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Quantification of plasma-derived blood coagulation factor VIII by real-time biosensor measurements

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Abstract

Plasma-derived blood coagulation factor VIII was analyzed in real time using biosensor technology. Monoclonal antibodies directed against the heavy and against the light chain of factor VIII were immobilized on different carboxymethyl dextran surfaces. Different factor VIII concentrations were injected over the antibody surfaces in parallel and response levels were determined from the dissociation phase at a fixed time after sample injection. Serial dilutions of plasma-derived factor VIII with known concentrations determined by a commercial FVIIIC:Ag ELISA were used as standards. A quantification limit of 0.9 I.U./ml with antibody 530p and 1.5 I.U./ml with antibody 531p was calculated. Intra-assay precision expressed as percent coefficient of variation was below 10% for concentrations above 0.6 I.U./ml. Inter-assay precision for antibody 530p was below 20% for concentrations higher than 0.6 I.U./ml. For 531p, inter-assay precision was below 10% for concentrations higher than 2 I.U./ml. A sensor chip lifetime in respect to regeneration of at least 100 cycles for both antibodies was found. The small sample requirement of 35 μl allows fast analysis of different FVIII products and the use of two monoclonal antibodies directed against two different FVIII domains provides additional information about the integrity of the FVIII molecule. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Real-time biosensor; Factor VIII; Monoclonal antibody

1. Introduction

Coagulation factor VIII (FVIII) is a large glycoprotein consisting of a light chain and a heavy chain, linked by a metal bridge [1]. Based on homology studies, the unproteolysed 2332-amino acid FVIII–polypeptide chain is divided into three different domains with the structure A1–A2–B–A3–C1–C2. The light chain has a molecular mass of about 80 kDa, the heavy chain varies in size from 90 to 200 kDa, as a result of proteolysis starting from the C-terminus. In plasma, FVIII circulates in complex with its carrier protein von Willebrand Factor (vWF) [2,3]. This complex functions as a cofactor for the activation of factor X by activated factor IX in the extrinsic blood coagulation cascade in the presence of phospholipid and calcium. Reduced levels or lack of FVIII result in a severe bleeding disorder, haemophilia A [4,5]. Haemophilia A is treated by infusion of plasma-derived FVIII (pdFVIII) concentrates or recombinant FVIII produced in Chinese hamster ovary cells [6] and baby

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hamster kidney cells [7,8]. Plasma-derived FVIII is isolated from cryoprecipitate using different procedures. It is common that after an aluminum hydroxide adsorption step to remove vitamin K-dependent clotting factors, various refining steps were added to improve purity [9–15] and immunological and virus safety of the product [16]. These procedures can also give a significant portion of modified or degraded FVIII, that has to be identified, characterized and separated [17–20].

For immunological characterization, antibodies against FVIII have been used in Western Blot, ELISA, and RIA. Several monoclonal antibodies were developed against the heavy and the light chain of FVIII, such as monoclonal antibody 038 [21,22], ESH8 [23], T2 [24] and monoclonal antibodies No. 8 and 5 developed by Rotblat et al. [25]. Antibody 8 recognizes an epitope in the A2 domain of the heavy chain. Antibody 5 binds to the light chain of FVIII. Antibody 038 is directed against the acidic region 2 on the light chain. Antibody ESH8 is directed against the C2 domain of the light chain. Antibody T2 binds to an epitope in the A1 domain of the heavy chain.

A biosensor for real-time monitoring of protein interaction based on surface plasmon resonance was developed in the early 1990s [26–29]. This system has been used for studying biomolecular interactions like protein–protein interaction and DNA–protein interaction [30]. One reaction partner is immobilized on a dextran sensor chip surface. The other interaction partner is injected and pumped over the immobilized surface. Upon binding, the angle of the evanescent wave changes. This is recorded by a diode array detector and transformed into response units (RU). An empirical correlation between response and thickness of the adsorbed layer could be found [31]. Therefore, it is possible to measure the concentration of the immobilized reaction partner and the concentration of adsorbed molecules [29,32,33]. Both can be performed in real time.

For characterization of FVIII, Raut et. al. [34] and Saenko et al. [19,35] described a method using surface plasmon resonance exploiting the phospholipid binding capability of FVIII and the binding to von Willebrand factor. This assay has not been designed for quantitative analysis.

For quantitative analysis, we have immobilized monoclonal antibodies against FVIII to the biosensor and samples of partially purified FVIII were injected. A standard curve was created using FVIII preparations with known concentrations. From these analyses, information on sensitivity, detection limit, quantification limit, precision, recovery, and chip lifetime were extracted.

2. Material and methods

2.1. Plasma-derived FVIII concentrates

For surface stability measurements, lyophilized plasma-derived FVIII concentrate batch No. 3235 (Octapharma, Vienna, Austria) was reconstituted in water to a concentration of 100 or 50 I.U./ml and diluted 1:2 with 20 m*M* Hepes, pH 7.4, containing 10 m*M* $CaCl_2$, 200 m*M* NaCl, 4% (w/v) sucrose, 20 m*M* glycine, and 0.1% (v/v) Tween 20 (2-fold stabilizing buffer). Aliquots of 200 μl were frozen at −20°C and thawed immediately before injection. For determination of assay parameters, FVIII concentrate Octanate batch No. 935581A120/U (Octapharma) was reconstituted in water to a concentration of about 200 I.U./ml. After a 1:2 dilution with 2-fold stabilizing buffer composition, three aliquots were frozen at −20°C. Each day when Biacore assays were performed, an aliquot was thawed and diluted to the respective concentrations with stabilizing buffer. From the same samples, FVIII:C Antigen ELISA was performed the same day.

All buffer components were purchased from Merck (Darmstadt, Germany).

2.2. Measurement of FVIII concentration by ELISA

FVIII concentrations of the standards used for creation of a calibration curve were determined by a commercially available enzyme immunoassay (Asserachrom VIIIC:Ag, Dagnostica Stago, Asnières-sur-Seine, France) designed for measurement of FVIII concentrations in plasma [36]. This assay contains two mouse anti-VIIIC:Ag monoclonal antibodies directed against two different antigenic determinants on the light chain of FVIII. Monoclonal antibody 833 is used for coating and FVIII capture,

monoclonal antibody D4H1 conjugated with peroxidase is used for detection of bound FVIII.

