



Magnetic bead-based detection of autoimmune responses using protein microarrays

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In the present study, a magnetic bead-based detection approach for protein microarrays is described as an alternative approach to the commonly used fluorescence-based detection system. Using the bead-based detection approach with applied magnetic force, it was possible to perform the detection step more rapidly as a result of the accelerated binding between the captured analyte in the microspot and the detection antibody, which was coupled to the magnetic beads. The resulting strong opacity shift on the microspots could be recorded with an ordinary flatbed scanner. In the context of autoimmunity, a set of 24 serum samples was analyzed for the presence of antibodies against 12 autoantigens using standard fluorescence and magnetic bead-based detection methods. Dynamic range, sensitivity, and specificity were determined for both detection methods. We propose from our findings that the magnetic bead-based detection option provides a simplified and cost effective readout method for protein microarrays.

Introduction

A multitude of microarray-based assay systems have been developed over the past ten years that enable the detailed measurement of hundreds or even thousands of molecular parameters within a single experiment. Nowadays, microarrays are well-established high-output tools for genomics, transcriptomics, and proteomics applications [1–6].

Protein microarray assays involve the immobilization of numerous capture agents and enable the analysis of multiple analytes from a single sample. In forward-phase arrays, capture agents such as proteins [7], antibodies [8,9], or antigens specifically detect the presence of target analytes. The application of protein microarrays for the detection of antibodies has been demonstrated in several contexts, such as infectious diseases [10–12], allergies [13], or autoimmune diseases [14]. At present, high-density protein micro-

arrays are mainly used for the analysis of protein–protein interactions and the screening of patient samples in order to discover new types of disease-specific antibodies. In addition, protein arrays consisting of focused panels of multiplexed sandwich immunoassays are already widely used in basic and applied research [15]. Within the field of autoimmune disease, Joos *et al.* employed protein microarrays to detect up to 18 different rheumatic disease-specific autoantibodies in human sera [16]. Sensitivities and specificities of the assays were similar to standard ELISA methods. To enable a detailed characterization of a patient's autoimmune status, this concept was further developed by including peptide antigens in the microarray [17]. Sharp and Utz suggested the application of autoantibody profiling to improve the diagnosis and prediction of disease onset and severity. Protein microarrays offer valuable diagnostic tools for disease monitoring and therapy since new clinical information can be gained from comprehensive autoantibody profiling, especially when combined with other plasma parameters, for example, cytokine levels [14].

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Zhu and Snyder recently reviewed the detection methods that were previously used for protein microarrays; for example, chemiluminescence detection methods and radio isotope-labeled probes that were adopted from established immunoassays [18]. Such detection methods have since been replaced to a large extent by fluorescence detection methods, as the latter are very sensitive, safe, and simple. However, their use requires special biochip readers that enable the discrete excitation and measuring of fluorophores at specific wavelengths. Lebrun and co-workers used a colorimetric readout system from a standard ELISA on protein microarrays, which enabled the cost-efficient detection of several autoantibodies in patient sera using commercial flatbed scanners [19]. Morozov and co-workers have demonstrated the implementation of magnetic beads as detection agents for protein microarray analysis in combination with on-chip electrophoresis [20]. It was further shown for the identification of viral pathogens [21] and in the genotyping of SNP variations [22] that the use of magnetic beads could reduce assay time and simplify the readout from DNA microarrays. In conjunction with PCR-based target amplification magnetic bead detection appeared to offer similar performance as compared to suspension bead arrays and electrophoresis techniques in terms of specificity and sensitivity [21]. To our current knowledge, no study has investigated magnetic beads as detection reagents on protein microarrays using an already established assay of pre-characterized patient seras with the focus to estimate inter-assay and intra-assay variability.

Here, we describe the use of magnetic bead-labeled antibodies to assess the autoimmune response against 12 autoantigens. Twenty-one serum samples derived from patients suffering from autoimmune diseases and samples from healthy donors were applied to an antigen microarray in order to detect the individual response patterns of disease-specific autoantibodies. This magnetic bead-based detection set-up was compared to the assay performance of an established fluorescence-based detection protocol with particular emphasis on speed, sensitivity, and specificity.

Materials and methods

Microarray fabrication

The following 12 antigens were provided by Phadia, Freiburg, Germany: thyroglobulin (TG), thyroid peroxidase (TPO), centromer antigen B (CENP-B), 100 kDa autoantigen associated with polymyositis and scleroderma (PmScl100), 70 kDa antigen associated with scleroderma (Scl70), double-stranded deoxyribonucleic acid (dsDNA), Sjögren syndrome antigen B (SSB/La), ribonucleoprotein (RNP) A and C, Sjögren syndrome antigen A (SSA/Ro-52 and SSA/Ro-60), and Sm autoantigen.

