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ARTICLE

Evaluation of ELISA procedures to detect von Willebrand Factor with monoclonal antibodies

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ABSTRACT: (Evaluation of ELISA procedures to detect von Willebrand Factor with monoclonal antibodies). The von Willebrand Factor is a multimeric glycoprotein responsible for the promotion of platelet adhesion and aggregation at sites of vascular injury, and for FVIII stabilization. Abnormalities on this protein are responsible for diverse types of von Willebrand Disease. In the present study, monoclonal antibodies against human von Willebrand Factor were developed as a means to improve von Willebrand Disease research and diagnosis. Monoclonal antibodies were tested for their ability to bind to purified and plasmatic von Willebrand Factor. Monoclonal antibodies vW22 and vW23 were found to bind only purified von Willebrand Factor, and monoclonal antibodies vW18 and vW21 were found to bind purified and plasmatic von Willebrand Factor. Antibodies vW18 and vW21 were used to perform a sandwich-enzyme-linked immunosorbent assay to detect and quantify von Willebrand Factor concentration in plasma samples from 143 coagulopathy patients and 12 healthy blood donors. The assay showed high performance, with strong correlation and agreement in results, when compared to electroimmunoassay (Rs = 0.843 and K = 0.691 with $p<0.001$) and a commercial ELISA (Rs = 0.930 and K = 0.819 with $p<0.001$). S-ELISA proved to be a useful tool in vWF quantification tests in Brazilian specialized laboratories as an alternative to imported tests.
Key words: von Willebrand Factor, von Willebrand Disease, Monoclonal antibodies, ELISA.

RESUMO: (Avaliação de procedimentos ELISA para detectar o Fator von Willebrand com anticorpos monoclonais). O Fator von Willebrand é uma glicoproteína multimérica responsável pela promoção da adesão e agregação de plaquetas nos locais de lesão vascular e pela estabilização do FVIII. Anormalidades na função ou estrutura desta proteína são responsáveis por diversos tipos de doença de von Willebrand. Para auxiliar na pesquisa e diagnóstico da doença de von Willebrand, este estudo desenvolveu anticorpos monoclonais contra fator von Willebrand humano. Os anticorpos monoclonais foram testados quanto à sua capacidade de se ligar ao fator von Willebrand purificado e plasmático. Os anticorpos monoclonais vW22 e vW23 ligaram-se somente ao fator von Willebrand purificado, e anticorpos monoclonais vW18 e vW21 ligaram-se tanto ao fator von Willebrand purificado como ao plasmático. Anticorpos vW18 e vW21 foram utilizados no desenvolvimento de um ELISA *sandwich* para detectar e quantificar a concentração de fator von Willebrand no plasma em uma amostra de 143 pacientes com coagulopatias e 12 doadores de sangue saudáveis. O teste mostrou alto desempenho, com forte correlação e concordância quando comparado com uma imunoeletrofose (Rs = 0,843 e K = 0,691 $p<0,001$) e um ELISA comercial (Rs = 0,930 e K = 0,819 $p<0,001$). O ELISA-S desenvolvido no presente trabalho mostrou um bom desempenho para ser usado como teste de quantificação vWF em laboratórios especializados no Brasil como alternativa para testes importados.
Palavras-chave: fator von Willebrand, Doença de von Willebrand, Anticorpo Monoclonal, ELISA.

INTRODUCTION

The von Willebrand Factor (vWF) is a multimeric glycoprotein synthesized by endothelial cells (Jaffe *et al*. 1973) and megakaryocytes (Nachman *et al*. 1977), being stored in the Weibel-Palade bodies of endothelial cells and in α-granules of platelets to be released upon stimulation (Wagner 1990). In plasma, vWF levels are in the 5-10-µg/mL range, where it performs two major functions in hemostasis: (i) promotion of platelet adhesion and aggregation at sites of vascular injury, and (ii) stabilization and protection of the Factor VIII (FVIII) against proteolytic degradation, forming a non-covalent complex with this protein (Ruggeri 1999). Besides alterations in vWD concentration, structural and functional abnormalities are responsible for the several types of von Willebrand Disease (vWD) (Sadler *et al*. 2000). vWD is the most common of the hereditary bleeding diseases; its prevalence is estimated to be as high as 1% (Nilsson 1977, Holmberg & Nilsson 1985, Rodeghiero *et al*. 1987, Bloom 1991, Werner *et al*. 1993). vWD type 1 is a quantitative deficiency with a reduced plasma level of vWF. In turn, vWD type 2 (2A, 2B, 2M, and 2N) are qualitative deficiencies, while vWD type 3 is characterized by almost complete deficiency of vWF (Nichols & Ginsburg 1997).