2.3. Real time biosensor measurements

For surface plasmon resonance measurements a Biacore 2000 instrument (Biacore, Freiburg, Germany) was used. Sensor chips and the amine coupling kit were purchased from Biacore, buffer components were from Merck. Monoclonal antibodies 530p and 531p (Harlan Sera-Lab, Loughborough, UK), initially described by Rotblat et al. as monoclonal antibodies No. 8 and 5 [25], were covalently coupled through the primary amine groups to the carboxymethyl dextran (CM) surface of a sensor chip CM5 (research grade, Biacore). The running buffer was 10 m*M* Hepes, 5 m*M* $CaCl_2$, 100 m*M* NaCl, 0.1% Tween 20, pH 7.4. The CM surface was activated with 80–120 μl of a 1:2 mixture of *N*-hydroxy-succinimide/*N*-ethyl-*N*′-(3-dimethyl-aminopropyl)-carbodiimide hydrochloride (NHS/EDC, Biacore). Immediately after surface activation, 250–300 μl of the respective monoclonal antibody solution (80–100 μg/ml) were injected. Prior to immobilization, the monoclonal antibody solutions were exchanged to 10 m*M* sodium acetate, pH 4.5, by a PD10 column (Amersham–Pharmacia, Uppsala, Sweden). Finally, two 50-μl portions of ethanolamine (Biacore) were applied to remove unspecifically adsorbed antibodies.

For concentration measurements, 5-μl portions of the respective solutions were injected. A calibration curve was created and concentrations were calculated using the following equation,

$$\text{Response} = R_{\text{high}} - (R_{\text{high}} - R_{\text{low}})/1 + (\text{Conc}/a)^b \quad (1)$$

where R_{high} is the highest response, R_{low} is the lowest response, Conc is the concentration of the FVIII solution, and *a* and *b* are mathematical parameters.

For regeneration of 530p surfaces, 5-μl portions of 2 *M* guanidine hydrochloride (GuaHCl), pH 7.0, were used. For regeneration of 531p surfaces, 5 μl portions of 1.5 *M* sodium thiocyanate (NaSCN) in 0.1 *M* sodium citrate, pH 7.9, were applied.

2.4. SDS–PAGE and Western blot

SDS–PAGE and Western blot were performed as alternative methods for characterization of FVIII. For electrophoretic separation of FVIII, an Xcell Mini-Cell system (Novex, San Diego, CA, USA) was used. Separation with 4–20% Tris–glycine Novex pre-cast gels was performed under reducing conditions (2% dithiothreitol).

Proteins, separated electrophoretically, were transferred to a nitrocellulose membrane (Schleicher and Schuell, Dassel, Germany) using the Xcell Mini-Cell system. After blocking with 3% skim milk powder in washing buffer, FVIII and its fragments were detected using anti-FVIII monoclonal antibodies 038 (Chemicon Int., Temecula, CA, USA), 531p (Harlan Sera-Lab), both directed against the light chain of FVIII, or 530p (Harlan Sera-Lab) directed against the heavy chain. Washing buffer was phosphate-buffered saline with 0.1% (v/v) Tween 20. Bound antibodies were detected using alkaline phosphatase-conjugated anti-mouse monoclonal antibody (Sigma–Aldrich, Vienna, Austria) with nitroblue-tetrazolium chloride and 5-bromo-4-chloro-3-indolylphosphate (Sigma–Aldrich) as substrates for color development. vWF was detected by an anti-vWF peroxidase conjugate (Dako, Glostrup, Denmark) and horseradish peroxidase color development reagent from Bio-Rad Laboratories (Hercules, CA, USA).

3. Results

For immunological quantification of FVIII an approach analogously described for measurement of a humanized protein by an anti-human IgG antibody was selected [37]. The antibody reaction was monitored in real time by a biosensor based on surface plasmon resonance as detection principle. We chose monoclonal antibodies 038, 530p, and 531p directed against FVIII. Fig. 1 shows the pattern of these antibodies in a Western Blot. Assaying serial dilutions of FVIII showed that 530p detects FVIII down to a concentration of 25 I.U./ml, 531p detects FVIII to 10 I.U./ml.

The respective antibody was immobilized using NHS/EDC chemistry. Despite its high affinity, anti-