Antigens were printed onto aldehyde-coated glass slides (CEL Associates, Pearland, Texas, USA) using a non-contact spotting system (BioChip Arrayer, PerkinElmer, Rodgau, Germany). Printing was performed under clean room conditions at 20 °C and a relative humidity of 65%. 300 pL of each antigen solution was printed in 14 replicates per array. Goat anti-human IgG (Dianova, Hamburg, Germany) (500 mg/L) was immobilized in 28 replicate spots per array. A mixture of Cy3-labeled and Cy5-labeled BSA (200 mg/L) was included to provide guiding spots to facilitate orientation during image analysis. Each microscopic slide contained 12 subarrays in a layout allowing the separation of the arrays by a framing grid to generate 12 individual cavities, each

containing one subarray (ProPlate™ Multi-Array Slide System, Grace Bio-Labs, Bend, OR, USA). After the printing process, the slides were incubated in a humid chamber at 23 °C for 12 h, followed by a blocking procedure involving a blocking buffer containing 15 g/L BSA (Roth, Karlsruhe, Germany), 50 g/L low-fat dried milk (Heirler, Radolphzell, Germany) and 1 mL/L polysorbate 20 (Tween 20; Merck, Darmstadt, Germany) in PBS (137 mmol/L NaCl, 2.7 mmol/L KCl, 11.9 mmol/L PO₄, pH 7.4; Fisher Scientific, Schwerte, Germany). The buffer was nebulized onto the slides as previously described [23]. Antigen arrays were dried at 23 °C and were stable for several months when dry stored at 4 °C (data not shown).

Magnetic bead coupling

Five hundred microliters of 10 g/L streptavidin-coated magnetic beads (Dynabeads® MyOne™ Streptavidin T1, Invitrogen, Carlsbad, CA, USA) 1 µm in diameter were washed three times with 500 µL PBS using a magnetic bead concentrator (Invitrogen) before coupling. After the final washing step, the supernatant was removed and 182 µL of 1.1 g/L biotin-conjugated goat anti-human IgG (Dianova) was added to achieve a final coupling density of 40 µg biotin-conjugated goat anti-human IgG per mg beads and incubated for 1 h at 23 °C. The coupled beads were washed five times with 500 µL PBS and resuspended in 278 µL PBS.

Assay protocol

The slides were mounted into a framing slide system to create individual subarray subcompartments and were initially rehydrated for 1 h at 23 °C with 50 µL assay buffer (15 g/L BSA, 25 g/L low-fat dried milk and 1 mL/L Tween 20 in PBS) per well. The rehydrated slides were washed once with 100 µL washing buffer (1 mL/L Tween 20 in PBS). The serum samples were kindly provided by Phadia, Freiburg, Germany. Fifty microliters of a 200-fold dilution of serum in an assay buffer was incubated on each array for 45 min at 23 °C followed by six washing steps using 150 µL of washing buffer.

The magnetic bead-based detection (MBD) method was performed using one part of magnetic beads conjugated to goat anti-human IgG and adding nine parts of PBS. Seventy-five microliters of this solution were added to each individual microarray. Slides were placed onto a magnet (LifeSep™ 96F, Dexter Magnetic Technologies Inc., Elk Grove Village, IL, USA). After 10 s, the slides were lifted off the magnet and placed into a trough containing 250 mL PBS. Subsequently, the magnet was placed on top of the slide carrier to attract and collect unbound beads (supplementary Figure 3). Finally, the slides were dismounted from the framing slide system, while still immersed in the PBS-filled trough, and gently washed three times with PBS in 50 mL plastic tubes. For fixation, the slides were incubated in 40 mL PBS containing 5% glutaraldehyde (Sigma-Aldrich, Hamburg, Germany) for 5 min before they were rinsed with distilled water (40 mL).

Fluorescence-based detection (FBD) was performed using a Cy5-conjugated goat anti-human IgG (Jackson Dianova) antibody. Fifty microliters of the detection antibody (3 mg/L in assay buffer) was added into each compartment and incubated at 23 °C for 45 min. Each compartment was washed six times with 150 µL washing buffer and the slide was dismounted from the framing slide system. Finally, the slide was washed with distilled water and dried by centrifugation at 1500 g.

Readout and data analysis

A flatbed scanner (CanoScan 8800F, Canon Europa, Amstelveen, The Netherlands) was used to visualize the captured magnetic beads in each individual microspot within the microarrays. Four hundred microliters of distilled water were placed onto the scanner window and one slide was laid onto the drop with its functionalized side facing down. The scanner was set to negative transmission light mode and 16-bit TIFF grayscale images with a resolution of 2400 dpi were generated. The images obtained were analyzed with standard microarray image analysis software (ScanArray Express, PerkinElmer).

In the case of FBD, the slides were scanned with a confocal laser scanner (ScanArray Express; PerkinElmer) at 635 nm and a laser power of 90% while the photomultiplier gain was adjusted to 45%. The resolution of the resulting 16-bit TIFF grayscale images was 10 μm (2540 dpi). The images were analyzed as described above.

The language R was used as a statistical environment for unsupervised hierarchical clustering. Euclidean distances between all sera from both investigated detection methods were calculated from log2-transformed signal to negative control values [24], and the hclust-algorithm available by default in R was subsequently employed to construct the resulting tree-structure projections.

Results

The present study describes a MBD method for the analysis of autoimmune responses using protein microarrays. The binding of autoantibodies to different antigens was visualized using magnetic bead-labeled or fluorescence-labeled anti-human IgG antibodies. A panel of 21 serum samples from patients suffering from autoimmune diseases and from healthy donors, used as negative controls, was applied to antigen microarrays in order to detect individual IgG response patterns to the immobilized antigens. The performance of the MBD procedure applied in the protein microarray assay was compared to a standard FBD system.