Due to the heterogeneity of vWD and the difficulties of its diagnosis, several methods to determine vWF levels

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have been developed in order to understand the mechanisms involved in this pathology, and to improve diagnosis (Favaloro 2001, Laffan *et al*. 2004). Standard diagnostic screening assays for vWD diagnosis include the analysis of Factor VIII activity, vWF Antigen (vWF:Ag) mensuration, and the evaluation of Ristocetin Cofactor Activity (vWF:Rco) (Favaloro *et al*. 2005). In the past, vWF:Ag mensuration based on Laurell rocket electrophoresis was widely used (Electro immunoassay – EIA) (Laurell 1966, Zimmerman *et al.* 1975). However, the technique has been largely replaced by the ELISA method, a more sensitive, specific, and standardized protocol (Budde & Schneppenheim 2001). Standardized ELISA protocols have been developed as a tool to quantify vWF and evaluate its functionality (Cejka 1982, Ingerslev 1987, Siaka *et al*. 2003, Caron *et al*. 2006, Ghosh *et al*. 2006); however, because of the heterogeneous nature of ELISA protocols, one single test does not afford a precise vWD diagnosis. Although the laboratory diagnosis of vWD type 2 and vWD type 3 is reasonably well defined, the laboratory diagnosis of vWD type 1 still poses a challenge. Several studies have tried to elucidate the complex etiology of this disease and determine the best approach to diagnose it (Pruthi 2006). Laboratory tests results are compatible with vWD type 1 if the levels of both vWF:RCo and vWF:Ag are <50 % of normal values (measured by a standardized calibrator) in at least two determinations (Laffan *et al*. 2004). However, since there is some degree of overlap between results from healthy subjects and patients with mild vWD type 1, a set of more dependable tests is needed to differentiate healthy individuals from vWD patients. In this context, monoclonal antibodies (MAbs) may be a useful tool in research and immunological assays involving vWD, as a means to improve diagnosis. This work describes the development and production of MAbs against vWF:Ag, and their performance in an alternative sandwich-ELISA (S-ELISA) for quantification of vWF.

MATERIAL AND METHODS

Plasma Samples

Plasma samples were obtained from patients referred for screening of hereditary coagulopathies in Laboratório de Hemostasia do Departamento de Genética da Universidade Federal do Rio Grande do Sul. The ethical aspects of the research were respected, and the project is approved by the Research and Ethics Committee of UFRGS (number 2008092). Samples from 143 patients with different disorders were used in this study to represent the real diversity in hereditary coagulopathy screening. Samples utilized were from patients with vWD type 1, vWD type 2, vWD type 3; patients with hemophilia A or B, patients with some bleeding symptoms and family history of vWD type 1, but with normal results in screening tests (PvWD type 1); patients with other bleeding diseases (Factor VII deficiency or combined FVIII/Factor V deficiency); and patients with normal results in screening tests (no coagulopathies). Plasma samples obtained from 12 healthy blood donors were used as control plasma (Table 1). Plasma from patients and healthy blood donors were collected by venipuncture in tubes containing 3.8% trisodium citrate (1:10, V:V). Platelet-poor plasma was obtained by centrifugation at 2,500 g for 15 min. Plasma samples were stored at -80 °C and thawed in water bath at 37 °C before use. In all experiments the vWF calibrator provided by Diagnostica Stago (Diagnostica Stago, Asnières-sur-Seine, France) was used as standard plasma.

