Biosensors and Bioelectronics 29 (2011) 18–22

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Protein biomarker enrichment by biomarker antibody complex elution for immunoassay biosensing

Gwenola Sabatte, Harma Feitsma, Toon H. Evers, Menno W.J. Prins*

Philips Research, High Tech Campus 11, 5656 AE Eindhoven, The Netherlands

ARTICLE INFO

Article history:
Received 11 April 2011
Received in revised form 27 June 2011
Accepted 28 June 2011
Available online 24 August 2011

Keywords:
Enrichment
Protein
Biomarker
Sample preparation
Immunoassay
Magnetic particles
Biosensor

ABSTRACT

It is very challenging to perform sample enrichment for protein biomarkers because proteins can easily change conformation and denature. In this paper we demonstrate protein enrichment suited for high-sensitivity integrated immuno-biosensing. The method enhances the concentration of the biomarkers and simultaneously removes matrix components that could interfere with the immunoassay. Biomarkers are captured using antibody coated magnetic particles and the biomarker antibody complexes are released by enzymatic elution. The eluted complexes are subsequently detected in a sandwich immunoassay biosensor. A scaling study of the enrichment process demonstrates an enrichment factor of 15 in buffer and plasma. We analyze the enrichment factor in terms of the three basic steps of the assay (capture, concentration, elution) and we quantify their respective efficiencies. The process is suited for integration into bio-analytical tools.

1. Introduction

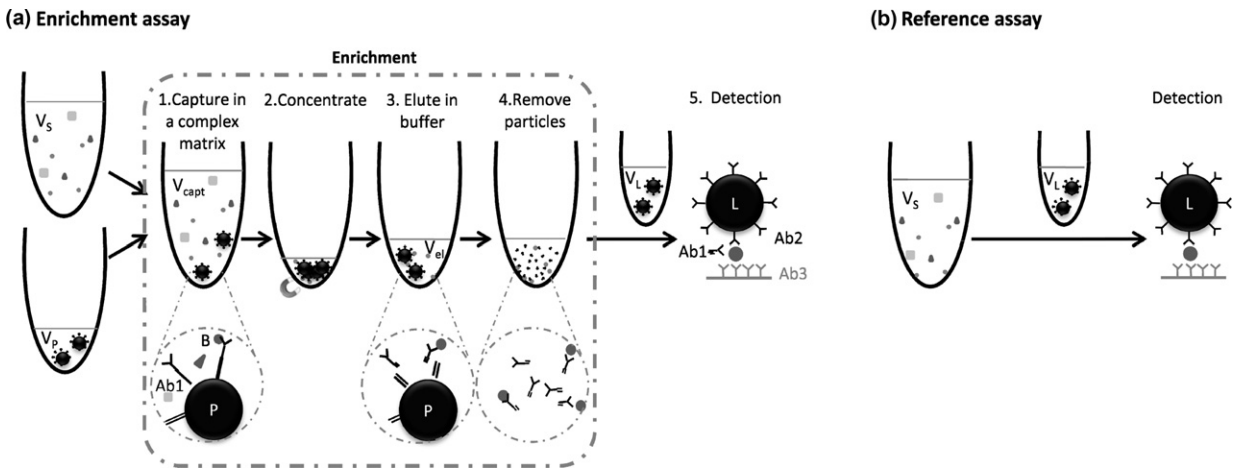
In vitro diagnostics have seen the emergence of many bioanalytical tools and methods aiming at fast, accurate and specific detection (Lenshof et al., 2009; Rissin et al., 2010; Vo-Dinh and Cullum, 2000; Yang et al., 2008). The methods of sample preparation are often specific to the detection technology, to the matrix (Baltussen et al., 2002; Chen et al., 2008) and to the class of analyte, e.g. nucleic acids (Chen and Ismagilov, 2006; den Dulk et al., 2010; Du et al., 2009; Lehmann et al., 2006; Lei et al., 2009; Liu et al., 2004; Pipper et al., 2008), cells (Baudoin et al., 2007; Guerquin-Kern et al., 2005; Herring et al., 2007; Walther and Mann, 2010; Zimmerman et al., 2008) or proteins (Hennion and Pichon, 2003). Sample preparation methods for nucleic acids use large differences in pH, salt concentrations or temperature to extract and purify the analyte. Such conditions strongly affect protein conformation, so cannot be applied for the enrichment of protein biomarkers in immunoassays (Reek et al., 1995). Whole cell sample preparation methods often exploit the size and mass of biological cells (Kielhorn et al., 2002). Immunoprecipitation is applied to prepare samples for protein mass spectrometric analysis. It relies on the specific capture of biomarkers and a release *via* an extreme pH or a salt concentration change, which often denatures the proteins and gives variable recoveries. Therefore these methods, which all have their merits, are not generally applicable for protein immunoassay biosensing (Firestone and Winguth, 1990).