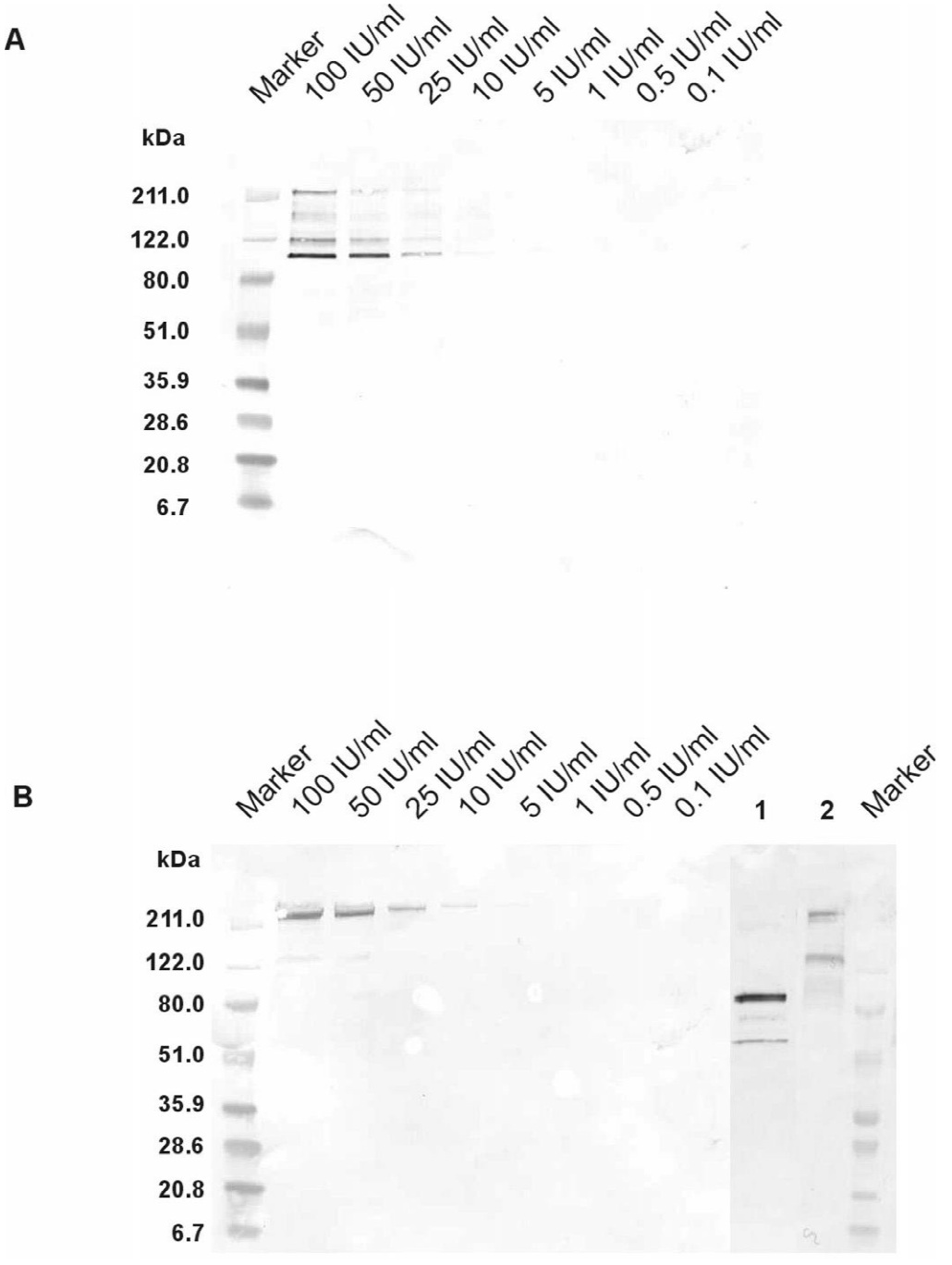

Fig. 1. Western blots of serial dilutions of FVIII concentrate containing vWF. (A) Developed with monoclonal antibody 530p, directed against the heavy chain. (B) Developed with monoclonal antibody 531p, directed against the light chain. Lanes denoted as (1) and (2) contain FVIII at a concentration of 100 I.U./ml. (1) Developed with monoclonal antibody 038 directed against the light chain; (2) with an antibody directed against vWF.

body 038 could not be immobilized in a way to produce a layer suited for real-time measurements. Ligand density for immobilized 530p ranged from 14 500 to 15 800 RU and for 531p from 15 200 to 15 800 RU. Five μl of serial dilutions of FVIII concentrate were injected at a flow-rate of 5 μl/min. The difference of response value between the baseline and the obtained response 100 s after the end of injection was calculated. A sensorgram of a single experiment is shown in Fig. 2.

3.1. Regeneration of the monoclonal antibody surfaces

Baseline stability after regeneration of the immobilized monoclonal antibody is a prerequisite for assay reproducibility as well as accuracy and sensitivity. For antibodies 530p and 531p, an efficient regeneration procedure was developed. Although the baseline decreases after each regeneration cycle, the antibody surface maintains its FVIII-binding capacity for at least 100 cycles. For the 530p surface, this was demonstrated by injecting 5 μl of a FVIII solution of about 50 I.U./ml and regeneration with 5 μl 2 *M* GuaHCl, pH 7.0. For the 531p surface, 5 μl portions of a FVIII solution of about 100 I.U./ml were injected and regeneration was performed with 5 μl of 1.5 *M* NaSCN in 0.1 *M* sodium citrate, pH 7.9 (see Fig. 3).

The most significant contribution to the variability of this method stems from the instability of the FVIII molecule. The surface with the immobilized antibodies is extremely stable, it has a life time of more than 100 cycles. In order to get representative samples containing the same amount of FVIII, a stock of 50 I.U./ml was prepared, aliquoted to 200-μl portions and stored at −20°C. Immediately before injection, an aliquot was thawed and placed in the precooled autosampler. Each aliquot was analyzed as quadruplet. From Fig. 3 it can be clearly seen that FVIII is degraded during storage in the autosampler. The response value of the first measurement in each series was always higher compared to the following ones. This is the explanation for the jigsaw shape of the plot showing the long-term stability of the surface (Fig. 3). After the 62nd cycle with antibody 530p and the 42nd cycle with antibody 531p, the

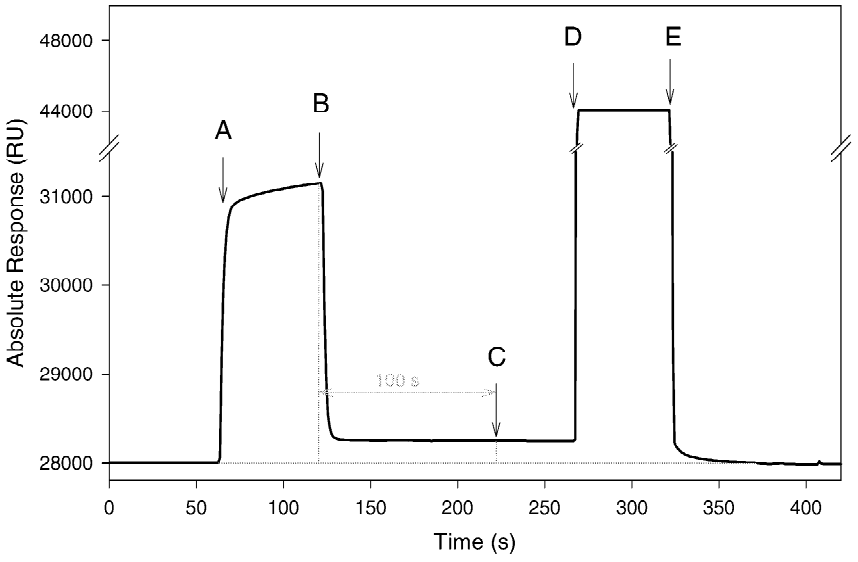

Fig. 2. Sensorgram for concentration measurements of FVIII by a real-time biosensor using immobilized monoclonal antibodies. (A) Indicates the injection of FVIII, (B) the end of injection, (C) the time point for measuring bound FVIII, (D) the beginning of regeneration and (E) the end of regeneration.