Purification of vWF:Ag

The partially purified FVIII/vWF antigen used for immunization and antibody production was prepared by Fisher and colleagues in the Laboratório de Hemostasia do Departamento de Genética da Universidade Federal do Rio Grande do Sul, based on the study by Yang *et al*. (1977) and as described elsewhere (Fischer *et al*. 1996). The vWF:Ag was purified from lyophilized commercial FVIII concentrate (LanderlanTM, Madrid, Spain). The concentrate was reconstituted with imidazole buffer pH 7.3 containing sodium azide, and centrifuged at 650 g for 20 min at 10 ºC. The supernatant was applied to a 65.0 cm x 2.6 cm Sepharose 4BTM column (Pharmacia,Uppsala, Sweden). vWF:Ag fractions were assayed by EIA using commercial anti-vWF:Ag serum. Purified aliquots of vWF:Ag adjusted to 225 µg/mL were stored at -20ºC.

Production of MAbs against human vWF

To generate MAbs against vWF, adult female BALB/c mice were intraperitoneally inoculated with 20 µg of partially purified vWF emulsified with complete Freund's adjuvant (Sigma-Aldrich, St. Louis, USA). Four weeks later, mice received an additional injection of antigen with incomplete Freund's adjuvant, and another week later the animals were intravenously boosted with 10 µg of the antigen without adjuvant. After three days, spleen cells were fused with SP2/0 mouse myeloma cells using polyethylene glycol as fusing agent, according to the method described by Köhler & Milstein (1975).

Table 1. Plasma samples utilized in ELISA development and standardization.

Samples	N	vWF levels
Controls	12	Normal
Haemophilia A or B	26	Normal
vWD Type 1	23	Decreased[a]
PvWD Type 1	17	Normal[a]
vWD Type 2	3	Normal/Decreased[a]
vWD Type 3	4	Marked decreased/absent[a]
Other coagulopathy	2	Normal
No coagulopathy	68	Normal
Total	**155**	

a. The vWD type 1 and 2 have the levels of vWF:Ag <50 % of normal values and vWD type 3 has the levels of vWF:Ag <3 % of normal values (measured by a standardized calibrator). Expected levels modified from Pruthi (2006).

Supernatants of hybridoma were screened for antibodies against the partially purified vWF antigen by indirect ELISA using anti-mouse IgG antibody conjugated with peroxidase (Sigma-Aldrich, St. Louis, USA) as signal-generating component. Cloned hybridoma cells were injected into pristane-sensitized BALB/c mice for production of ascites, and the immunoglobulins were purified by sepharose-protein G affinity chromatography (GE Healthcare Wisconsin, USA). Immunoglobulin subclass was determined by MAb isotyping kit (Sigma-Aldrich, St. Louis, USA).

Polyclonal antiserum purification

Antiserum against vWF utilized in standard EIA and ELISA was produced as described by Fischer *et al*. (1996). Polyclonal antiserum was produced by immunization of rabbits by subcutaneous injections of vWF:Ag emulsified in complete Freund's adjuvant. The antiserum was titrated and standardized for EIA (Fischer *et al*. 1996). The polyclonal antiserum used in the ELISA experiments was purified by sepharose-protein G affinity chromatography (GE Healthcare Wisconsin, USA). The antiserum was dialyzed with 20 mM sodium phosphate buffer pH 8.4, and applied to a protein G-Sepharose column equilibrated with the same buffer and eluted with 100 mM glycine-HCl buffer, pH 2.4. The fractions containing IgG were dialyzed against phosphate buffered saline (PBS).