In this paper we describe a widely applicable sample preparation method for protein biomarkers, based on biomarker capture onto antibody coupled magnetic particles and enzymatic elution of the biomarker antibody complexes into a small volume. The use of magnetic particles allows enrichment of the biomarker molecules in the fluid, while the use of a mild enzymatic elution process allows subsequent detection of the protein biomarkers by an affinity assay. The assay relies on the following steps (Scheme 1): specific capture by magnetic particles, then concentration into a small volume, and then purification and elution. Finally, the biomarker is recaptured in an immunoassay for detection. In the first step, antibody coated magnetic particles (P) in a volume V_P are added to the sample volume (V_S) containing the biomarker molecules (B). In the combined volume V_{capt}, the biomarker is captured by the antibodies Ab_1 which are immobilized on the particles *via* a DNA linker. The particles are then transferred to a clean solution with a smaller volume (V_{el}), where the biomarker antibody complexes (Ab_1–B) are eluted from the particles by restriction or degradation of the DNA linker. As a last step, the eluted complex is detected in a sandwich immunoassay employing two antibodies Ab_2 and Ab_3, binding to different epitopes than Ab_1.

The enrichment assay in Scheme 1 is compatible with a wide range of immunoassay detection methods, e.g. labeled as well as label-free detection (Stern et al., 2008). Philips Research has developed an optomagnetic biosensor technology in which superparamagnetic particles are used as detection label (Bruls et al.,

* Corresponding author. Fax: +31 40 27 42944.
E-mail address: menno.prins@philips.com (M.W.J. Prins).

0956-5663/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2011.06.046



Scheme 1. Enrichment assay (panel a) and reference assay (panel b). Particles *P* coated with antibodies (Ab_1) capture the biomarker proteins (*B*). The particles are concentrated into a clean matrix of a smaller volume (V_{el}), wherein the capturing antibodies are eluted from the particles by enzymatic elution. The biomarkers are detected in a sandwich immunoassay in step 5. In our experiment, the biomarkers are detected using an antibody coated sensor surface and antibody coated magnetic particles as labels (*L*). The labels are detected by optical scattering in an f-TIR biosensor (Bruls et al., 2009).

2009). The particles are actuated by magnetic fields to allow rapid and specific biological binding of the nanoparticles onto the sensor surface, while unbound or weakly bound particles are removed by magnetic forces. Particles bound at the sensor surface are detected by scattering and absorption of an evanescent optical field, so-called frustrated total internal reflection (f-TIR). We have used the optomagnetic immunoassay biosensor in the enrichment assay because of the small sample volume (7 μL) and the short time to result (5 min). The enrichment assay was investigated using total prostate specific antigen (PSA) as the biomarker. Prostate cancer is one of the leading causes of death in men and PSA is clinically used for screening and diagnostics (Botchorishvili et al., 2009).

