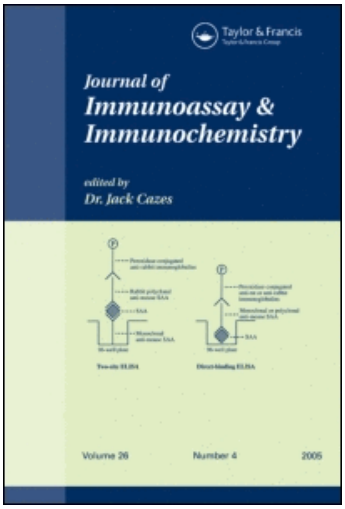

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A Suspension Array Immunoassay for the Toxin Simulant Ovalbumin

Hardeep S. Bhogal,[1] Mark Snodgrass,[2] Lori J. McLaws,[1] Terrina Dickinson-Laing,[2] David C. Mah,[2] and R. Elaine Fulton[1]
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Abstract: A microsphere-based suspension array (SA) system was used for the development and characterization of an immunoassay for the toxin simulant ovalbumin. Results obtained by SA immunoassay were compared with those obtained by enzyme-linked immunosorbent assay (ELISA) using the same immunoreagents. The limit of detection (LOD) for the SA ovalbumin assay was 4.9 ng/mL, compared to a LOD of 0.01 ng/mL for the ovalbumin ELISA. Although the ELISA LOD exceeded that of the SA assay, the SA assay was simple and rapid to perform, with assays being completed in half the time of the traditional ELISA. The well-to-well reproducibility (coefficient of variation (CV)) of the ELISA and the SA assay was 4.9% and 5.1%, respectively. The ELISA and SA assay plate-to-plate reproducibility was 14.8% and 6.1%, respectively. The protocols used to develop the SA assay for ovalbumin may be used as a template for development of other SA assays for toxins, bacteria, and viruses.

Keywords: ELISA, Immunoassay, Microsphere, Multiplex, Ovalbumin, Suspension array

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INTRODUCTION

The development of biological weapons by Iraq during the Gulf War[1] and the occurrence of bioterrorism incidents, such as the attack on the US postal system with *Bacillus anthracis*-laden letters in 2001,[2] highlight the fact that exposure to biological threat (BT) agents has emerged as a significant potential threat to military, first responder, and civilian populations. There is, therefore, a need to develop systems that are sufficiently sensitive and specific for the rapid detection and identification of a broad range of potential BT agents, including bacteria, viruses, and toxins.

Due to the high sensitivity, specificity, selectivity, and adaptability of antibody-based technologies, the enzyme-linked immunosorbent assay (ELISA) is currently the most widely used method of detection and identification of BT agents.[3,4]

Unlike other detection and identification technologies, such as the polymerase chain reaction (PCR), the ELISA does not require extensive sample processing prior to analysis.[5] However, conventional microplate ELISAs have a number of limitations,[6] one of which is that they require separate assays for each antigen of interest.[4] Hence, multiple ELISAs must be performed to test for a panel of BT agents, an approach which is time-consuming, reagent-intensive, and problematic if limited sample volumes are available.

A relatively new alternative to the traditional ELISA is the suspension array (SA) immunoassay. This immunoassay format utilizes Luminex xMAP bead technology[5] in which 5.5 μm polystyrene microspheres are embedded with precise ratios of two (red and near infrared) fluorescent dyes. By varying the ratio of dyes, up to 100 bead classes are available, each with a unique spectral signature. This allows multiplexed identification of up to 100 discrete analytes in a single sample using a flow cytometry-based dual laser detector system.[5,7] Beads can be utilized in a capture immunoassay format in which spectrally unique bead sets are covalently coupled with different capture antibodies (CAb) (Figure 1). Thus, each bead set acquires the capability to 'capture' the corresponding specific antigen. Beads are mixed and incubated with the test sample in a well in a filtration plate. To detect each of the captured antigens in the sample, detector antibodies (DAb) are introduced that specifically bind to the antigens, followed by indicator antibodies (IAb) labeled with a fluorescent phycoerythrin (PE) reporter tag. For analysis, the bead complexes are acquired by the suspension array reader and are channeled through a flow cell where two lasers excite the fluorophores in the beads.[7] The red classification laser excites the dyes in each microsphere, identifying its unique fluorescence signature.[4,7] The green reporter laser excites the PE reporter molecule associated with the bead, which allows quantitation of the antigen by its fluorescence intensity (FI).[4,7] To exclude bead aggregates from