Sandwich ELISA-based assay for vWF (S-ELISA)

S-ELISA was performed in microtitre plates coated with 50 ng MAbs per well in 20 mM carbonate-bicarbonate buffer (pH 9.6) by overnight incubation at 4 ºC. The plates were washed three times with 0.5% BSA-PBS buffer and incubated for 1 h at 37 ºC with 0.5% BSA-PBS as blocking solution. Plasma to be tested was diluted 1:4 in 0.5% BSA-PBS buffer, and the plate was incubated again for 1 h at 37 ºC. Then, plates were washed three times with 0.5% BSA-PBS buffer and the second antibody conjugated with peroxidase was added and incubated for 1 h at 37 ºC. Following three washes in 0.5% BSA-PBS, the chromogen and the substrate were added (o-phenylenediamine, Sigma-Aldrich, St. Louis, USA), the reaction was stopped after 15 min with 12.5% H_2SO_4, and absorbance (*A*) at 492 nm was determined in a spectrophotometer (SPECTRAmax 250, Molecular Devices, Toronto, Canada). The control curve was obtained by serial dilution of vWF calibrator from 1:2 to 1:128. Each plasma was assayed in duplicate, and reference standard plasma was used as positive control.

The performance of the S-ELISA developed in this work was compared with the performance of a commercial ELISA (ASSERACHROM VWF:Ag ELISA, Diagnostica Stago, Asnières-sur-Seine, France). Both tests were run in parallel. The plasmas were tested in one dilution (1:102), and control plasmas were tested in two dilutions (1:51 and 1:102).

Precision test

The precision of the present S-ELISA was evaluated by intra-assay and inter-assay tests. To determine variability within a plate and among different plates and operators, three plasma samples with high, medium, or low vWF levels were tested. Six 16-well plates for the assay were tested by two operators. Results from precision analysis were given as coefficient of variation (CV).

Laurell Electro immunoassay (EIA)

The EIA first described by Laurell (1966) is one of the standard methods for vWF quantification. EIA is currently used in Laboratório de Hemostasia do Departamento de Genética da Universidade Federal do Rio Grande do Sul as a standard assay in the investigation of hereditary coagulopathies (Fischer *et al*. 1988). EIA is performed in glass plates (10 cm x 8 cm) with 1% agarose gels in tris-tricine buffer pH 8.6 containing polyclonal serum against vWF:Ag (Zimmerman *et al*. 1975, Monthony *et al*. 1978). Plasma dilutions (1:2) were prepared in saline solution (NaCl 0.9%). The standard plasma was diluted 1:2, 1:4 and 1:8 in saline solution to establish the control curve. EIA results were determined by two independent tests for each sample, and the mean was used for analyses. When discrepancies in results between tests were found, both tests were repeated.

Statistical analysis

Statistical analyses of Spearman's correlation and Kappa coefficient were performed with software SPSS 12.0 for Windows (IBM Corporation, IBM Corporation, Chicago, USA). The CV was determined using the software Microsoft Excel (Microsoft Corporation, Redmond, USA). To compare the results between vWF quantification by ELISA and EIA, we considered the means of two independent tests. Spearman's correlation was conducted to evaluate the intensity of correlation between results from different methods of vWF quantification. The Kappa coefficient was calculated to examine the level of agreement of positive or negative results found in the different tests.

RESULTS

Production of MAbs against human vWF

To generate MAbs capable of recognizing vWF, mice were immunized with partially purified vWF. Hybridomas secreting mouse IgG were initially identified by ELISA. Fourteen hybridomas producing MAbs to vWF were identified from a fusion procedure and, of those, five were cloned. Twenty-five clones obtained were isotyped. Eighteen MAbs were isotyped as IgG1, four as IgM, and three as IgG2a. MAbs vW18 (IgG1), vW21 (IgG1), vW22 (IgG2a), and vW23 (IgG2a), which exhibited higher reactivity to vWF, were selected to be tested in ELISA and conjugated with horseradish peroxidase reagent as described by Nakane & Kawaoi (1974).

Sandwich ELISA-based assay for vWF (S-ELISA)

Before testing the application of MAbs to quantify vWF, the most suitable conditions for use in ELISA were standardized. Four buffers were tested as blocking solution: saline solution with 5% non-fat dry milk (blotto), PBS (0.15 M sodium chloride plus 0.01 M sodium phosphate buffer; pH 7.2), 0.02% tween 20 in PBS, and 0.5% bovine serum albumin fraction V (BSA) in PBS. The BSA-PBS buffer showed the best performance to be used in ELISA.