2. Materials and methods

All buffer materials were supplied by Sigma Aldrich Corporation unless otherwise stated. Superparamagnetic particles functionalized with carboxylic acid groups or streptavidin (MasterBeads 500 nm diameter) were purchased from Ademtech. Monoclonal anti-PSA antibodies were purchased from Fujirebio (α-PSA-10, α-PSA-36, and α-PSA-66). Calibrators were prepared from human PSA complex (Fujirebio PSA kit) by diluting either in pure human heparin plasma pool from 20 apparently healthy donors (purchased from Innovative) or in 5% BSA in PBS (for buffer experiments). DNase was purchased from Qiagen and diluted in sterile RNase free water. All DNA strands were purchased from IBA GmbH. NEB EcoRI buffer (10× concentrated from NEBiolabs) was used as elution buffer.

Antibodies were conjugated to a single strand of DNA using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, Sigma Aldrich) chemistry in 1× phosphate buffered saline (PBS) for 1 h at room temperature with shaking (Hermanson, 2008). The conjugates were purified with a TSK-Gel SuperSW 3000 column (Tosoh Biosciences, 300 × 4.6 mm, 4 μm particles, 25 nm mean pore size), run at a flow rate of 0.25 mL of 60 mM phosphate buffer, pH 6.8, per minute at ambient temperature, monitored at 260 and 280 nm. The concentration of antibodies coupled with the single strand of DNA was assessed by UV–vis spectroscopy. The single strand of DNA coupled to α-PSA-10 was hybridized in PBS with its biotinylated complementary strand prior to addition to the particles. Capture particles were prepared as follows: a mixture of 1:1 streptavidin particles at 2 mg/mL and biotinylated DNA coupled α-PSA-10 IgG at 500 nM was incubated for 20 min. Then, the particles were washed and re-suspended at 2 mg/mL in 5% BSA in PBS. 50 μL capture particles at 2 mg/mL were mixed with PSA sample for 15 min, then the particles were washed magnetically and re-suspended in 1:1 DNase (1500 units in RNase free water) and NEB EcoRI buffer 1×. After 10 min elution, the particles were removed magnetically. 3.5 μL eluate and 3.5 μL α-PSA-36 coated magnetic particles at 2 mg/mL were mixed and directly injected into the disposable f-TIR biosensor cartridge. The cartridges were printed and assembled as explained in the article from Dittmer et al. (2010).

3. Results and discussion

3.1. Biomarker capture by magnetic particles

The efficiency of biomarker capture is a key factor in the enrichment assay. Capture particles were used as depicted in the inset of Fig. 1. The particles are streptavidin coated magnetic particles

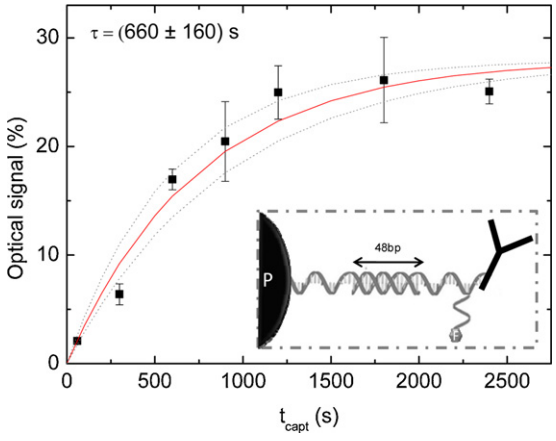


Fig. 1. Determination of the capturing time constant. A sandwich immunoassay was performed with α-PSA-66 coated on the sensor surface and α-PSA-10 on the capture particles. The following assay parameters were used: m_P = 0.01 mg, V_P = 5 μL, V_S = 45 μL, 5 pM PSA in the sample, and V_{capt} = 50 μL. In this assay $m_P = m_L$ due to the absence of elution. The solid line represents a curve of Eq. (1) with τ = 660 s; the upper dashed lines is a curve with $\tau = (660 - 160) = 500$ s, the lower dashed line is a curve with $\tau = (660 + 160) = 820$ s. The inset shows a sketch of the molecular architecture of the linker on the capture particles. A streptavidin coated magnetic particle is coupled to biotinylated double stranded DNA coupled to an antibody. The double-stranded DNA was formed from one biotinylated strand and one complementary strand conjugated to the antibody with EDC/NHS chemistry.