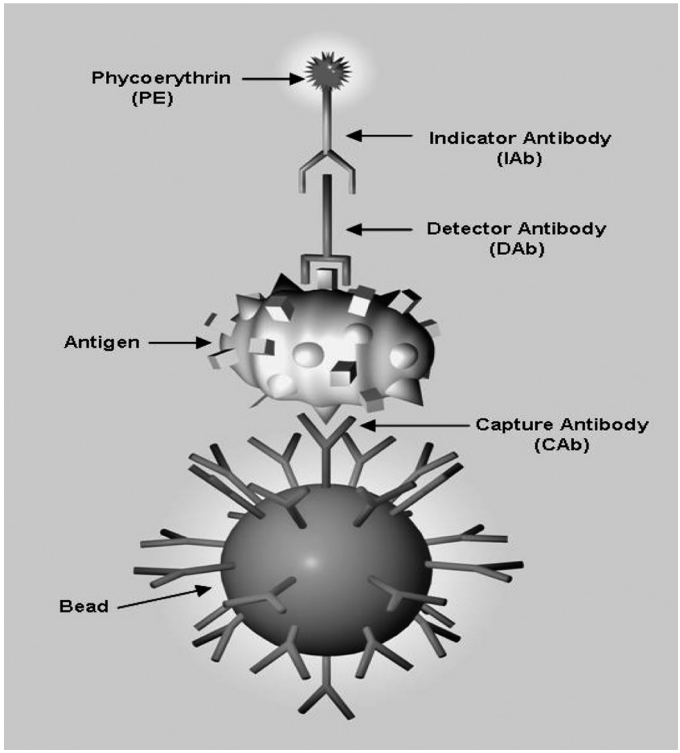

Figure 1. Schematic of a Suspension Array Immunoassay. Carboxyl-coated 5.5 μm beads are embedded with varying ratios of two fluorescent dyes, resulting in an assortment of beads with unique spectral signatures. In a typical assay an individual bead set is covalently coupled to CAb. The coupled beads are used to probe for antigen in an unknown sample. Subsequently, DAb is added which binds specifically to the antigen. This is followed by addition of a PE-conjugated IAb. The complex is analyzed in a flow cytometer where the classification laser excites the dyes in the bead to identify its unique fluorescence signature. The reporter laser excites the PE molecules associated with the complex to allow quantitation based on fluorescence intensity.

the analysis, the microsphere size is measured by light scatter of the classification laser. Bead complexes that lie within the size range of individual beads are included and only individual beads are read by the reader.[7] High-speed digital signal processors and software record the fluorescence signals simultaneously for each bead, translating the signals into data for each bead-based assay in real-time.

The SA immunoassay has several advantages over the ELISA, the most significant being that the SA immunoassay can test up to 100 different antigens simultaneously. This not only conserves the amount of test

sample and reagents required for analysis, but also saves time required to perform multiple assays for each antigen.[6,8] In addition, the beads used in SA assays are suspended in solution during incubation, thus shortening the diffusion path of antigen to antibody binding sites on the beads.[3] Binding in free solution is favoured kinetically relative to binding to a solid phase. Therefore, incubation times can be reduced compared to ELISA; respectable sensitivity with incubation times as little as 30 minutes per step have been reported.[3] Furthermore, the antibody concentrations and assay volumes used in SA assays are less than those used in ELISA, resulting in a lower overall cost per SA assay compared to ELISA.

In this study, a SA assay was developed and characterized for ovalbumin, a 45 kDa glycoprotein found in chicken egg whites. Ovalbumin is often used in immunoassay development as a simulant for protein toxins such as ricin, botulinum toxin or staphylococcal enterotoxins. Suspension array immunoassays have been reported for several BT agents, including aerosolized *Bacillus anthracis*, *Yersinia pestis*, and botulinum toxoid,[9,10] thus demonstrating proof of principle for this detection approach. However, these reports did not provide a detailed methodology for optimization of each component step in the development of a novel SA assay. In this study, we describe the detailed procedures for development, optimization, and characterization of ELISA and SA immunoassays for ovalbumin. We also compare the sensitivity of the developed SA assay to the ELISA for detection and identification of ovalbumin, using the same component reagents. The methodology described herein will serve as a template for the subsequent development of SA assays for other BT agents.