Magnetic bead-based detection

Magnetic beads, loaded with anti-human IgG, were used as the detection reagent in the assay. After sample incubation and washing of the protein arrays, incubation in the MBD system only took around 10 s, as the magnetic beads were actively attracted and directed toward the microspots by magnetic force, which accelerates the binding to the captured human IgGs (Figure 1). It was possible to easily remove unbound magnetic beads from the surface by applying magnetic force from the top of the microarray. The captured magnetic beads within each microspot resulted in a strong opacity shift visible to the naked eye, which could be recorded with a standard flatbed scanner (Figure 2).

Performance evaluation

The MBD approach was evaluated by testing the same sera with fluorescently labeled anti-human IgG and a confocal biochip reader. Benefits and drawbacks of the presented MBD approach for autoantigen array analysis were evaluated by comparing the results generated using the MBD approach with the results obtained from conventional FBD protocol and with the results obtained from an established diagnostic test, namely the Varelisa® assay from Phadia [25].

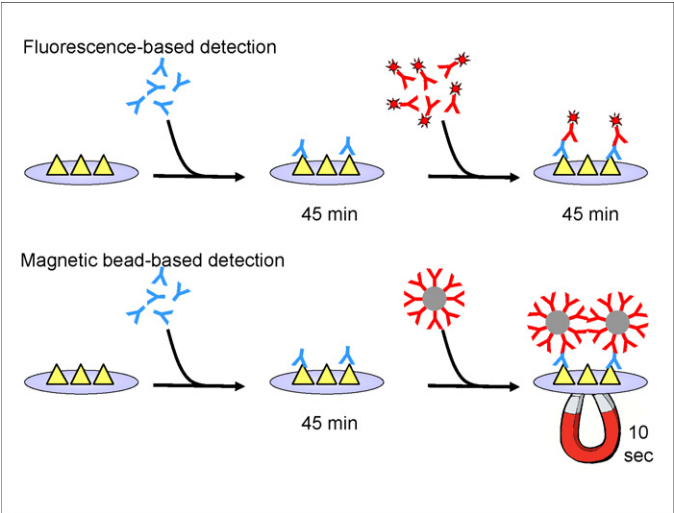


FIGURE 1
Readout systems. The serum samples were applied to the autoantigen microarray and bound autoantibodies were detected with an anti-human IgG antibody that was either fluorescently labeled or coupled to magnetic beads. With the assistance of magnetic force to direct the magnetic beads onto the slide surface, bead-based detection was achieved in less than 1 min.

Reproducibility and variability

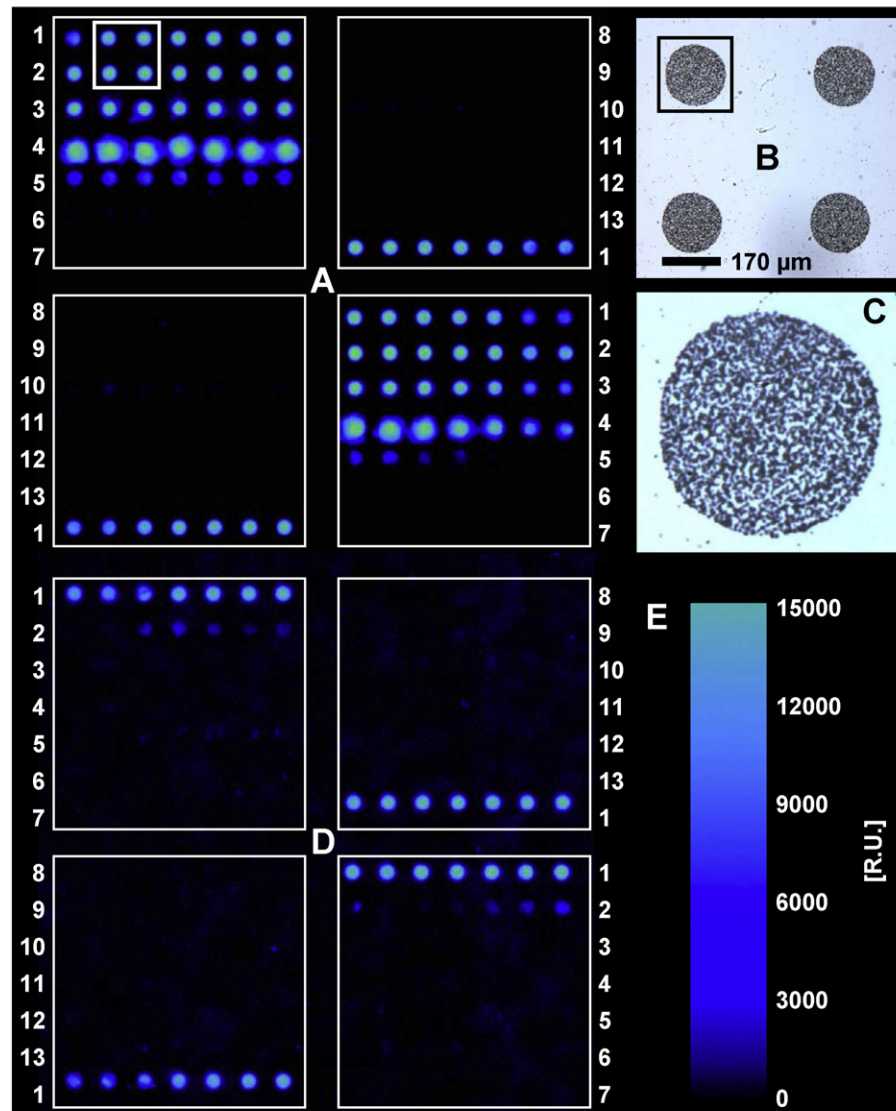
Four sera were analyzed at a single dilution on three subarrays per slide and on three different slides, respectively, to compare the reproducibility and variability of the two different detection methods.