coupled with a fixed amount of DNA conjugated antibodies. The use of magnetic particles is advantageous for a high surface-to-volume ratio, the possibility to manipulate the particles by magnetic fields, and the possibility to use the particles directly in the optomagnetic f-TIR detection system. When the concentration of antibodies is much larger than the concentration of biomarker molecules, the capture process can be modeled as a first order reaction with a capture efficiency ε_{capt} (Irwin et al., 2002, 2003):

$$\varepsilon_{capt} = 1 - \exp\left(\frac{-t_{capt}}{\tau}\right) \quad \text{with} \quad \tau = \frac{1}{k_{on}[Ab]} \propto \frac{1}{k_{on}[P]} \tag{1}$$

where t_{capt} is the incubation time for the capture step (step 1 of the enrichment process), τ is the capture time constant, k_{on} is the association rate constant (in $M^{-1}\,s^{-1}$), [Ab] is the concentration of antibodies in the fluid (in M), and [P] is the concentration of particles in the solution (in M). τ is the time required to capture 63% of the biomarker molecules. τ is inversely proportional to the concentration of antibodies, so also inversely proportional to the amount of particles as the amount of antibodies per particle was fixed in our experiments. When $t_{capt} \gg \tau$, then ε_{capt} approaches 100% and the sample is depleted of biomarker molecules during the capture step. When $t_{capt} \ll \tau$, then ε_{capt} is low and can be approximated by $\varepsilon_{capt} \approx t_{capt}/\tau$, so it is proportional to the amount of magnetic particles in solution.

Fig. 1 shows data on the time-dependence of the capture process, for an amount of 0.01 mg magnetic particles suspended in 50 μL, i.e. a particle concentration of 0.2 mg/ml. The molecular architecture on the particles is shown in the inset of Fig. 1. Antibodies were provided with biotinylated DNA (details have been provided in Section 2) and thereafter coupled to the 500 nm streptavidin coated magnetic particles. The capture of biomarker proteins by the particles was immediately followed by detection in an f-TIR cartridge (so without an intermediate elution step). The data show an increase of signal as a function of incubation time until saturation of the signal, in agreement with Eq. (1). The curves in Fig. 1 give a time constant τ of 660 ± 160 s, which we will use to calculate the capture time constants and capture efficiencies in other experiments.

3.2. Three epitopes assay and elution conditions

The enrichment assay depicted in Scheme 1 targets three epitopes on the biomarker. Therefore we have evaluated the potential hindrance of the sandwich immunoassay by the presence of a third antibody. We compared sandwich assays with and without DNA coupled Ab_1 spiked into the PSA solution. The results are shown in Fig. 2. The dose–response curves are very similar and both show a detection limit of about 1 pM. Therefore, we conclude that the three epitopes binding is a valid detection format for the PSA enrichment assay.

Elution was carried out by enzymatic cleavage of the linker between particle and antibody. The DNA strand in the linker contains 10 nucleotides of single stranded DNA on each side of a 48 bp of double stranded DNA chain (see the inset of Fig. 1). The two single-stranded extensions enhance the molecular flexibility and give the DNase space to access the cleavage segment. The formulation of the elution buffer was determined by analyzing DNase cleavage products with gel electrophoresis. PBS (pH 7.2), Tris buffer (pH 7.4) and NEB EcoRI buffer (pH 7.2) were shown to yield equal degradation of DNA linker molecules. Five minutes at room temperature with DNase in NEB EcoRI buffer were sufficient to effectively degrade the DNA linker (93–100% as determined by gel electrophoresis). Furthermore, we investigated potential interferences with the detection immunoassay. It appeared that the f-TIR immunoassay is not impacted by the presence of the DNA linker, the DNase, or the elution buffer components.