EXPERIMENTAL

Antigens and Antibodies

The CAb, mouse monoclonal anti-ovalbumin antibody OVA-14, was purchased from Sigma (St. Louis, MO). The DAb, rabbit anti-ovalbumin polyclonal antibody (ion-exchange purified) was purchased from Chemicon International Inc. (Temecula, CA). The IAb utilized in the SA immunoassay, PE-conjugated goat F(ab')2 anti-rabbit IgG (H + L), was obtained from Cedarlane Laboratories Ltd. (Hornby, ON). The IAb utilized in the ELISA, goat anti-rabbit IgG (H + L) horseradish peroxidase (HRP)-conjugated antibody, was obtained from Bethyl Laboratories Inc. (Montgomery, TX). A PE-conjugated goat anti-mouse IgG (H + L) (Cedarlane) was used for validation of antibody coupling to SA microspheres. Albumin from chicken egg white, grade V, minimum 98% purity, was obtained from Sigma.

Antibody Purification

Mouse monoclonal anti-ovalbumin antibody was purified using a NAb™ Spin Purification Kit (Pierce, Rockford, IL) according to the manufacturer's directions. Briefly, the method used was as follows. ImmunoPure Immobilized Protein G Plus™ gel slurry was mixed and 200 μL was dispensed into a Handee Spin Cup™ column. The column was placed in a microcentrifuge collection tube. Three hundred μL of Binding Buffer was added and the uncapped cup/tube assembly was centrifuged at 5000 × g for 1 minute. The filtrate was discarded and 400 μL of Binding Buffer was added. The uncapped cup/tube assembly was centrifuged as above and the filtrate was discarded. The antibody sample was added and the capped cup/tube assembly was agitated for 1 hour at 4°C. The cup/tube assembly was uncapped and centrifuged again as above. The column was washed three times with 400 μL of Binding Buffer, then transferred to a new collection tube. Elution Buffer (400 μL) was added. The cup/tube assembly was agitated for 5 minutes and then centrifuged as above. The spin column was transferred to a new collection tube and the filtrate was saved as the first elution fraction. The elution and centrifugation steps were repeated three times to collect four fractions total. Protein concentrations of each fraction were determined by measuring the absorbance at 280 nm in a UV500 UV-Visible spectrophotometer (Unicam, Cambridge, UK).

ELISA

Buffers and Reagents

Coating buffer (0.05 M carbonate-bicarbonate, pH 9.6), wash buffer (phosphate buffered saline, pH 7.4 (PBS), 0.1% bovine serum albumin (BSA), 0.1% Tween-20), and blocking buffer (PBS, 2% BSA) were used in ELISA. Diluent buffer (PBS, 2% BSA, 0.1% Tween-20) was used for dilutions of antibodies and antigens. Carbonate-bicarbonate (0.05 M, pH 9.6) capsules, PBS, and Tween-20 were obtained from Sigma. BSA fraction V was obtained from EMD Chemicals (Gibbstown, NJ). ABTS Peroxidase Substrate System was purchased from KPL (Gaithersburg, MD).

Assay Protocol

The CAb was diluted in ELISA coating buffer and 100 μL was added to wells of a Nunc Maxisorb 96-well flat bottom plate (VWR, Mississauga, ON). The plate was incubated overnight at 4°C. For each washing step, wells were washed five times with ELISA wash buffer, using an ELX50

Auto Strip Washer (Bio-Tek Instruments Inc., Nepean, ON). After washing, ELISA blocking buffer (300 μL) was added to block open protein-binding sites on the plate. The plate was incubated for 1 hour at 37°C, washed five times with ELISA wash buffer, and the blocking step was repeated. Plates were washed as above. Antigen was diluted with ELISA diluent buffer and 100 μL was added to the wells. The plate was incubated for 1 hour at 37°C and then washed as above. DAb diluted in ELISA diluent buffer (100 μL per well) was added to the wells. The plate was incubated for 1 hour at 37°C and washed as above, followed by the addition of IAb diluted in ELISA diluent buffer (100 μL per well). The plate was incubated for 1 hour at 37°C and washed again as above. For detection, 200 μL of ABTS HRP substrate solution was added to all wells and incubated for 30 minutes at room temperature in light-free conditions. Absorbance of reaction products was read at 405 nm ($A_{405\,nm}$) in a Molecular Devices Thermomax^{R} automated plate reader (Fisher Scientific, Ottawa, ON).

Assay Optimization

The CAb was optimized by titration of varying concentrations of CAb in the presence of a fixed concentration of IAb. The optimal CAb concentration was defined as the lowest concentration of CAb giving the maximum absorbance signal. The DAb was optimized by titration of varying concentrations of DAb in the presence of the optimal concentration of CAb and fixed concentrations of antigen and IAb. The optimal DAb was defined as the concentration that yielded the maximum absorbance. The IAb was optimized by titration of varying concentrations of IAb in the presence of the optimal concentrations of CAb and DAb and a fixed concentration of antigen. The optimal concentration of IAb was the concentration yielding the highest signal to background (S/B) ratio.