A common practice in FBD analysis for protein microarrays is to calculate the mean intensity and standard deviation from replicate spots. The prerequisite for this procedure is that the data must have a Gaussian distribution. In general, this is the case for the FBD system. However, for the MBD system, the intensity distribution across replicate spots was not usually within a Gaussian distribution. Instead, it was often observed that 4 to 5 of the 14 replicate spots displayed a high and consistent signal, while the remaining spots emitted a weak signal, or had no beads attached at all. Owing to the shape of the magnetic bead array intensity distribution, which was observed to be non-Gaussian, a weighted mean was selected as an alternative numerical strategy for data processing.

The measured variability scores for the two detection methods are summarized in Table 1. The intra-slide and inter-slide % coefficients of variation (CVs) were higher for the MBD than for the FBD, but still lower than 15%. Furthermore, no obvious correlation was found showing that any particular antigen was

TABLE 1 Assay variability			
%CV	Method	Weighted mean CM	Arithmetic mean
Intra-array	MBD	NA	9.7%
	FBD	NA	12.4%
Intra-slide	MBD	11.3%	10.7%
	FBD	6.4%	6.7%
Inter-slide	MBD	14.6%	13.8%
	FBD	9.0%	9.5%

The intra-array and intra-slide and inter-slide variability of the two methods was calculated using the weighted mean and arithmetic mean of replicate spot intensities.

**FIGURE 2**

Visualization of magnetic beads captured in microarray spots. For simplified readout purposes, the imaging of captured magnetic beads could easily be done with an ordinary flatbed scanner. The reactivity of a patient serum is shown as a false color image of an array (A), the signal intensity is related to the density of beads that are captured in a spot. Bead-covered spots are also visible to the naked eye and exemplified by light microscopy magnification (B,C). Row 1 represents spots of goat anti-human IgG and rows 2–13 spots of autoantigens (TG, TPO, CENP-B, PmScl100, Scl70, dsDNA, SSB/La, RNP A and C, SSA/Ro-52 and SSA/Ro-60 and Sm). Additionally, a negative serum is shown (E) and a color scale for quantitative interpretation of false colors (E).

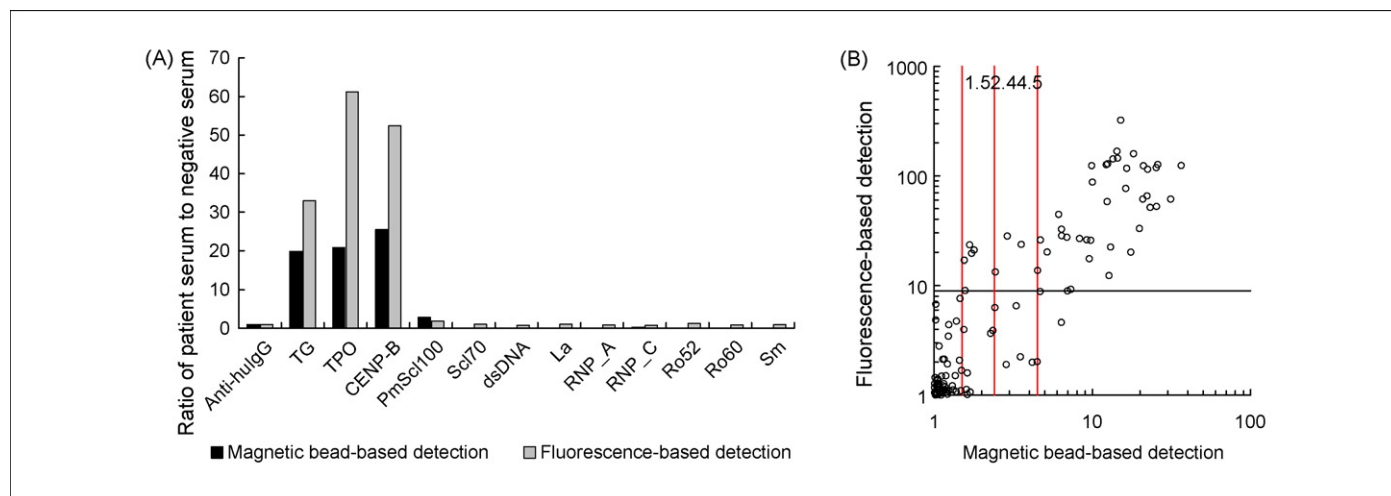
susceptible to increased variability for either of the two methods (data not shown). A magnitude amplitude (MA) plot ([Supplementary Figure 1](#)) revealed a deviation in the correlation between intensity and variance between the two detection methods. While FBD had an increasing variability for intensities approaching background levels, the MBD showed a distinct increase in the low intensity regimes, while the variability was low when approaching background intensities. This phenomenon could result from a binary tendency of the MBD, where nearly all beads are removed below a certain threshold in bound autoantibody.