To determine whether the MAbs could be used in the development of an ELISA for vWF quantification, we tested plasma vWF calibrator as antigen in a S-ELISA, using rabbit IgG anti-vWF polyclonal antibodies as the capture antibody, four MAbs (vW18, vW21, vW22 or vW23) as the secondary antibody, and peroxidase--conjugated anti-mouse IgG. In contrast to vW18 and vW21, vW22 and vW23 MAbs did not recognize the vWF present in the plasma (Fig. 1).

To determine the best combination between capture and detection antibodies, the polyclonal rabbit and monoclonal mouse antibodies were tested as both capture and as peroxidase-labeled detection antibodies. The highestresponse was obtained with 50 ng/well of MAb as the captureantibody and as detection antibody (Fig. 2).

After the assay conditions for a sandwich ELISA were optimized, a standard curve was constructed. vWF calibrator dilutions ranging from 1:2 to 1:128 were used to establish the standard curve. The best conditions for the ELISA were found to be: plates coated with 50 ng/well of MAb, plasma diluted 1:4 in 0.5 % BSA-PBS buffer, and antibody labelled with peroxidase as detecting antibody.

The precision of ELISA was evaluated by intra-assay and inter-assay comparisons. The results of the three plasmas evaluated are shown in Table 2. The CV for the high-level plasma ranged from 3.80% to 6.42%, between different plates and operators. For medium- and low-level plasmas, CV varied from 4.69% to 4.74% and 28.35% to 33.79% respectively. The overall CV of these samples was 5.37%, 4.69% and 31.24% for high-, medium- and low- level samples, respectively.

Comparison between S-ELISA and EIA

To further study the capacity of the MAbs to detect and quantify the concentration of vWF in blood plasma, all plasmas used in this study were tested by Laurell EIA. In the comparative experiments, because of the great inter-individual variation on vWF levels for symptomatic vWD , agreement was considered as vWF quantifications above or below 50% of the normal value. The normal range of vWF is broad, with 95% of values between 50% and 200% on average (Gill *et al.* 1987, Manucci *et al.* 1985). In the first analysis, 149 plasmas were compared using S-ELISA and EIA. The results from this quantification test showed 138 plasmas in agreement and 11 in disagreement. Four of 11 donors returned after a recall for a new blood sample. Plasma from these four patients were retested with the same parameters used before, and vW18 MAb was also tested as pre-coated antibody and as signal generating component. In this new analysis, of the four samples tested, one was normal in both tests and three confirmed discrepancy across results. Also, no significant difference between MAbs vW21 and vW18 quantification was found. The final result of the comparison experiment showed that the tests evaluated produced similar results for 139 samples, while 10 samples had discordant results (Table 3). The results indicate that S-ELISA and EIA are highly correlated (Rs = 0.843 and $p<0.001$ by Spearman's correlation). In addition, the Kappa coefficient of agreement between tests was 0.691 with $p<0.001$.

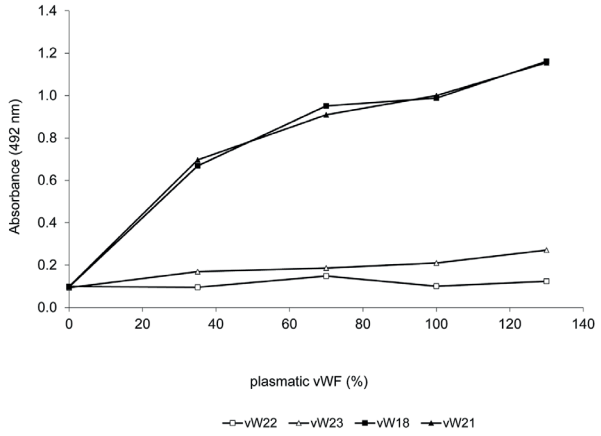

Figure 1. Binding of anti-von Willebrand Factor monoclonal antibodies to plasmatic von Willebrand Factor. Serial dilutions of plasma containing 0, 37, 70, 100 and 130% of the normal concentration of plasmatic von Willebrand Factor were tested using rabbit IgG anti-vWF polyclonal antibodies as capture antibody in combination with monoclonal antibodies (vW18, vW21, vW22 or vW23) as detection antibody.