Suspension Array Immunoassay

Buffers and Reagents

The buffers used in antibody coupling to beads (Wash Buffer, Activation Buffer, PBS, Blocking Buffer, Storage Buffer, and Staining Buffer) were provided in an Amine Coupling Kit purchased from Bio-Rad Laboratories Inc. (Hercules, CA). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysulfosuccinimide (S-NHS), used in the coupling process, were obtained from Pierce. PBS-BSA (PBS, 1% BSA) was used for all washing and dilutions of antibodies and antigens in the SA assays.

Bead Coupling

A single set of microspheres (COOH Bead Region 010 at a concentration of 1.25×10^7 beads/mL) was purchased from Bio-Rad Laboratories Inc. Protein G-purified mouse monoclonal anti-ovalbumin antibodies were covalently coupled to COOH Bead 010 using the Amine Coupling Kit protocol provided by the manufacturer. Briefly, the method was as follows. Beads were vortexed and sonicated (VWR Ultrasonic Bath Model 75HT) for 30 seconds. A 100 μL aliquot was added to the Amine Coupling Reaction Tubes. The tubes were centrifuged at 14000 × g for 6 minutes at 4°C; these same conditions were used for all subsequent centrifugation steps. Supernatants were removed and beads were resuspended in 100 μL of Wash Buffer and centrifuged. The supernatants were removed and the beads were resuspended in 80 μL of Activation Buffer. Ten μL of 50 mg/mL EDC was added to each tube, followed by the addition of 10 μL of 50 mg/mL S-NHS. The tubes were wrapped in foil and agitated at room temperature for 20 minutes; PBS (150 μL) was then added, and the tubes were centrifuged. The supernatants were removed and the beads were resuspended in 100 μL of PBS. Varying amounts of CAb were added to each reaction tube and the volume was adjusted to 500 μL with PBS; final concentrations of CAb ranged between 0.25–11 μg/mL. The tubes were wrapped in foil and agitated overnight at 4°C. The next day, the tubes were centrifuged and the supernatants were removed. The beads were washed in 500 μL of PBS and centrifuged. The supernatants were removed and the beads were resuspended in 250 μL of Blocking Buffer. The tubes were wrapped in foil and agitated for 30 minutes at room temperature. After blocking, the tubes were centrifuged and the supernatants were removed. The beads were then washed once in 500 μL of PBS and centrifuged. The supernatants were removed and the beads were resuspended in 150 μL of Storage Buffer. Final bead counts were performed using a hemacytometer. The tubes containing beads were stored in light-free conditions at 4°C.

Assay Optimization

The CAb-coupled beads were validated as follows. Beads were vortexed for 15 seconds, then 10,000 beads were added to 50 μL aliquots of 1 μg/mL PE-labeled anti-CAb antibody diluted in Staining Buffer. As a negative control, a set of CAb-coupled beads was added to 50 μL of Staining Buffer only. The tubes were covered in foil and agitated for 30 minutes at room temperature. The beads were centrifuged at 14000 × g for 6 minutes at 4°C and the supernatants were removed. The beads were resuspended in 125 μL of Storage Buffer and transferred to a 96-well flat bottom plate. The plate was placed in the Bio-Plex 100 SA assay reader (Bio-Rad), where 50 μL of each

sample was analyzed by the reader to determine median FI from 100 bead complexes. Detector antibody and IAb concentrations were optimized as previously described in the assay optimization section for ELISA.

Assay Protocol

Assays were conducted in 96-well filtration plates with a pore size of 1.2 μm (Millipore, Bedford, MA). A 50 μL aliquot of CAb-coated beads (5000 coupled beads) was mixed with 50 μL of test antigen. The filtration plate was then shaken on a microplate shaker (Wallac PerkinElmer, Woodbridge, ON) at 500 rpm for 30 minutes at room temperature in light-free conditions. Fluid was vacuum aspirated from the wells using a Millipore 96-well vacuum manifold. Wells were washed four times with 100 μL of PBS-BSA to remove unbound antigen and the beads were resuspended in 50 μL of PBS-BSA. Aliquots (50 μL) of DAb were added to the wells and the filtration plate was shaken as described above. Wells were washed as described above to remove excess DAb and the beads resuspended in 50 μL of PBS-BSA. Fifty μL of IAb was added to the wells and the filtration plate was shaken as described above. Wells were washed four times with 100 μL of PBS-BSA and the beads resuspended in 100 μL of PBS-BSA. The filtration plate was then read in the Bio-Plex 100 SA assay reader. Fifty μL of sample from each well was analyzed by the reader to determine the median FI from 100 bead complexes.