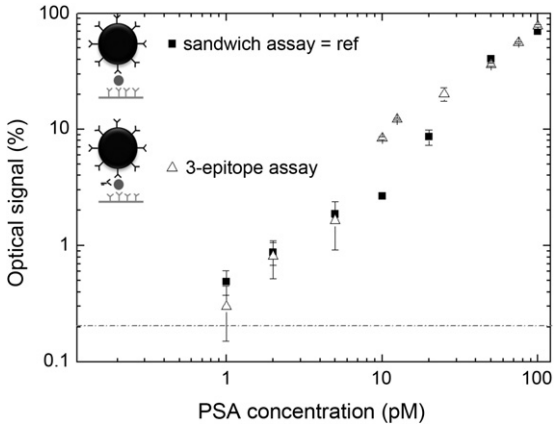


Fig. 2. Interference investigation of the three epitopes binding in the sandwich assay for detection. The reference assay was performed as depicted in Fig. 1 with α-PSA-66 antibodies on the sensor surface and α-PSA-36 antibodies on the label particles. In the 3-epitope assay, an additional α-PSA-10 antibody was spiked into the PSA sample for 10 min at a concentration of 400 nM. In these assays $m_P = m_L$ due to the absence of elution. The dashed lines represent the blank plus three times the standard deviation of the blank. The dashed lines are similar for the two dose–response curves.

3.3. The enrichment assay

The enrichment assay was investigated using a total assay time of 30 min, consisting of 15 min for the capture step, 10 min for the elution, and 5 min for the detection immunoassay. Dose–response curves were measured for different ratios of sample volume to elution volume (V_S/V_{el}) and compared to the reference assay, as shown in Fig. 3. Experimental details are listed in Table 1. The sample volume varied from 5 to 400 μL. A fixed amount of magnetic particles (0.1 mg) was added to the sample for the capturing process.

The capture time constant τ was derived from the time constant in Fig. 2 and scaled with the magnetic particle concentration used in the experiment (m_P/V_{capt}). After the capture step, the particles were magnetically concentrated and subsequently the biomarker antibody complexes were eluted into a volume of 5 μL. In the final step, the eluate was detected in an f-TIR immunoassay biosensor cartridge. Fig. 3 shows data points as well as linear fits to the data. For increasing volumetric ratios, the curves show an increase of slope on linear–linear scales (panel a) and a shift to lower concentrations on logarithmic–logarithmic scales (panel b). Over the total range of volumetric ratios, the dose–response curves show an increase of the slope and a shift to lower concentrations by a factor 50.

For every dose–response curve, we determine the enrichment factor (ξ) by dividing the slope of the curve by the slope of the reference curve. The steepest curve shows an enrichment factor of 15 with respect to the reference assay. The maximum enrichment factor of 15 is lower than the applied volumetric ratio of ×80. In order to be able to quantitatively understand the values of the enrichment factors, we express the enrichment factor as a product of the underlying processes, namely capture, concentration and elution:

$$\xi = \varepsilon_{capt}.\varepsilon_{conc}.\varepsilon_{el} = \varepsilon_{capt}.\frac{V_S}{V_{el}}.\varepsilon_{el} \tag{2}$$

where ε_{capt} is the capture efficiency (ranging between 0 and 1), ε_{conc} is the concentration efficiency which equals the ratio of the sample volume to the elution volume (V_S/V_{el}), and ε_{el} is the elution efficiency (ranging between 0 and 1). The capture efficiency of each reaction could be determined from Eq. (1). Consequently the elution efficiency could be derived from Eq. (2). The parameters and results are summarized in Table 1.

Table 1
Experimental parameters and experimental results for the enrichment assays shown in Fig. 3. The total assay time was 30 min (15 min for capture, 10 min for elution, and 5 min for detection). The slope of the curves was derived from a linear fit to the data in Fig. 3. The enrichment efficiency ξ is the ratio of the slope of the enrichment dose–response curve divided by the slope of the dose–response curve of the reference. The capture time constant τ was derived from the time constant in Fig. 1 scaled with the respective magnetic particle concentration (m_P/V_{capt}). Using the value of τ, the capture efficiency (ε_{capt}) was calculated from Eq. (1) with t_{capt} equal to 15 min. The elution efficiency (ε_{el}) was derived from Eq. (2), using the values of ε_{conc}, ξ, and ε_{capt}. The error on the slopes was determined by a linear fit. The error on ε_{capt} was derived from Eq. (1). The error on ε_{el} was derived from Eq. (2).

