Detection of prostate specific antigen (PSA) in the serum of radical prostatectomy patients at femtogram per milliliter levels using digital ELISA (AccuPSA™) based on single molecule arrays (SiMoA)

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Objective

The aim of this study was to detect prostate specific antigen (PSA) in the serum of patients who had undergone radical prostatectomy (RP). To achieve this objective, the most sensitive ELISA for PSA (AccuPSA™) based on single molecule detection was developed and evaluated.

Relevance

The ability to detect PSA in RP patients 4-6 weeks after surgery (nadir PSA levels) has potential prognostic value, and monitoring of PSA at very low concentrations could also enable earlier detection of biochemical relapse. Using existing immunoanalyzers, however, PSA is often not detectable in the serum of RP patients for several years post-surgery until levels exceed a threshold determined by the analytical sensitivity of commercially available assays. New, highly sensitive assays are needed to measure PSA at femtogram per milliliter concentrations in order to detect PSA in all of these patients shortly after surgery. The single molecule detection approach described here fulfills this requirement.

Methodology

We have developed a method for detecting single immunocomplexes formed in the enzyme-linked immunosorbent assay (ELISA) using single molecule arrays (SiMoA). This digital ELISA for PSA (AccuPSA™) method is based on isolating single immunocomplexes labeled with an enzyme in arrays of femtoliter wells, sealing the arrays in the presence of the enzyme substrate, and fluorescently imaging the array. Fluorescent product molecules of the enzyme-substrate reaction are confined in the femtoliter volume, giving rise to a local high concentration that can be easily detected using a standard fluorescent microscope. By using high density arrays of femtoliter wells, hundreds to thousands of single immunocomplexes can be detected simultaneously. Isolation of single immunocomplexes using SiMoA gives rise to a dramatic increase in sensitivity over bulk, ensemble detection methods. The most sensitive digital ELISA for detecting PSA was developed that has a limit of detection (LOD) of 6 fg/mL (200 aM) in serum. This assay was used to measure PSA in the sera of thirty RP patients.

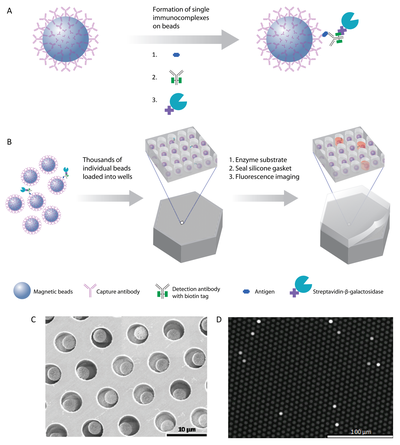


Figure 1. AccuPSA™ based on arrays of femtoliter wells. (A) Capturing and labeling single protein molecules on beads using standard ELISA reagents. (B) Loading of beads into femtoliter well arrays for isolation and detection of single molecules. (C) SEM image of a small section of a femtoliter well array after bead loading. 2.7-µm-diam. beads were loaded into an array of wells with diameters of 4.5 µm and depths of 3.25 µm. (D) Fluorescence image of a small section of the femtoliter well array after signals from single enzymes are generated.

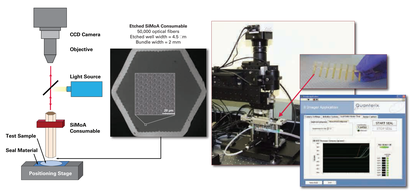


Figure 2. Prototype SiMoA Instrumentation. A prototype SiMoA instrument has been developed for assay validation and to support pharmaceutical and diagnostics collaborations. The SiMoA consumable is manufactured by etching tens of thousands of reaction vessels into the end of an optical fiber bundle. Strips of 8 fiber bundles are sized to sample one column of a microtiter plate, enabling convenient processing of up to 96-samples. The current bench-top instrument automatically seals the reaction vessels and concurrently reads the array of over 50,000 single molecule fluorescent assays. Sample preparation is done on a separate automated fluid handling workstation capable of processing hundreds of samples per shift.

Results

A) Sub-femtogram per milliliter detection of PSA

We developed AccuPSA™ based on detection of single enzyme labels using SiMoA. Figure 3 shows data from AccuPSA™. The human form of PSA was spiked into 25% bovine serum to be representative of clinical test samples. Using AccuPSA™ to detect PSA in 25% serum, an LOD was 10 fg/mL determined from this experiment. Figure 4 illustrates the predicted CV profile, which shows an LOQ of 18 fg/mL. For comparison, a leading commercial PSA assay (ADVIA Centaur, Siemens) reports an LOD of 3 pM (0.1 ng/mL) in human serum, and the most sensitive previously reported assay for PSA had an LOD of 10 fM (2). The single molecule assay reported here is, therefore, more sensitive than the commercial assay by a factor of 15000, and more sensitive than other ultra-sensitive methods by a factor of at least 50.