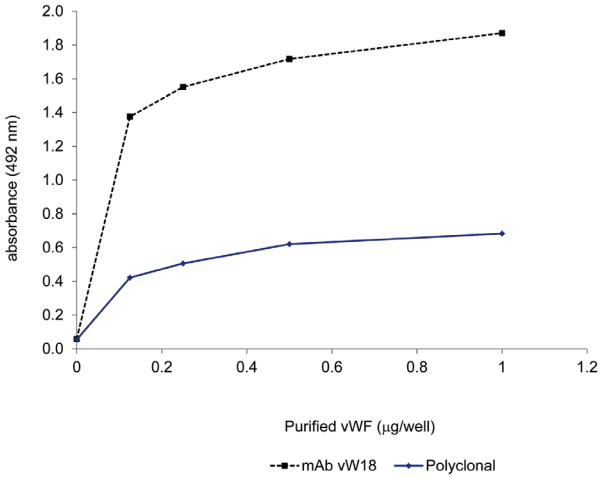

Figure 2. Sandwich ELISAs with polyclonal or monoclonal antibodies. Purified vWF (0, 0.125, 0.25, 0.5 and 1 µg) was tested by S-ELISA with 500 ng/well of polyclonal antibodies as the capture antibody, and polyclonal antibodies labeled with peroxidase as the detection antibody (1:2,000); and by S-ELISA with 50 ng/well of vW21 MAb for capture and vW18 MAb labeled with peroxidase as the detectionantibody (1:20,000).

Table 2. CV from three plasma samples tested by different plates and operators.

	Operator 1		Operator 2		Overall	
vWF	**absorbance**	**CV (%)**	**absorbance**	**CV (%)**	**absorbance**	**CV (%)**
High	0.9948 ± 0.0639	6.42	0.9751 ± 0.0370	3.80	0.9850 ± 0.0529	5.37
Medium	0.9098 ± 0.0427	4.69	0.9084 ± 0.0430	4.74	0.9091 ± 0.0426	4.69
Low	0.1778 ± 0.0600	33.79	0.1700 ± 0.0481	28.35	0.1739 ± 0.0543	31.24

Values for absorbance are expressed as mean ± standard deviation.

Comparison between ELISAs

To establish the potential and accuracy of the standardized S-ELISA, we tested its results against a commercial ELISA used for vWF quantification in diagnosis and research (Remy *et al*. 1995, Fischer *et al*. 1998, Biron *et al*. 1999, Siaka *et al*. 2003). For the comparison experiment, the same definition of agreement used before was adopted. The comparative evaluation of the two ELISAs in this study is shown in Table 4. The data shows that 34 out of 37 human blood plasmas yielded similar results in both ELISAs. Two of the three discordant plasmas were samples which also showed disparity in the EIA comparison, and the third one was plasma from a patient with vWD type II. Both ELISAs gave good results in terms of specificity and accuracy. Spearman's method showed a strong correlation between ELISAs (Rs = 0.930 and $p<0.001$). The Kappa coefficient of agreement between tests was 0.819 with $p<0.001$.

DISCUSSION

In our study, we obtained two MAbs (vW18 and vW21) with a strong reaction to the vWF from human plasma, and two MAbs (vW22 and vW23) which only reacted against purified vWF. The vWF is strictly linked to the FVIII in plasma (Koedam *et al*. 1990, Wise *et al*. 1991, Koppelman *et al*. 1996, Vlot *et al*. 1996); so, it is possible that the epitopes recognized by vW22 and vW23 in purified vWF may be unavailable when this protein is associated with FVIII in human plasma.

Since polyclonal antibodies produced by Fischer *et al*. (1996) are a set of distinct immunoglobulins capable of recognizing different epitopes from vWF, a stronger reactivity was expected with this serum. Nevertheless, due to the multimeric nature of vWF (Lynch *et al*. 1983, Fretto *et al*. 1986, Wagner *et al*. 1987), a large number of repeating units of this protein may work as epitopes for MAbs, which can explain the stronger reaction of MAbs observed in our study, compared with polyclonal antibodies. Since MAbs showed a higher response to vWF, with high specificity, and would prevent batch-to-batch variation of polyclonal antibodies when used in ELISAs, the vW21 was utilized as pre-coating antibody and as signal-generating component to improve ELISA.