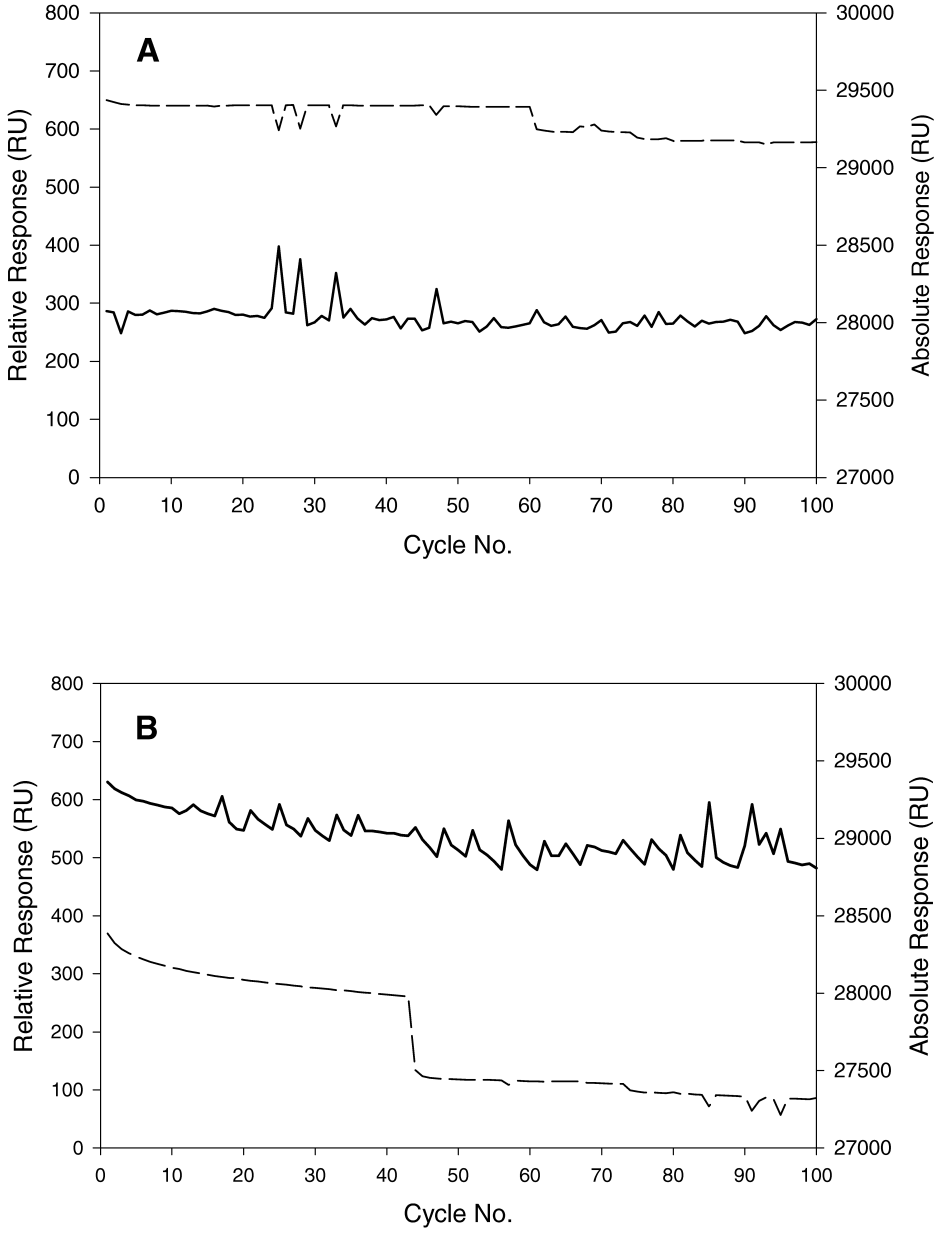

Fig. 3. Stability of immobilized anti FVIII antibodies during 100 injection cycles. (A) Indicates the surface generated with antibody 530p and (B) the surface generated with antibody 531p. (- - -) absolute response of the baseline; (—) relative response of bound FVIII.

baseline dropped, because of using a new buffer lot with a slightly different refractive index. This change did not influence the actual measurement of FVIII concentrations.

3.2. Assay parameters

For antibodies 530p and 531p calibration curves were generated by injecting increasing concentra-

tions of FVIII. The amount of FVIII in the standard was assayed by a commercially available FVIII:C ELISA test kit. The calibration curve for quantification was generated by regression of the data by the four parameter logistic function given in Eq. (1). The curves representing assays on three different days are shown in Fig. 4. The intra-assay precision expressed as percent coefficient of variation (C.V.) was calcu-