Curves	Experimental parameters							Experimental results				
	V_S (µL)	V_P (µL)	m_P (mg)	V_{capt} (µL)	V_{el} (µL)	m_L (mg)	$\varepsilon_{conc} = V_S/V_{el}$	Slope (% per pM)	ξ	τ (s)	ε_{capt}	ε_{el}
Ref	5	–	–	–	–	0.01	–	0.41 ± 0.01	–	–	–	–
×1	5	5	0.1	10	5	0.01	1 ± 0.02	0.12 ± 0.01	0.29 ± 0.02	36 ± 3	1.0 (0.999 < ε < 1)	0.29 ± 0.02
×10	50	50	0.1	100	5	0.01	10 ± 0.2	1.19 ± 0.08	2.91 ± 0.22	264 ± 32	0.97 (0.93 < ε < 1)	0.30 ± 0.06
×40	200	50	0.1	250	5	0.01	40 ± 1	5.07 ± 0.71	12.4 ± 1.3	367 ± 80	0.91 (0.74 < ε < 1)	0.34 ± 0.13
×80	400	50	0.1	450	5	0.01	80 ± 2	6.09 ± 0.52	14.8 ± 1.1	594 ± 144	0.78 (0.3 < ε < 1)	0.24 ± 0.16

Fig. 3. Enrichment assay for various volumetric ratios in buffer. The total assay time was 30 min (15 min capture, 10 min elution and 5 min detection). The optical signal is plotted versus the sample concentration of PSA. The sample volume varied from 5 to 400 µL while the elution volume was fixed at 5 µL. See details in Table 1. Panel a) shows the data on a linear–linear scale. Panel b) shows the data on log–log scale. The lines represent linear fits to the data, fitted by a least squares method in panel a).

For increasing sample volume, the capture time constant τ increases and therefore the capturing efficiency ε_{capt} decreases. The decrease becomes apparent when the value of t_{capt} approaches the value of τ. The derived values of the elution efficiency are in good agreement over the different assays, which gives support that the relationship in Eq. (2) is indeed applicable to the assays. The elution efficiency of biomarker antibody complexes from the magnetic particles is about 0.3, which is significantly lower than the cleavage efficiency of the pure linker molecules (>0.93 as was determined by gel electrophoresis). We attribute the difference to steric hindrance by the magnetic particles; the diffusion of enzymes may be hindered by the volume of the particles, or the particle surface may hinder the molecular cleavage process itself. We have performed a ×1 enrichment assay as described in Table 1 and one with a ten times lower amount of magnetic particles (m_P = 0.01 mg); the latter assay showed an elution efficiency of about 0.7, which indicates that particle-induced hindrance of enzyme transport indeed plays a role (data not shown).

It is important to consider the effectiveness of the enrichment assay in complex biological samples. To this end, we have also performed a ×80 enrichment assay using a heparin plasma pool as sample input. To avoid interferences due to native PSA, the plasma was first depleted of PSA by exposing the sample to particles with anti-PSA. Thereafter an accurate concentration of PSA was spiked into the sample, which dilutes the plasma by only 2%. To minimize further dilution of the matrix, the first step of the enrichment assay (i.e. the addition of capturing particles) was performed with

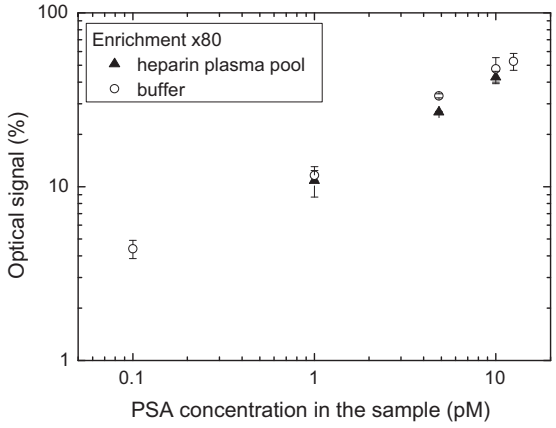


Fig. 4. Dose–response curves of the enrichment assay (×80) in a heparin plasma pool compared to the dose–response curve of the same assay carried out in buffer. Experimental parameters are listed in Table 1 (with the only difference that $V_P \approx 0$ in the plasma assay).

minimal carry-over volume ($V_P \approx 0$). Fig. 4 shows the dose–response curves for the enrichment assay in the plasma pool and in buffer. The curves are in very good agreement, which demonstrates the compatibility of the enrichment assay steps with the use of a complex biological matrix.

