based assay using an AcuStar.
VWF:RCo	• Assessment of VWF activity level utilizing ristocetin and (usually) an 'agglutination' assay. • Historically performed by agglutination using an aggregometer (originally) or BSC instrument (from 2008). • Reference assay performed in this study by automated agglutination assay using a CS-5100 instrument. • Comparator assays were automated chemiluminescent based VWF:RCo assay using an AcuStar and Innovance VWF Ac assay on CS5100 (see below).
VWF:CB	• Assessment of VWF activity level utilizing collagen. • Historically performed by manual ELISA. • Reference assay performed in this study by automated ELISA using an ELISA workstation (Best 2000). • Comparator assay was automated chemiluminescent based assay using an AcuStar.
VWF Ac	• Siemens INNOVANCE VWF 'activity' assay. • Assessment of VWF activity level utilizing a direct VWF-Glycoprotein Ib binding method. • The system employs two gain of function Glycoprotein Ib mutations within a recombinant molecule that facilitates VWF binding. • Performed in this study by automated LIA using a CS-5100.

Abbreviations: ELISA, enzyme linked immunosorbant assay; LIA, latex-particle immunoassay; VWF, von Willebrand factor; Ag, antigen; RCo, ristocetin cofactor; CB, collagen binding; Ac, activity.

2.2. Reference and historical methods

FVIII:C was assessed for patient samples as part of their original analysis within our standard VWD diagnostic test panel, using a one stage clot-based assay on either a Behring BCS or (more recently) CS-5100 analyzers (both from Siemens Healthcare, Marburg, Germany) and Siemens reagents, but has not otherwise been formally assessed in the current report. Our standard VWF:Ag was performed as an in house sandwich ELISA assay, essentially as previously extensively reported by our laboratory (refer to [1,7,8,12] as key references), but now performed on a BioKit BEST 2000 ELISA workstation (Biokit, Lliçà d'Amunt, Barcelona, Spain), using polyclonal antibodies from Dako (Glostrup, Denmark; rabbit anti-human VWF; catalogue no. A0082) for coating 96-well plates (Linbro Titertek EIA plate; ICN Biomedicals, Aurora, OH, USA) and Dako horse radish peroxidase labeled rabbit anti-human VWF (catalogue no. P0226), for VWF detection. Historically, a similar approach was used to perform manual/semi-automated ELISAs. Our current standard VWF:CB was performed in parallel with VWF:Ag on the same ELISA workstation, also as an in house sandwich ELISA assay and essentially as previously extensively reported by our laboratory (refer to [1,7,8,12] as key references), currently using bovine collagen (reflecting a type I/III collagen mixture) from ICN Biomedicals (catalogue no. 193492) for coating 96-well plates (Pierce Maleic Anhydride activated plates; catalogue no. 15110; Thermo Scientific, Rockford, USA) and Dako horse radish peroxidase labeled rabbit anti-human VWF (catalogue no. P0226) for VWF detection. Historically, a similar approach was used to perform manual/semi-automated ELISAs, but using alternate ELISA plates (Linbro Titertek EIA plate; ICN Biomedicals, Aurora, OH, USA). Our standard VWF:RCo was performed as an agglutination assay, essentially as also previously extensively reported by our laboratory (refer to [1,7,8,12] as key references), currently on a CS-5100 analyzer, using Siemens reagents (BC VWF reagent; catalogue no. 10446425). Historically, we originally used in-house fixed platelets on a platelet aggregometer, and later the same system we currently employ but with testing performed on a Behring BCS analyzer. For the

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