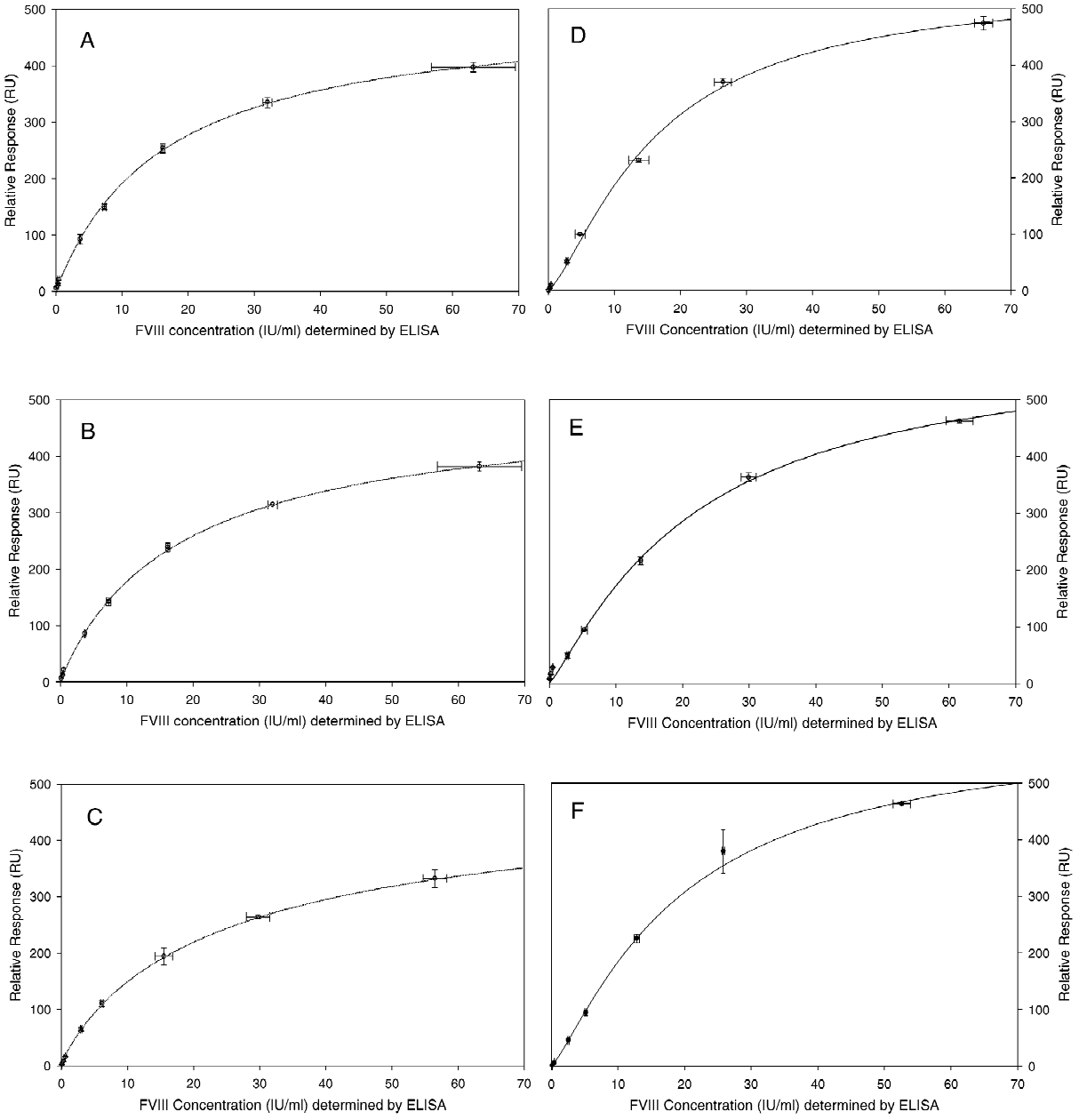

Fig. 4. Calibration curves using a logistic function for determination of FVIII with real time biosensor technology. (A–C) Indicate measurements on three different days with antibody 530p and (D–F) with antibody 531p. (⊕) Experimental data; (—) fit.

Table 1
Intra-assay precision data given as relative response values for quantification of FVIII by monoclonal antibody 530p

Intra-assay precision	FVIII concentration determined by FVIII:C antigen ELISA in IU/ml (mean)							
	0.1	0.3	0.6	3.3	6.7	15.8	30.9	59.9
Day 1								
Relative	8.0	14.2	20.7	101.7	155.9	263.5	341.4	407.2
response	5.5	10.5	20.4	88.7	147.2	251.7	328.1	391.3
(RU)	4.9	10.5	19.7	86.2	147.2	247.0	329.5	393.6
Mean	6.1	11.7	20.3	92.2	150.1	254.1	333.0	397.4
SD	1.6	2.1	0.5	8.3	5.0	8.5	7.3	8.6
%C.V.	26.2	17.9	2.5	9.0	3.3	3.3	2.2	2.2
Day 2								
Relative	6.6	11.7	23.9	86.3	146.7	247.2	314.1	391.2
response	11.4	10.7	19.9	88.6	137.1	232.8	315	379.1
(RU)	3.4	15.2	22	81.5	137.3	236.5	308.9	376.5
Mean	7.1	12.5	21.9	85.5	140.4	238.8	312.7	382.3
SD	4.0	2.4	2.0	3.6	5.5	7.5	3.3	7.8
%C.V.	56.3	19.2	9.1	4.2	3.9	3.1	1.1	2.0
Day 3								
Relative	3.3	8.6	17.3	69.2	114	209.5	266.3	347.4
response	2.2	8	16.3	64.8	107.2	193.2	262.1	333.8
(RU)	2.2	7.8	14.3	62.3	104.5	180.4	249.6	314.6
Mean	2.6	8.1	16.0	65.4	108.6	194.4	259.3	331.9
SD	0.6	0.4	1.5	3.5	4.9	14.6	8.7	16.5
%C.V.	23.1	4.9	9.4	5.4	4.5	7.5	3.4	5.0

lated from the relative response values and is shown in Tables 1 and 2. For concentrations above 0.6 I.U./ml, the C.V. was below 10%. The significant influence is the degradation of FVIII due to its extreme instability. The inter-assay precision was calculated from relative response values derived from assays performed on three different days. For antibody 531p, the C.V. was below 10% for concentrations higher than 2 I.U./ml, for antibody 530p, the C.V. was below 20% for concentrations above 0.6 I.U./ml. The values for the various concentrations are given in Tables 3 and 4. The limit of detection described as the response value bigger than three times the standard deviation of baseline noise was 1.5 RU. The intercept of the calibration curve was also in the same range. Three RU can be assigned as a response due to FVIII-binding. The detection limit is about 0.05 I.U./ml for 530p and 0.1 I.U./ml for 531p. The limit of quantification was determined from the calibration curve of day 1. From the intercept of the abscissa generated by the upper line describing the prediction limit (95%), a horizontal line to the line describing the lower prediction limit was drawn. From this point of intersection a vertical line was drawn to the ordinate. The intersection was defined as limit of quantification (see Fig. 5). A limit of quantification of 0.88 I.U./ml was determined for antibody 530p and 1.46 I.U./ml for antibody 531p.

Then four unknown FVIII samples, A, B, C, and D, dissolved in stabilization buffer, were analyzed by ELISA and the real-time biosensor method using antibody 530p as well as 531p. Interestingly, with antibodies 530p and 531p a higher amount of FVIII compared to ELISA was detected (see Fig. 6).