4. Conclusions

We have designed and investigated a protein enrichment assay based on biomarker antibody elution from magnetic particles. The biomarker antibody complexes are eluted by enzymatically cleaving dsDNA linker molecules between the antibodies and magnetic particles. The process is very mild and preserves the proteins, so that these can be sensitively and specifically detected in a subsequent immunoassay. The method performs two functions at the same time, namely the enrichment as well as the purification of the sample. Enrichment is achieved by molecular capture and subsequent volume reduction. Purification is achieved by transfer to a clean elution buffer, wherein cleavage takes place and the biomarker antibody complexes are released. We have analysed the assay in terms of the basic steps (capture, concentration and elution) and studied dose–response curves for a range of sample volumes (5–400 μL). Over the range of sample volumes, the dose–response curves show a shift to lower concentrations and an increase of the slope by a factor 50. Compared to a reference assay, the net enrichment obtained for an 80-fold volume reduction was a factor 15, determined by a limited capture efficiency (about 0.8) and elution efficiency (about 0.3) in the assay. We have demonstrated that the enrichment process is also effective when plasma is used as input.

The enrichment assay is very flexible. The method can be applied to multiplexing assays when different antibodies are used in combination with sequence specific elution of the DNA linkers. The present paper has focused on a three-epitope format. An alternative format is an assay in which the linker contains a tag molecule, which can be used to recapture and detect the biomarker antibody complex, for example by using anti-tag antibodies. The enrichment assay is suited for labeled detection as well as for label-free detection. The sample purification achieved by our enrichment assay can be particularly beneficial for label-free detection principles which are generally very sensitive to matrix properties. We conclude that the protein enrichment method described in this paper is well suited for use in a wide range of bio-analytical platforms.

Acknowledgements

The authors thank G. Lucassen and S. Neerken for many helpful discussions, E. Dirksen for purification of the conjugates, B. van Meerbergen and R. Wimberger-Friedl for critical reading of the manuscript, and the molecular diagnostics group for general support in the research. The work of G.S. was supported by the European Commission through the 6th framework Human Potential Programme (Marie Curie ToK-DEV fellowship, MTKD-CT-2006-042410 LISA).