Weighted mean-based data processing allowed the estimation of intra-array tendencies and the comparison of MBD and FBD in terms of variability and reproducibility. This showed that both

methods exhibited inter-slide and intra-slide variabilities within the range of 15%.

Congruence

The individual congruence between the two detection methods was then evaluated. The negative serum was employed in order to normalize patient-specific responses and a similar response pattern was observed ([Figure 3A](#)). The intensity ratios of all 21 positive autoimmune patient sera used for MBD and FBD were combined to assess an inter-method correlation ([Figure 3B](#)), which resulted in $R^2 = 0.49$. The dynamic range was found to be larger for FBD (>100) than for MBD (<40). In addition, appropriate thresholds or cut-off lines defining a positive or a negative autoantibody

**FIGURE 3**

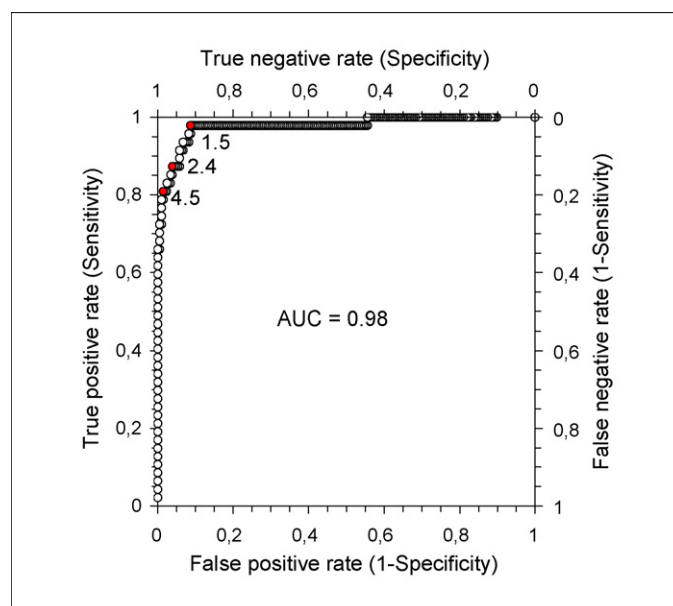
(A) Response pattern of a serum sample for fluorescence and magnetic bead readout. The intensity ratios of a single patient serum and negative serum for detection of autoantibodies are shown for magnetic bead (array shown in Figure 2) and fluorescence readout. (B) Intensity ratios between patient sera and control sera obtained from fluorescence-based detection were plotted against magnetic bead-based detection. The vertical red lines represent the indicated thresholds from ROC analysis (Figure 5) while the horizontal black line indicates a threshold that had been introduced to assign results as positive or negative for a fluorescence-based detection.

response were introduced, and used to assess the specificity and sensitivity of MBD. For FBD, a threshold was defined, which was ninefold higher than the signals derived from negative control patients (horizontal line). For the datasets generated with the MBD method, vertical lines were introduced to reflect different thresholds. These different thresholds were deduced from a receiver-operating characteristics (ROC) analysis, described below. The upper right quadrant created by both horizontal and vertical lines represents the events that were positive in both readout methods.

Sample classification

In a ROC analysis, the area under the curve serves as an estimate of the probability that MBD will rank a randomly chosen positive test result higher than a randomly chosen negative test result [26]. In the comparison of the MBD derived data with the FBD datasets that was conducted, an area under the curve of 97% was obtained (Figure 4), indicating a high degree of concordance between the two detection methods. Sensitivity and specificity scores for the three chosen classifier values are shown in Table 2. With a classifier of 1.5, a signal with MBD was predicted as positive when the patient serum signal was 1.5-fold higher than the negative control serum. In this case, 98% of all positive results were correctly classified as truly positive (252 data points). In addition to this, 91% of all negative results were correctly classified as truly negative using the same classifier. When selecting a classifier value of 2.4,

the specificity increased to 96%, while the sensitivity decreased to 87%. A further increase of the classifier value to 4.5 resulted in a sensitivity decrease to 81% and to a specificity increase to 99%. When signals were grouped dichotomously into positive or negative scores, the ROC analysis revealed concordance between the FBD and MBD methods.