4. Discussion

By direct immobilization of the monoclonal antibodies 530p and 531p directed against FVIII heavy or light chain, respectively, we were able to quantify FVIII. These antibodies were selected, because they could easily be immobilized and they are commercially available in a sufficient concentration and

Table 2
Intra-assay precision data given as relative response values for quantification of FVIII by monoclonal antibody 531p

Intra-assay precision	FVIII concentration determined by FVIII:C antigen ELISA in IU/ml (mean)							
	0.04	0.2	0.5	2	5	13	25	60
Day 1								
Relative	−1.2	3.9	9.3	52.0	100.3	227.3	377.4	484.2
response	1.6	4.7	9.2	55.4	99.2	233.0	365.9	477.8
(RU)	1.3	5.2	9.4	49.2	100.4	232.9	364.6	460.2
Mean	0.6	4.6	9.3	52.2	100.0	231.1	369.3	474.1
SD	1.5	0.7	0.1	3.1	0.7	3.3	7.0	12.4
%C.V.	250.0	15.2	1.1	5.9	0.7	1.4	1.9	2.6
Day 2								
Relative	9.1	17.1	29.8	54.2	96.2	224.4	372.4	458.5
response	8.4	16.5	27.8	47.9	94	213.8	358.9	464.8
(RU)	8	16.7	27.2	45.9	93	212.1	358.9	463.2
Mean	8.5	16.8	28.3	49.3	94.4	216.8	363.4	462.2
SD	0.6	0.3	1.4	4.3	1.6	6.7	7.8	3.3
%C.V.	7.1	1.8	4.9	8.7	1.7	3.1	2.1	0.7
Day 3								
Relative	−0.7	1.4	24.7	45.9	98.1	233.8	363.2	460.5
response	−0.4	0.4	5.9	46.6	96.1	225.1	423.5	465.6
(RU)	−0.8	1.1	6.6	45	89.5	218.5	352.2	464.6
Mean	8.5	16.8	28.3	49.3	94.4	216.8	363.4	462.2
SD	0.6	0.3	1.4	4.3	1.6	6.7	7.8	3.3
%C.V.	7.1	1.8	4.9	8.7	1.7	3.1	2.1	0.7

Table 3
Inter-assay precision data given as relative response values for quantification of FVIII by monoclonal antibody 530p

Inter-assay precision	FVIII concentration determined by FVIII:C antigen ELISA in IU/ml (mean)							
	0.1	0.3	0.6	3.3	6.7	15.8	30.9	59.9
Day 1 (RU)	6.1	11.7	20.3	92.2	150.1	254.1	333.0	397.4
Day 2 (RU)	7.1	12.5	21.9	85.5	140.4	238.8	312.7	382.3
Day 3 (RU)	2.6	8.1	16.0	65.4	108.6	194.4	259.3	331.9
Mean	5.3	10.8	19.4	81.0	133.0	229.1	301.7	370.5
SD	2.4	2.3	3.1	13.9	21.7	31.0	38.0	34.3
%C.V.	45.3	21.3	16.0	17.2	16.3	13.5	12.6	9.3

Table 4
Inter-assay precision data given as relative response values for quantification of FVIII by monoclonal antibody 531p

Inter-assay precision	FVIII concentration determined by FVIII:C antigen ELISA in IU/ml (mean)							
	0.04	0.2	0.5	2	5	13	25	60
Day 1 (RU)	0.6	4.6	9.3	52.2	100.0	231.1	369.3	474.1
Day 2 (RU)	8.5	16.8	28.3	49.3	94.4	216.8	363.4	462.2
Day 3 (RU)	−0.6	1.0	12.4	45.8	94.6	225.8	379.6	463.6
Mean	3.3	6.9	16.7	49.1	96.3	224.5	370.8	466.6
SD	4.5	8.9	10.2	3.2	3.2	7.2	8.2	6.5
%C.V.	136.4	129.0	61.1	6.5	3.3	3.2	2.2	1.4

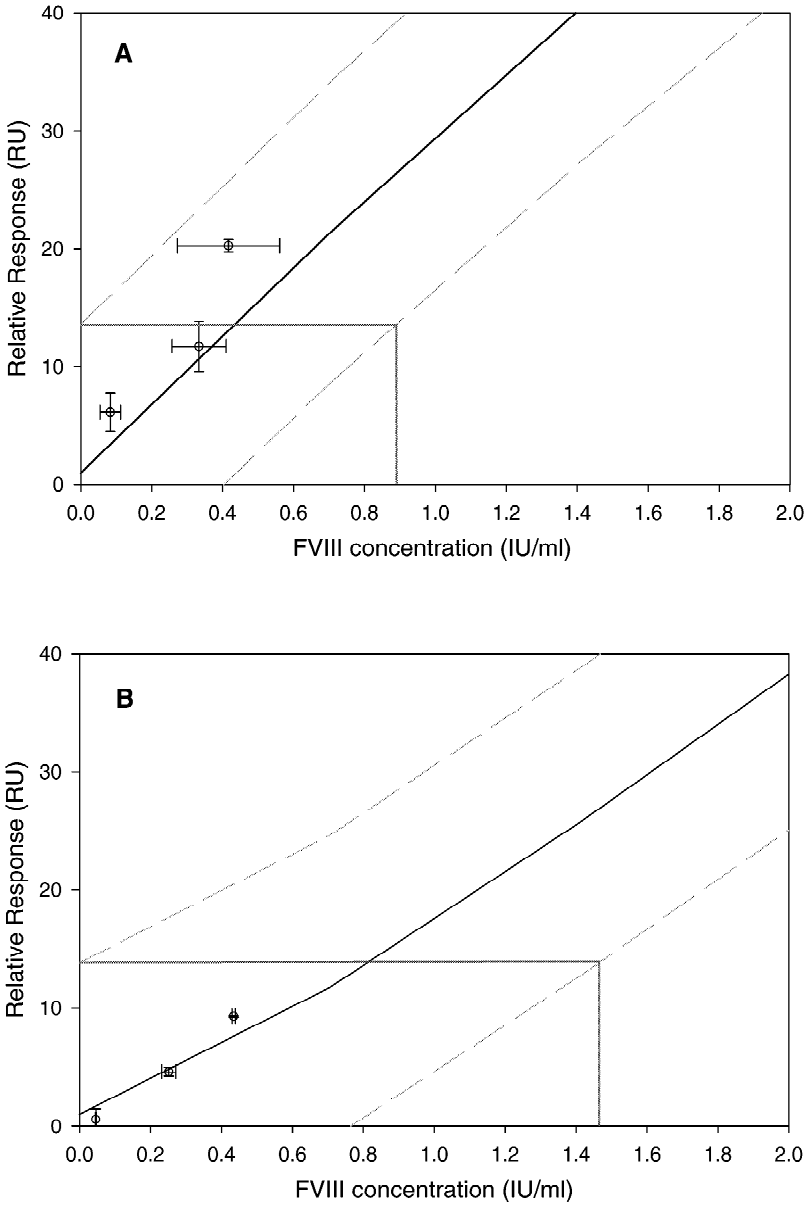

Fig. 5. Graphic determination of the quantification limit. (A) The quantification limit for immobilized antibody 530p, (B) for antibody 531p. (⊕) Experimental data; (—) fit; (- - -) prediction limit (95%).