References

Baltussen, E., Cramers, C.A., Sandra, P.J.F., 2002. Anal. Bioanal. Chem. 373 (1–2), 3–22.
Baudoin, R., Corlu, A., Griscom, L., Legallais, C., Leclerc, E., 2007. Toxicol. In Vitro 21 (4), 535–544.
Botchorishvili, G., Matikainen, M.P., Lilja, H., 2009. Curr. Opin. Urol. 19 (3), 221–226.
Bruls, D.M., Evers, T.H., Kahlman, J.A.H., van Lankvelt, P.J.W., Ovsyanko, M., Pelssers, E.G.M., Schleipen, J.J.H.B., de Theije, F.K., Verschuren, C.A., van der Wijk, T., van Zon, J.B.A., Dittmer, W.U., Immink, A.H.J., Nieuwenhuis, J.H., Prins, M.W.J., 2009. Lab Chip 9 (24), 3504–3510.
Chen, D.L., Ismagilov, R.F., 2006. Curr. Opin. Chem. Biol. 10 (3), 226–231.
Chen, Y., Guo, Z.P., Wang, X.Y., Qiu, C.G., 2008. J. Chromatogr. A 1184 (1–2), 191–219.
den Dulk, R.C., Schmidt, K.A., Gill, R., Jongen, J.C.B., Prins, M.W.J., 2010. Proc. 14th Int. Conf. on Miniaturized Systems for Chemistry and Life Sciences , Groningen, The Netherlands, pp. 449–451.
Dittmer, W.U., Evers, T.H., Hardeman, W.M., Huijnen, W., Kamps, R., de Kievit, P., Neijzen, J.H., Nieuwenhuis, J.H., Sijbers, M.J., Dekkers, D.W., Hefti, M.H., Martens, M.F., 2010. Clin. Chim. Acta 411 (11–12), 868–873.
Du, W.B., Li, L., Nichols, K.P., Ismagilov, R.F., 2009. Lab Chip 9 (16), 2286–2292.
Firestone, G.L., Winguth, S.D., 1990. Methods Enzymol. 182, 688–700.
Guerquin-Kern, J.L., Wu, T.D., Quintana, C., Croisy, A., 2005. Biochim. Biophys. Acta, Gen. Subj. 1724 (3), 228–238.
Hennion, M.C., Pichon, V., 2003. J. Chromatogr. A 1000 (1–2), 29–52.
Hermanson, G.T., 2008. Bioconjugate Techniques, Second ed. Elsevier.
Herring, K.D., Oppenheimer, S.R., Caprioli, R.M., 2007. Semin. Nephrol. 27 (6), 597–608.
Irwin, P., Damert, W., Brewster, J., Gehring, A., Tu, S.I., 2002. J. Rapid Methods Automat. Microbiol. 10 (2), 129–147.
Irwin, P., Damert, W., Tu, S.I., 2003. J. Rapid Methods Automat. Microbiol. 11 (4), 265–284.
Kielhorn, E., Schofield, K., Rimm, D.L., 2002. Cancer 94 (1), 205–211.
Lehmann, U., Vandevyver, C., Parashar, V.K., Gijs, M.A., 2006. Angew. Chem. Int. Ed. Engl. 45 (19), 3062–3067.
Lei, Y.J., Gao, C., Wang, C., Han, J., Chen, J.M., Xiang, G.C., Shi, Q., Jiang, H.Y., Zhou, W., An, R., Dong, C.F., Yuan, Y.K., Dong, X.P., 2009. Biomed. Environ. Sci. 22 (1), 55–61.
Lenshof, A., Ahmad-Tajudin, A., Jaras, K., Sward-Nilsson, A.M., Aberg, L., Marko-Varga, G., Malm, J., Lilja, H., Laurell, T., 2009. Anal. Chem. 81 (15), 6030–6037.
Liu, R.H., Yang, J.N., Lenigk, R., Bonanno, J., Grodzinski, P., 2004. Anal. Chem. 76 (7), 1824–1831.
Pipper, J., Zhang, Y., Neuzil, P., Hsieh, T.M., 2008. Angew. Chem., Int. Ed. 47 (21), 3900–3904.
Reek, F.H., Smits, M.A., Kamp, E.M., Smith, H.E., 1995. Biotechniques 19 (2), 282–285.
Rissin, D.M., Kan, C.W., Campbell, T.G., Howes, S.C., Fournier, D.R., Song, L., Piech, T., Patel, P.P., Chang, L., Rivnak, A.J., Ferrell, E.P., Randall, J.D., Provuncher, G.K., Walt, D.R., Duffy, D.C., 2010. Nat. Biotechnol. 28 (6), 595–599.
Stern, E., Steenblock, E.R., Reed, M.A., Fahmy, T.M., 2008. Nano Lett. 8 (10), 3310–3314.
Vo-Dinh, T., Cullum, B., 2000. Fresenius J. Anal. Chem. 366 (6–7), 540–551.
Walther, T.C., Mann, M., 2010. J. Cell Biol. 190 (4), 491–500.
Yang, J., Gunn, J., Dave, S.R., Zhang, M., Wang, Y.A., Gao, X., 2008. Analyst 133 (2), 154–160.
Zimmerman, T.A., Monroe, E.B., Tucker, K.R., Rubakhin, S.S., Sweedler, J.V., 2008. Methods Cell Biol. 89, 361–390 (Chapter 13).