Data Analysis

Preliminary analysis of ELISA data was performed using Softmax 3.0 software (Molecular Devices, Menlo Park, CA). Raw data was exported to Microsoft Office Excel 2003 for statistical analysis and plotting of graphs. Suspension array immunoassay data was exported from Bio-Plex Manager 4.0 software into Microsoft Office Excel 2003 for statistical analysis and plotting of graphs. Samples were run either in triplicate (ELISA) or in quadruplicate (SA assay).

RESULTS AND DISCUSSION

Ovalbumin ELISA Development

Down-Selection of Antibodies

The initial step in the development of a capture ELISA was to determine which antibody combinations produced the highest $A_{405\,nm}$ S/B ratio. To determine which anti-ovalbumin antibodies were the ideal capture and

detector antibodies, an indirect capture ELISA was performed using a number of commercially available anti-ovalbumin antibodies as capture and detector antibodies in different combinations (data not shown). The combination of antibodies that produced the highest S/B ratio (S/B = 11.4) was a mouse anti-ovalbumin CAb paired with a rabbit anti-ovalbumin DAb (data not shown).

Mouse monoclonal anti-ovalbumin antibody was purified to determine if purification would decrease the background and/or increase the signal. Purification of mouse CAb resulted in an average increase in absorbance of 60% compared to unpurified CAb (data not shown). A combination of protein G-purified mouse monoclonal antibody as CAb and commercially-purified rabbit polyclonal antibody as the DAb yielded a S/B ratio of 24.6 (data not shown).

Optimization of ELISA

Optimization of CAb, DAb and IAb concentrations for indirect ELISAs was performed using varying concentrations of each test antibody. The optimal concentration of CAb and DAb was 15 μg/mL (Figure 2a) and 3 μg/mL (Figure 2b), respectively. Using these optimized CAb and DAb concentrations, the optimal concentration of the IAb was 80 ng/mL (Figure 2c).

Limit of Detection of ELISA

To determine the limit of detection (LOD) of the optimized indirect capture ovalbumin ELISA, an antigen titration was performed using 2-fold serial dilutions of ovalbumin from 39 ng/mL to 0.0048 ng/mL. The LOD was defined as the lowest antigen concentration yielding an absorbance reading within one standard deviation of the assay cutoff (background plus two standard deviations). Using this approach, the LOD was 9.5 pg/mL (Figure 3). This ELISA LOD was 30-fold more sensitive than the ELISA LOD of 300 pg/mL for ovalbumin previously reported.[3] This difference in LODs is likely due to the different methods used (indirect versus direct ELISA). In addition, differences in antibody affinity, avidity, and/or purity may have also contributed to the difference in LODs.

Ovalbumin SA Immunoassay Development

Optimization of Antibody Coupling to Beads

The ovalbumin antibody selected and optimized as CAb for use in ELISA was used as CAb to develop the SA immunoassay for ovalbumin.