**FIGURE 4**

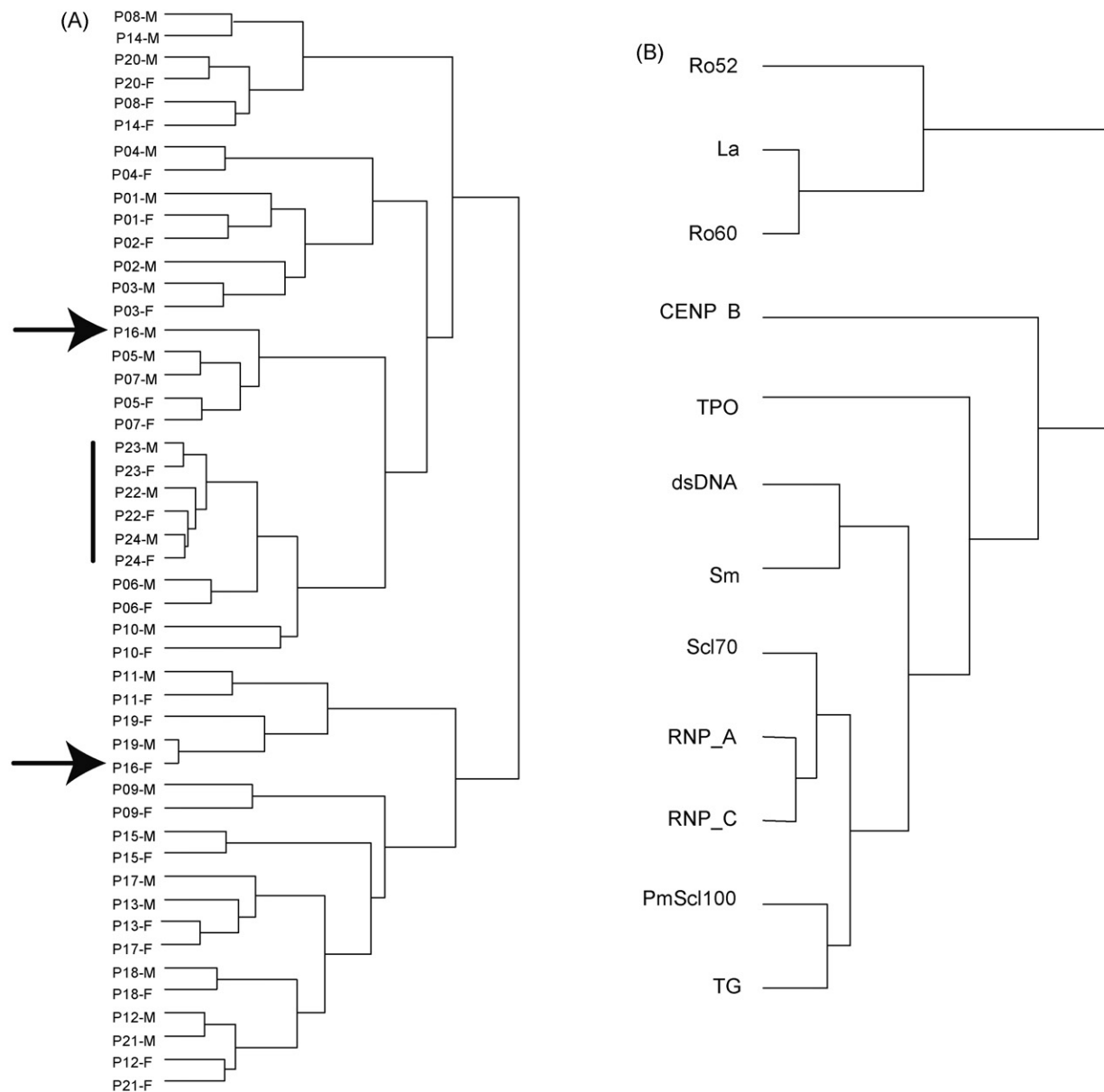
ROC analysis for specificity and sensitivity. The detection of autoantibodies by means of magnetic beads coupled to anti-human IgG or fluorescently labeled goat anti-human IgG antibodies were compared using receiver-operating characteristics (ROC). Here, the true positive rate was plotted against the false positive rate. Three examples for sensitivity and specificity at different classifiers (ratios between signals from patient samples and negative control samples) for magnetic bead-based detection are shown. Furthermore, the area under the curve was determined as a criterion for the discrimination of positive and negative signals.

TABLE 2

Sensitivity and specificity of several classifiers

Classifier	Sensitivity	95% CI	Specificity	95% CI
4.5	80.9%	0.67–0.91	98.5%	0.96–0.99
2.4	87.2%	0.74–0.95	96.1%	0.93–0.98
1.5	97.9%	0.89–0.99	91.2%	0.87–0.95

The sensitivity and specificity levels were calculated for each classifier and supplemented with their respective confidence intervals (CI).

**FIGURE 5**

Clustering analysis. The two dendrograms show the clustering of (A) patient sera and (B) antigen clustering. On the patient sera level, the hierarchical clustering indicates a concordance between fluorescence bead-based detection (-F) and magnetic bead-based detection (-M) with only a single patient serum (arrow) not grouping adjacent, in contrast to the negative controls (bar). In the antigen clusters, tight grouping of proteins may allow the revelation of co-autoimmunity toward certain substances and together with the heatmap ([Supplementary Figure 2](#)) could enable the scoring of antigens that drive the patient sera grouping.

To study the classification of each sample and antigen across both detection methods, an unsupervised clustering based on Euclidean distance was performed and presented in a dendrogram ([Figure 5A](#)). The clusters obtained suggested that both detection methods allowed the clustering of the samples according to their known disease status, as the vast majority of sera were grouped adjacent or close. Further, the negative control group samples all grouped tightly together in a subcluster. The dendrogram representing antigen clustering ([Figure 5B](#)) showed similar response patterns and facilitated sample grouping (see also [Supplementary Figure 2](#)). This information might allow the identification of key

antigens, antigens with functional similarities, as well as antigens with lower diagnostic power, but this was beyond the realms of the presented study. The results above revealed that magnetic bead-based detection is an interesting alternative to fluorescence-based detection.