purity. Monoclonal antibody 038 [21,22] was also tested, but a ligand density high enough to generate a significant signal was not obtained. The antibody might have been immobilized through the antigen binding site. Further optimization of the immobilization procedure was not pursued. With monoclonal antibodies 530p and 531p, a stable layer could be generated. After 100 cycles, the stability experiments were terminated. The most critical point is the extremely low stability of the analyte. Immediately after thawing degradation commences, although the samples are placed in a cooled autosampler. There-

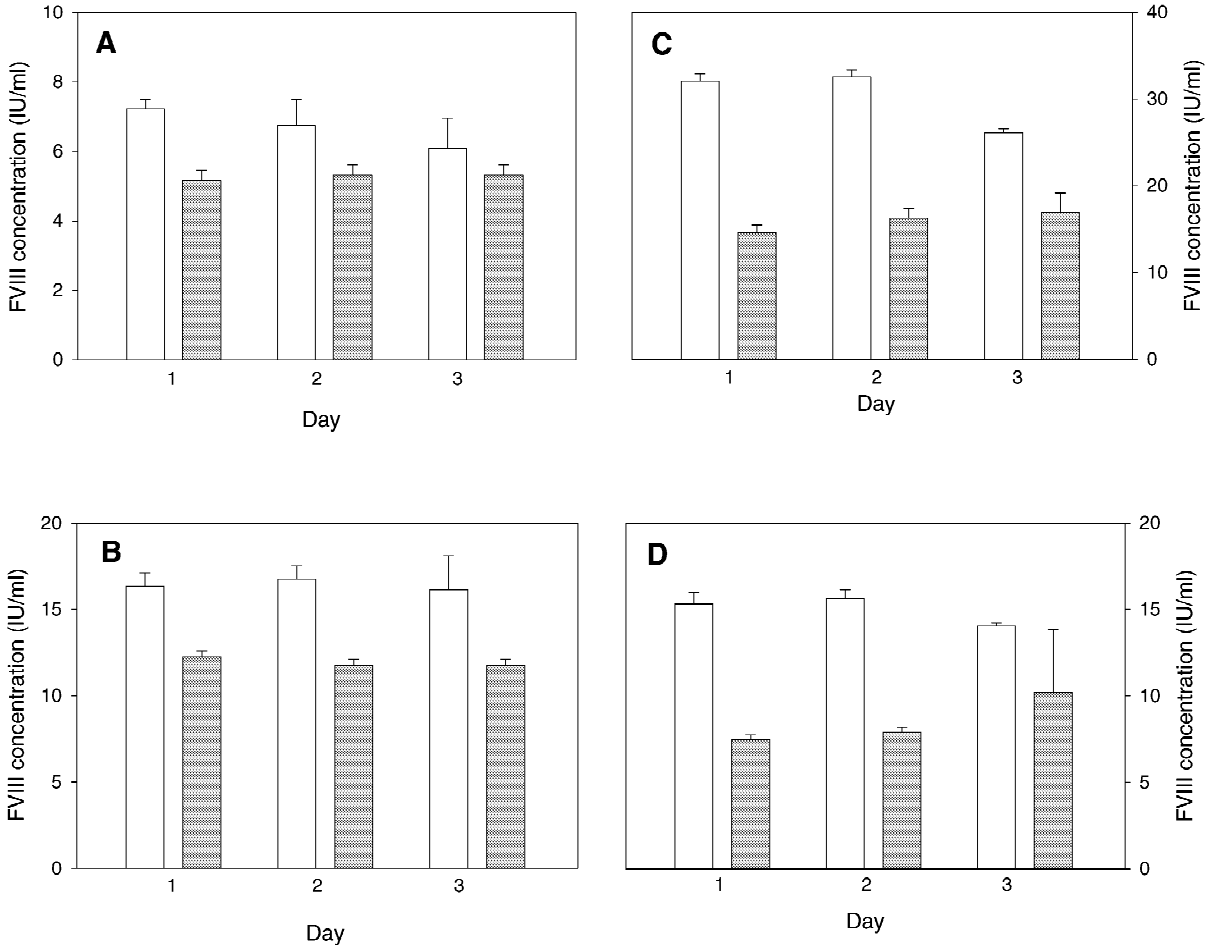

Fig. 6. Comparison of quantification of FVIII samples by FVIII:C antigen ELISA and real-time biosensor technology with four unknown samples. White columns indicate FVIII concentrations determined by real time biosensor technology, gray columns indicate FVIII concentrations determined by FVIII:C antigen ELISA. Samples in (A,B) were quantified with antibody 530p, directed against the heavy chain, samples in (C,D) with antibody 531p, directed against the light chain.

fore, it is of extreme importance to control the integrity of the sample. Only then a satisfactory accuracy can be achieved (for inter- and intra-assay precision see Tables 1–4). This drawback is compensated by the low requirements of sample volume. Only 5 μl were injected. The limit of quantification was 0.88 I.U./ml for antibody 530p and 1.46 I.U./ml for antibody 531p can be explained by the instability of the FVIII molecule. The regeneration and the actual condition of the surface layer is under control in contrast to other immunological methods such as ELISA or RIA. A positive control can be injected to ensure the integrity of the surface.

Christensen [33] and Richalet-Sécordel et al. [38] have described concentration measurements using real time biosensor technology by measuring the initial slope of the adsorption phase. This method is limited by minor differences in the refractive index between the sample solution and the running buffer, resulting in an uncontrolled response leap during the initial phase of adsorption. Such a minor refractive index difference can never be excluded. On the other hand, Richalet-Sécordel's method would allow quantification without the need of a standard curve, when a homogeneous analyte can be assumed. For quantification of FVIII this is not possible, since the

molecule is associated with its carrier protein vWF. It is still unclear to which extent FVIII is associated with this carrier protein and to which extent vWF is degraded or dissociated into its subunits. Thus a big variation in molecular mass and consequently in diffusion coefficients of this complex is observed.