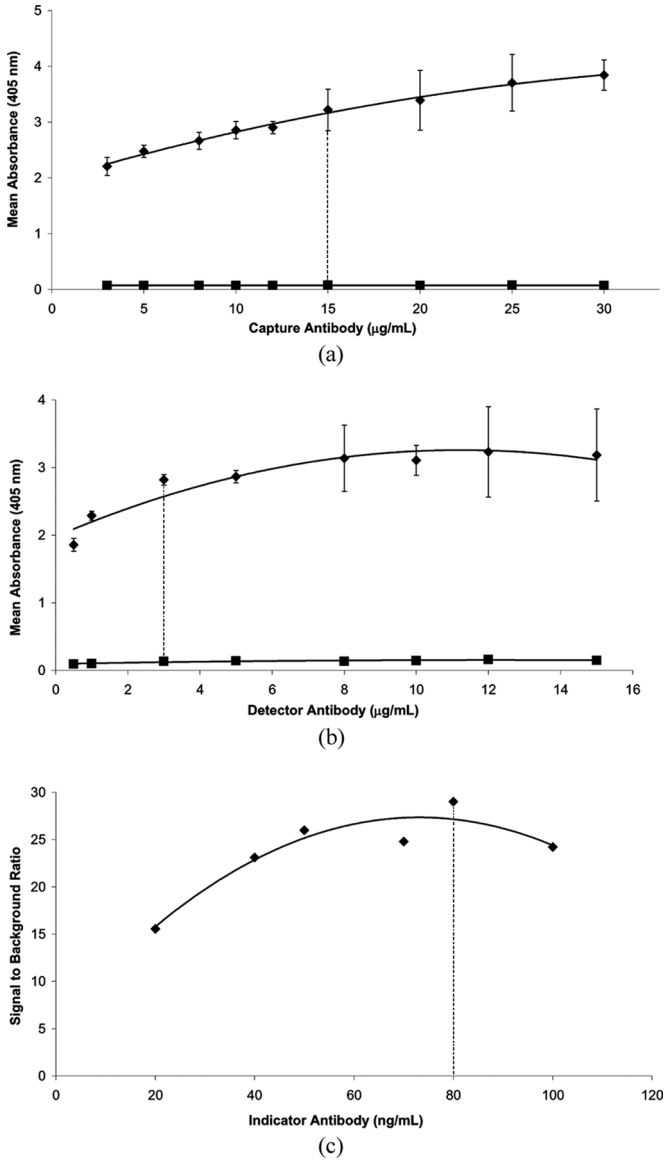

Figure 2. ELISA Antibody Optimization. (a) The optimal concentration of CAb (dotted line) was determined using IAb at 0.2 μg/mL (diamonds) or no IAb (squares). (b) The optimal concentration of DAb (dotted line) was determined by titration in the presence of 5 μg/mL ovalbumin (diamonds) or no ovalbumin (squares) using CAb at 15 μg/mL and IAb at 60 ng/mL. (c) The optimal concentration of IAb (dotted line) was determined by titration using CAb at 15 μg/mL, ovalbumin at 10 μg/mL, and DAb at 3 μg/mL.

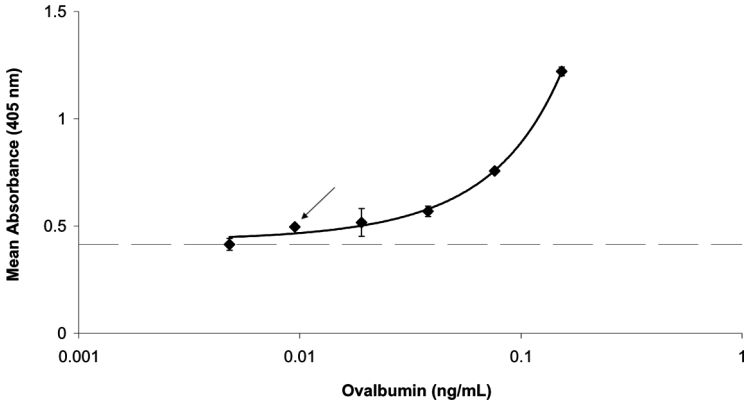

Figure 3. Limit of Detection of ELISA. The limit of detection for ovalbumin (represented by arrow) was determined using CAb at 15 μg/mL, DAb at 3 μg/mL, and IAb at 80 ng/mL. Dotted line represents background absorbance +2 standard deviations ($r^2 = 0.9945$).

To determine the optimal CAb concentration needed for coupling to microspheres, protein G-purified mouse anti-ovalbumin monoclonal antibody was coupled to beads using a range of protein concentrations from 0.25 to 7 μg/mL. After the coupling process, bead validations were conducted and it was concluded that the optimal CAb coupling concentration (lowest CAb concentration yielding the highest FI) was 5 μg/mL (Figure 4a). All other concentrations of coupled CAb, with the exception of 0.25 μg/mL, also yielded FI values over the recommended bead validation cutoff (FI > 10,000; Luminex bead coupling protocol). It is likely that these CAb-coupled beads could also be used for the SA immunoassay, however, this hypothesis remains to be tested.

SA Assay Optimization

The ovalbumin antibody optimized as DAb for use in ELISA was used as DAb for the SA immunoassay. To determine the optimal concentration of DAb, the SA immunoassay was performed with the optimized CAb-coupled beads, using concentrations of rabbit anti-ovalbumin DAb ranging from 0.5 to 12 μg/mL. The IAb, PE-conjugated goat anti-rabbit IgG, was used in excess at a constant concentration of 10 μg/mL. The concentration of ovalbumin used in the antigen positive wells was 10 μg/mL. Under these conditions, the optimal concentration of DAb (the lowest concentration yielding the highest FI) was 5 μg/mL (Figure 4b).