Discussion

The present study describes the use of a magnetic bead-based detection (MBD) approach to screen for autoantibodies using protein microarrays. Magnetic beads, loaded with goat anti-human antibodies, were used to visualize autoantibodies bound

to different antigens in a protein microarray. The presence of the magnetic beads in the microspot resulted in a strong opacity shift, which was possible to record with an ordinary flatbed scanner. In addition, it was possible to reduce the incubation time required for the species-specific detection of antibodies to less than 1 min owing to the rapid migration of beads toward the array surface when a magnetic force is applied. The number of magnetic beads on the microspots corresponds to the amount of captured analytes.

The detection of autoantibodies in patient sera was one of the earliest applications to be transferred from singleplex immunoassays to multiplex protein microarray assays. The reasons for this were obvious [16]: (i) there was a clinical demand for the identification of several autoantibodies to confirm the diagnosis of the type of rheumatic disease, (ii) autoantibodies are present in sufficiently high concentrations and do not require ultra-sensitive detection techniques, and (iii) the detection of the different autoantibodies requires only one species-specific detection antibody; therefore a semi-quantitative determination of autoantibody titers is sufficient. The first commercial application based on protein microarrays therefore targeted autoimmune diagnostics [27]. In the meantime, many other antigen microarray-based tests have been developed for autoimmune disease diagnostics as well as for allergy and infectious disease diagnostics [15]. Therefore, improvements in the detection of specific autoantibodies are always of particular interest.

Another obvious benefit of protein microarray assays is that only minute amounts of sample and reagents are needed. One of the current obstacles to the broader application of protein microarrays is that the conventional FBD systems typically involve advanced and expensive laser scanning equipment (also referred to as biochip readers). Therefore, magnetic beads loaded with detection antibodies were applied as detection reagents, which enabled readout using a low-cost standard flatbed scanner as the detection system. Since bound magnetic beads produce strong shifts in opacity on the surface of the glass, signals derived from the presence of antigen-bound autoantibodies within a microspot can be recorded using a standard flatbed scanner. In addition, it was possible to reduce the incubation time for the detection step from 30 min to less than 1 min. The subsequent procedure for the detection of bound autoantibodies using the MBD can be done in less than 10 min. Taken together, the overall assay time – starting with the incubation of the sample and ending with data acquisition – requires less than 60 min. With the present set-up, 12 serum samples can be analyzed on each slide and up to four slides can be processed manually in parallel without a significant increase in the total assay time. It is also possible to use even larger numbers of arrays per slide to increase throughput without substantially reducing the performance levels of the assay.

The MBD presented displayed advantages in terms of speed and readout whereas FBD generally achieved a higher dynamic range as well as a higher sensitivity. This is most probably due to the following: For the MBD approach, an average background of 20–50 beads within each microspot was observed, whereas a maximum of approximately only 3000 magnetic beads per spot can be achieved owing to the small size of the magnetic bead (1 μm) and the microspot (150 μm). This explains the lower dynamic range of the MBD approach compared to the FBD approach. This is due to the lower ratios between the maximum signal and the lowest signal

above background. The MBD system frequently yielded a higher fraction of spots with very low numbers of magnetic particles. We believe that this most probably is due to the magnetic beads being removed completely from spots by effects such as shear forces that might occur during slide rinsing. The applied magnetic force on the beads was sufficiently strong to allow rapid collection of beads on the array surface, and to remove unbound and only weakly associated beads during the washing step. Our estimations of the force magnitude lead to the conclusion that inhomogeneity or irregularities in the magnetic field would not be substantial as to produce the observed variability between spots. Further, no obvious geometric pattern or location of empty spots was observed, which suggests that turbulences occurring during the addition and removal of washing liquid over the array, may be the source of variability. Owing to the described binding pattern, a weighed mean concept was introduced in place of the arithmetic mean. Simple mean and standard deviation estimations are generally applied to FBD, but require the data to be Gaussian distributed. In the present case, they were rejected as a result of the non-Gaussian tendency of the magnetic bead data. The weighted mean concept was chosen owing to its non-dependence on particular data distributions and because the method produces a single intensity data point per replicate set in an unsupervised fashion that may serve as a representative estimate of the general tendency of the replicates. The non-Gaussian intensity distribution observed with the magnetic bead detection may be at least partly inherent owing to the magnetic beads experiencing larger volume forces from the fluid environment for instance. It might be feasible to reduce variability and approach a Gaussian distribution through future optimization efforts of particularly the washing protocol. Here, automated liquid handling or designing fluidic array masks would offer more robust solutions to reduce the impact of manual operations.

For the identification of autoantibodies involved in autoimmune, allergy, and infectious diseases, a semi-quantitative, binary detection or even just the presence of antibodies is often sufficient. An assay with limited dynamic range and sensitivity may therefore be sufficient for the qualitative analysis of the presence or absence of antibodies. In the autoantigen microarray detection system presented in this study, the sensitivity of the MBD assay was not a limiting factor. In order to validate MBD in terms of sensitivity and specificity, ROC analysis was employed as a binary classification model to evaluate the concordance and grouping between magnetic bead-based and fluorescence readout. Results were grouped in either positive or negative classes based on the classification of the sera on each particular autoantigen. The sensitivity and specificity achieved correlated well between MBD and FBD. Different thresholds for the MBD system were introduced to achieve optimal sensitivity and specificity.