Measuring four unknown FVIII samples, we found a higher amount of FVIII with the two antibodies immobilized to the sensor chip surface, compared to ELISA. The sandwich type ELISA is performed with two antibodies, monoclonal antibody 833 and monoclonal antibody D4H1, both directed to epitopes on the light chain of FVIII. This leads to the conclusion that partially degraded light chain is present in the FVIII preparation. It is clear that FVIII with partially degraded light chain is still recognized by the 530p surface. Obviously, the epitopes for monoclonal antibody 833 and monoclonal antibody D4H1 used in the ELISA are partially degraded, whereas antibody 531p still can bind to its epitope on the light chain. The interaction of the FVIII–vWF complex and its degraded form with these antibodies are not fully understood and different affinities of the complex may explain this behavior.

5. Conclusion

Real-time biosensor technology represents an alternative to other immunological methods for quantification of the complex FVIII molecule. The antibody surface layer is extremely stable. The method has a high sensitivity and accuracy.

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References

[1] L.O. Andersson, N. Forsman, K. Huang, K. Larsen, A. Lundin, B. Pavlu, H. Sandberg, K. Sewerin, J. Smart, Proc. Natl. Acad. Sci. USA 83 (1986) 2979.
[2] P. Lollar, Mayo. Clin. Proc. 66 (1991) 524.
[3] A.J. Vlot, S.J. Koppelman, B.N. Bouma, J.J. Sixma, Thromb. Haemost. 79 (1998) 456.
[4] I. Peake, Haemophilia 4 (1998) 346.
[5] A.J. Gale, J.L. Pellequer, E.D. Getzoff, J.H. Griffin, Thromb. Haemost. 83 (2000) 78.
[6] R.J. Kaufman, L.C. Wasley, A.J. Dorner, J. Biol. Chem. 263 (1988) 6352.
[7] B.G. Boedeker, Transfus. Med. Rev. 6 (1992) 256.
[8] F. Scheiflinger, F. Dorner, Wien. Klin. Wochenschr. 111 (1999) 172.
[9] T. Burnouf, M. Burnouf-Radosevich, J.J. Huart, M. Goudemand, Vox Sang. 60 (1991) 8.
[10] M. Griffith, Ann. Hematol. 63 (1991) 131.
[11] D. Josic, H. Schwinn, M. Stadler, A. Strancar, J. Chromatogr. B 662 (1994) 181.
[12] P.M. Mannucci, A. Gringeri, M. Cattaneo, Ric. Clin. Lab. 20 (1990) 227.
[13] R. Myers, M. Wickerhauser, L. Charamella, L. Simon, W. Nummy, T. Brodniewicz-Proba, Vox Sang. 60 (1991) 141.
[14] M.E. Hrinda, F. Feldman, A.B. Schreiber, Semin. Hematol. 27 (1990) 19.
[15] H. Schwinn, M. Stadler, D. Josic, F. Bal, W. Gehringer, I. Nur, R. Schutz, Arzneimittelforschung 44 (1994) 188.
[16] E. Berntorp, Ann. Hematol. 68 (1994) 35.
[17] I. Ramasamy, A. Farrugia, E. Tran, V. Anastasius, A. Charnock, Biologicals 26 (1998) 155.
[18] S. Raut, M. Di Giambattista, S.A. Bevan, A.R. Hubbard, T.W. Barrowcliffe, R. Laub, Thromb. Haemost. 80 (1998) 624.
[19] E.L. Saenko, K. Loster, D. Josic, A.G. Sarafanov, Thromb. Res. 96 (1999) 343.
[20] D. Josic, A. Buchacher, C. Kannicht, Y.P. Lim, K. Loster, K. Pock, S. Robinson, H. Schwinn, M. Stadler, Vox Sang. 77 (1999) 90.
[21] M. Arai, D. Scandella, L.W. Hoyer, J. Clin. Invest. 83 (1989) 1978.
[22] M. Arai, H. Inaba, M. Higuchi, S.E. Antonarakis, H.H. Kazazian Jr., M. Fujimaki, L.W. Hoyer, Proc. Natl. Acad. Sci. USA 86 (1989) 4277.
[23] D. Scandella, G.E. Gilbert, M. Shima, H. Nakai, C. Eagleson, M. Felch, R. Prescott, K.J. Rajalakshmi, L.W. Hoyer, E. Saenko, Blood 86 (1995) 1811.
[24] J. Ware, J.R. Toomey, D.W. Stafford, Thromb. Haemost. 61 (1989) 225.
[25] F. Rotblat, A.H. Goodall, D.P. O’Brien, E. Rawlings, S. Middleton, E.G. Tuddenham, J. Lab. Clin. Med. 101 (1983) 736.
[26] S. Lofas, B. Johnsson, J. Chem. Soc., Chem. Commun. 21 (1990) 1526.
[27] L.G. Fagerstam, A. Frostell, R. Karlsson, M. Kullman, A. Larsson, M. Malmqvist, H. Butt, J. Mol. Recognit. 3 (1990) 208.
[28] B. Johnsson, S. Lofas, G. Lindquist, Anal. Biochem. 198 (1991) 268.
[29] L.G. Fagerstam, A. Frostell-Karlsson, R. Karlsson, B. Persson, I. Ronnberg, J. Chromatogr. 597 (1992) 397.
[30] R.L. Rich, D.G. Myszka, Curr. Opin. Biotechnol. 11 (2000) 54.

[31] E. Stenberg, B. Persson, H. Roos, C. Urbaniczky, J. Colloid Interface Sci. 143 (1991) 513.
[32] R. Karlsson, L. Fagerstam, H. Nilshans, B. Persson, J. Immunol. Methods 166 (1993) 75.
[33] L. Christensen, Anal. Biochem. 249 (1997) 153.
[34] S. Raut, L. Weller, T.W. Barrowcliffe, Br. J. Haematol. 107 (1999) 323.
[35] E. Saenko, A. Sarafanov, N. Greco, M. Shima, K. Loster, H. Schwinn, D. Josic, J. Chromatogr. A 852 (1999) 59.
[36] J.P. Girma, E. Fressinaud, A. Houllier, Y. Laurian, J. Amiral, D. Meyer, Haemophilia 4 (1998) 98.
[37] R.L. Wong, D. Mytych, S. Jacobs, R. Bordens, S.J. Swanson, J. Immunol. Methods 209 (1997) 1.
[38] P.M. Richalet-Secordel, N. Rauffer-Bruyere, L.L. Christensen, B. Ofenloch-Haehnle, C. Seidel, M.H. Van Regenmortel, Anal. Biochem. 249 (1997) 165.