The optimal concentration of IAb was determined using concentrations of PE-labeled goat anti-rabbit IgG ranging from 0.031 to 1 μg/mL,

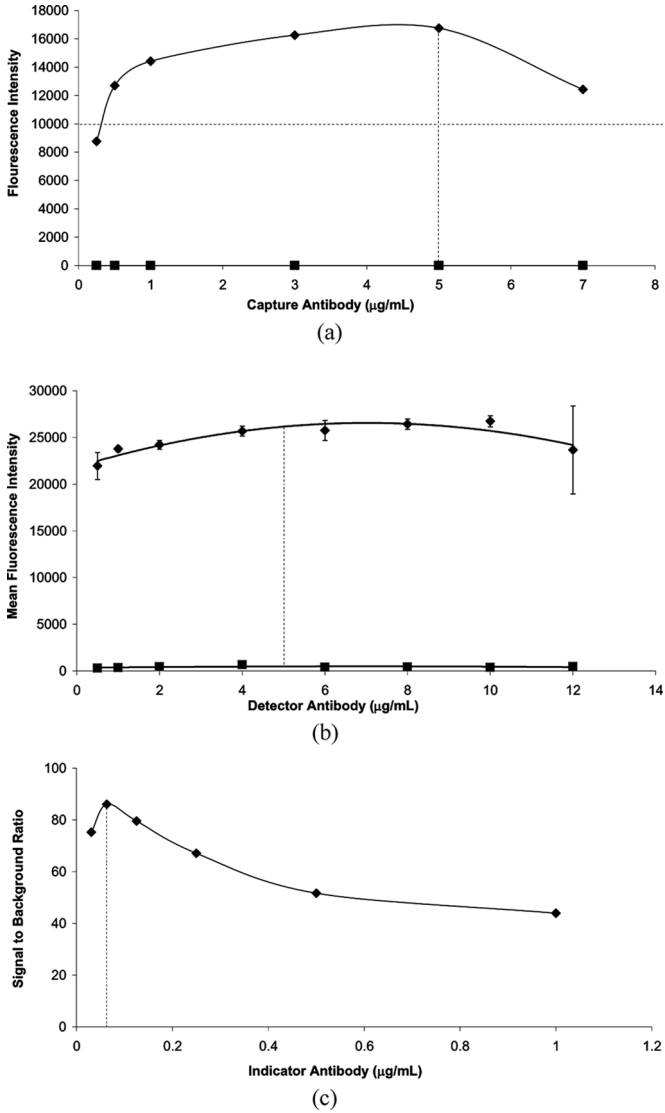

Figure 4. SA assay Antibody Optimization. (a) To determine the optimal CAb concentration (vertical dotted line) above the validation cutoff (horizontal dotted line) for coupling to the suspension array microspheres, various concentrations of CAb were used in the presence (diamonds) or absence (squares) of PE-conjugated anti-mouse IgG. Data is representative of two experiments. (b) The optimal concentration of DAb (vertical dotted line) was determined in the presence of ovalbumin (diamonds) or without ovalbumin (squares) using IAb at 10 μg/mL. Data is representative of two experiments. (c) The optimal concentration of IAb (vertical dotted line) was determined using ovalbumin at 10 μg/mL and DAb at 5 μg/mL.

optimized DAb at 5 μg/mL, and antigen in excess at 10 μg/mL. The optimal concentration of IAb was defined as the concentration that yielded the highest S/B FI ratio. As the concentration of IAb decreased, the S/B ratio increased. The maximal S/B ratio occurred at 0.063 μg/mL IAb (Figure 4c), but this concentration had a low signal strength of 7298.4 FI, compared to higher concentrations of IAb that had signal strengths over 20,000 FI (data not shown). Therefore, an optimal IAb concentration of 0.25 μg/mL was chosen, as this was the lowest concentration of IAb that had a signal strength over 20,000 FI while still maintaining a high S/B ratio (data not shown).

Limit of Detection of SA Immunoassay

Utilizing the optimized SA assay parameters, the LOD of the capture SA ovalbumin immunoassay was determined by titrating 2-fold serial dilutions of ovalbumin from 312 to 0.038 ng/mL. The LOD, defined as the lowest ovalbumin concentration with a FI reading within one standard deviation of the assay cutoff (background FI plus two standard deviations), was 4.88 ng/mL (Figure 5).