Commercial ELISAs from different manufacturers have inter-assay CV between 3.0% and 12.1%, and an intra-assay CV from 1.9% to 10% (Helena Laboratories, ELISARA vWF, REAADS vWF ELISA from Corgenix and STAGO). These parameters are similar to those obtained for our ELISA. In our Elisa, the low-level plasma shows more consistent variation for vWF (CV 28.35% - 33.79%); however, it is detected because low levels of vWF are very close to the detection limit of the S-ELISA (absorbance is almost equal to the blank samples), and do not represent a problem for quantification of vWD type 3 patient samples.

The great variation in vWF levels of patients with vWD type 1 makes it difficult to determine how much protein concentration in plasma has to fall in order to trigger the specific signs and symptoms of the disease (Pruthi 2006). In the test comparing our ELISA to the EIA utilized as standard protocol in Laboratório de Hemostasia, six samples from patients with vWD type 1 and three from PvWD type 1 had normal vWF quantification by S-ELISA; however, a decrease in vWF concentration was found in EIA quantifications. The higher sensitivity of ELISA could be the cause for the observed difference. However, these patients presented vWF levels near the limit of 50%, and complementary tests are recommended in such cases in order to reach a more comprehensive diagnosis. The low reproducibility in results and the great variation in EIA results (Budde & Schneppenheim 2001, Favaloro *et al*. 2004) could bring in more difficulties to determine vWF plasma levels. The difference found in these results may

Table 3. vWF quantification by EIA and S-ELISA methods.

Samples	N	EIA		S-ELISA	
		Normal	Decreased	Normal	Decreased
Controls	10	10		10	
Haemophilia A or B	26	26		26	
vWD Type 1	21	4	17	10	11
PvWD Type 1	16	13	3	16	
vWD Type 2	2	2		2	
vWD Type 3	4		4		4
Other coagulopathy	2	2		2	
No coagulopathy	68	68		67	1
Total	149	125	24	133	16

Table 4. vWF quantification by Commercial ELISA and S-ELISA methods.

Samples	N	Commercial ELISA		S-ELISA	
		Normal	Decreased	Normal	Decreased
Controls	7	7		7	
Haemophilia A or B	5	5		5	
vWD Type 1	12	2	10	4	8
PvWD Type 1	4	4		4	
vWD Type 2	1	1			1
vWD Type 3	3		3		3
Other coagulopathy	1	1		1	
No coagulopathy	4	4		4	
Total	37	24	13	25	12

be explained based on a difference in sensitivity of ELISA and the variation in the EIA quantifications. However, analytical statistics showed a strong correlation and a good level of agreement between the tests.

In the comparison between our protocol and the commercial ELISA used, three samples showed disagreement; two samples were near the cut-off values of the tests, and it is possible that some structural change in vWF hindered determination. The third sample in disagreement was diagnosed as vWD type 2. It showed low vWF level in S-ELISA and a normal level in commercial ELISA. vWD type 2 samples could have low or normal vWF levels, depending on the form of vWD type 2 involved (Holmberg & Nilsson 1992, Simon & Roisenberg 2004, Pruthi 2006).

The very strong correlation and good level of agreement between both ELISAs confirm the similar performance of S-ELISA, when compared with a commercial ELISA largely used for vWF quantification.

In this study, an S-ELISA with new MAbs anti-vWF as capture antibody and as second antibody with high specificity to vWF from human plasma was developed. This assay shows high performance, similar to standard methods used for vWF quantification and, together with tests of vWF functionality, could improve the diagnosis of vWD. Differences found between tests can be explained based on differences in quantification methods and in the arbitrary limit of 50% of vWF concentration utilized in comparison methods. In summary, the ELISA protocol developed here shows good promise as a high-performance tool in vWF quantification tests in specialized laboratories.

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