In the field of autoimmunity, allergy, and infectious disease diagnostics, multiplexed assays have recently entered the *in vitro* diagnostic market, and it is anticipated that tumor marker panels will be applied in monitoring therapy in the future [15]. While it is likely that protein microarrays will be accepted in the *in vitro* diagnostics market, some obstacles still have to be overcome: 'Medical need, combined with an overall cost reduction, must become the driving force behind protein arrays gaining a substantial share of the IVD market' [28]. The application of protein microarrays as diagnostic tools has huge potential, but there is a

need for additional technical solutions that simplify the procedure, decrease assay times, and reduce costs [29].

The application of a magnetic bead-based detection system combined with a low cost detection system may present such a solution within the field of autoimmune diagnostics in cases where an ultra-low limit of detection is not needed and qualitative detection is sufficient. While fluorescence readers are becoming smaller and more affordable, options for low-resource environments will be important to investigate to allow developing and employing home made solutions for protein microarray assays.

Acknowledgements

We would like to thank Dr. Peter Höpfl of Phadia, Freiburg, Germany for providing antigens and serum samples. This work was funded by The Swedish Governmental Agency for Innovation Systems (VINNOVA) and the Royal Swedish Academy of Science (KVA).

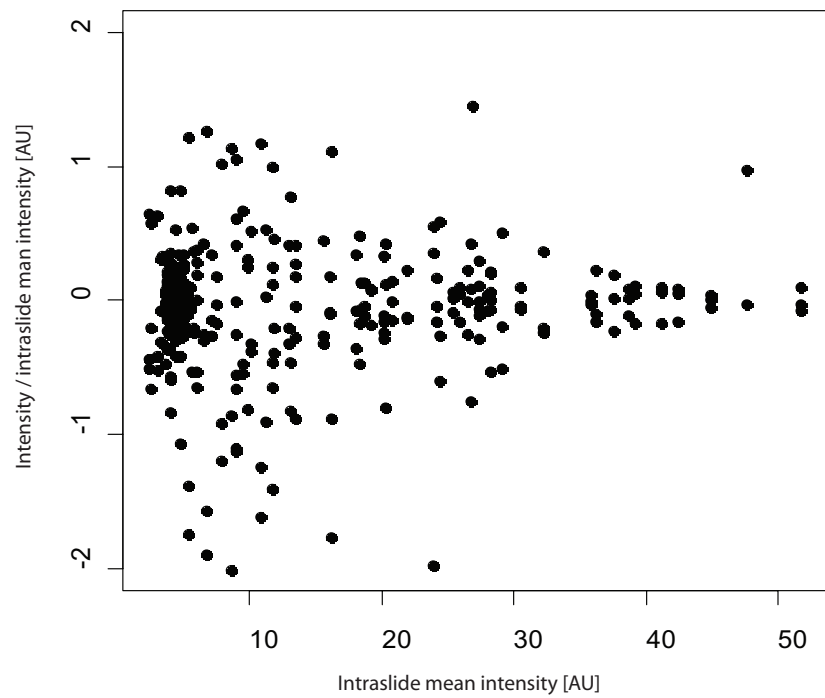
Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.nbt.2009.07.011](https://doi.org/10.1016/j.nbt.2009.07.011).

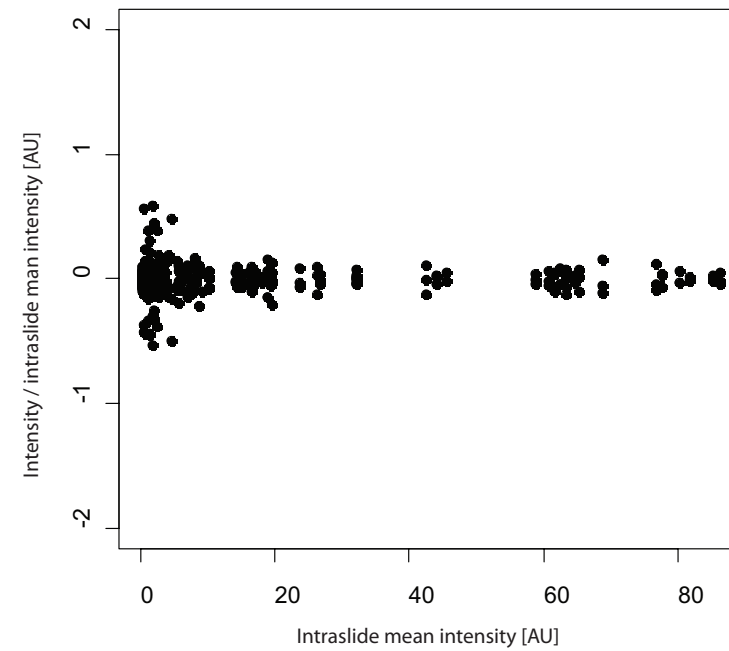
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MA - plot (Magnetic Bead Detection)



MA - plot (Fluorescence - based Detection)



Heatmap cluster of seras and antigens