The LODs of ovalbumin for ELISA (0.0095 ng/mL) and the SA assay (4.88 ng/mL) were compared and it was observed that the ELISA LOD was 500-fold more sensitive than the SA assay LOD. This observation is consistent with findings reported in a previous study,[3] where an ELISA LOD of 0.3 ng/mL and a SA assay LOD of 1 ng/mL were observed for ovalbumin. The 500-fold difference may be attributed, in part, to the longer incubation times for the ELISA (1 hour) versus

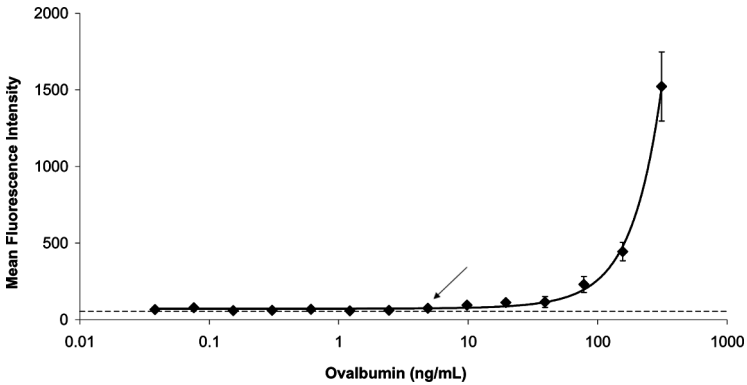

Figure 5. Limit of Detection of SA assay. The limit of detection for ovalbumin (represented by arrow) was determined using DAb at 5 μg/mL and IAb at 0.25 μg/mL. Dotted line represents background absorbance +2 standard deviations ($r^2 = 0.998$).

Table 1. Well-to-well reproducibility for SA immunoassay

Plate #	Mean FI	Standard deviation	CV (%)	Average CV (%)
1	20236.5	955.41	4.72	5.07
2	20273.5	792.42	3.91	
3	21960.3	2278.38	10.52	
4	22842.5	258.09	1.13	

Standard conditions for SA assay: 5 μg/mL CAb, 10 μg/mL ovalbumin, 5 μg/mL DAb, 0.25 μg/mL IAb. Four replicate wells per plate. %CV = SD/mean FI.

the SA assay (30 minutes). Also, it is possible that some antibody inactivation occurs during the antibody coupling step in the SA assay, due to chemical crosslinking of the antigen binding site, resulting in decreased sensitivity.

Reproducibility of SA Immunoassay and ELISA

The well-to-well and plate-to-plate reproducibility for the SA immunoassay and ELISA were determined. With the standard SA assays that have been performed thus far, the average percent coefficient of variance (%CV) of the FI between sample wells (well-to-well reproducibility) was 5.07% over four replicate wells (Table 1). In comparison, the ELISA had a well-to-well %CV of 4.9% over three replicate wells (Table 2). With respect to plate-to-plate reproducibility, the %CV of the average FI from one plate to the next was 6.05% for four assay plates run over 22 days for the SA immunoassay, and the average plate-to-plate %CV was 14.8% over three assay plates in an 8 day time span for the ELISA (Table 3). Therefore, well-to-well reproducibility appeared to be comparable for both ELISA and SA immunoassay formats, whereas plate-to-plate reproducibility was observed to be better for the SA immunoassay compared to the ELISA.

Table 2. Well-to-well reproducibility for ELISA

Plate #	Mean absorbance	Standard deviation	CV (%)	Average CV (%)
1	2.8023	0.1004	3.58	4.90
2	2.80847	0.1774	6.32	
3	2.1426	0.1026	4.79	

Standard conditions for ELISA: 15 μg/mL CAb, 10 μg/mL ovalbumin, 3 μg/mL DAb, 80 ng/mL IAb. Three replicate wells per plate. %CV = SD/mean absorbance.

Table 3. Plate-to-plate reproducibility for ELISA and suspension array assay

Type of assay	# of assay plates	# Wells per assay	Mean signal	Plate-to-plate standard deviation	Plate-to-plate CV (%)
SA assay	4	4	21328 FI	1290.6	6.05%
ELISA	3	3	2.5844 AU	0.3827	14.80%

Standard conditions for SA assay: 5 μg/mL CAb, 10 μg/mL ovalbumin, 5 μg/mL DAb, 0.25 μg/mL IAb. Standard conditions for ELISA: 15 μg/mL CAb, 10 μg/mL ovalbumin, 3 μg/mL DAb, 80 ng/mL IAb. %CV = SD/mean.

CONCLUSIONS

In this study, a SA immunoassay for ovalbumin was developed and determined to be rapid, sensitive and reproducible. Using the optimization procedures developed for the ovalbumin SA assay, SA assays for other BT agents such as toxins, bacteria, and viruses can now be developed. Once other individual SA assays are developed, they can be used to create multiplexed assays to detect multiple BT agents simultaneously in a single sample.

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