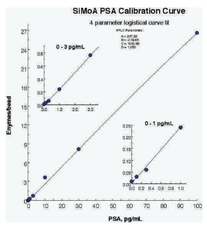


Figure 3. Sub femotgram per milliliter detection of PSA in serum

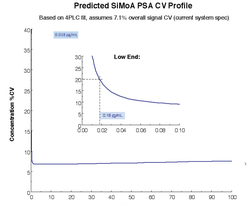


Figure 4. Predicted CV profile of SiMoA PSA

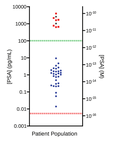
B) Detection of PSA in patients after radical prostatectomy

To demonstrate the possible diagnostic value of detecting very low concentrations of proteins in human clinical samples using AccuPSA™, PSA was measured in serum samples from patients who had undergone radical prostatectomy (RP) surgery. After RP, the vast majority of PSA is eliminated, and levels fall below the detection limit of standard commercial assays (3 pM or 0.1 ng/mL). Regular monitoring of these patients for return of PSA can detect recurrence of prostate cancer, but several years may pass post-surgery for biochemical recurrence to be detected by current immunoanalyzers. The ability to accurately quantify PSA levels in post-prostatectomy patients at very low concentrations (<3 fM or 100 fg/mL) should provide early indication of recurrence if PSA levels increase. First, we validated AccuPSA™ for specificity to PSA using control standards (Bio-Rad) and serum from healthy individuals (ProMedDx) that had been assayed using the ADVIA Centaur PSA assay (Figure 5).

	Centaur ng/mL	SiMoA ng/mL
Bio-Rad Control 1	0.838	1.06 ± 0.21
Bio-Rad Control 2	2.47	2.66 ± 0.36
Normals		
ProMedDx S376	2.1	1.60
ProMedDx S378	2.3	1.70
ProMedDx S381	2.9	2.14
ProMedDx S388	4.1	3.95
ProMedDx S395	0.93	0.63
ProMedDx S396	0.9	0.77
ProMedDx S397	1.2	0.66

Figure 5. Comparison of AccuPSA™ and commercial immunoanalyzer (ADVIA Centaur, Siemens). PSA samples from Bio-Rad (controls) and ProMedDx (serum from healthy individuals) that had previously been tested on the Centaur were tested using AccuPSA™. The PSA concentrations of the healthy serum samples determined using SiMoA were (24±12)% lower than those originally determined on the ADVIA Centaur. The systematic bias between the two technologies can be explained by a difference in the PSA used to generate calibration curves or the cycle of freeze-thaw that the samples experienced before being tested with AccuPSA™.

We then used AccuPSA™ to measure PSA in the sera of patients who had undergone radical prostatectomy. Figure 6 shows PSA levels measured using AccuPSA™ in the serum of 30 RP patients (age 60–89) whose blood was collected at least six weeks post-surgery. The PSA levels in the sera of all 30 patients were below the detection limit of commercial assays. PSA was successfully detected in all 30 patients using AccuPSA™, with concentrations ranging from 14 fg/mL to 9.4 pg/mL, with an average of 1.5 pg/mL. Further clinical studies are required to establish the diagnostic benefit of measuring PSA at fg/mL levels in RP patients.



Patient ID	[PSA] (pg/mL)	Dose %CV	Patient ID	[PSA] (pg/mL)	Dose %CV	Patient ID	[PSA] (pg/mL)	Dose %CV
S600	9.39	6%	S590	0.22	22%	S580	0.30	4%
S599	0.75	10%	S589	0.85	17%	S579	1.22	15%
S598	2.71	12%	S588	2.33	3%	S578	0.090	91%
S597	1.79	12%	S587	1.06	13%	S577	1.92	6%
S596	2.46	17%	S586	1.29	22%	S576	0.014	286%
S595	0.32	21%	S585	0.49	84%	S575	0.79	63%
S594	1.63	15%	S584	0.056	136%	S574	1.82	20%
S593	1.15	12%	S583	1.33	26%	S573	0.22	32%
S592	3.46	9%	S582	4.76	9%	S572	1.04	20%
S591	0.21	25%	S581	1.57	31%	S571	0.24	21%

Figure 6. Digital detection of PSA in serum samples of patients who had undergone radical prostatectomy. The concentrations of PSA were determined using digital ELISA in serum samples from RP patients (●), healthy control samples (■), and Bio-Rad PSA control samples (▲). RP patient samples were obtained from SeraCare Life Sciences (Milford, MA), and all had undetectable PSA levels as measured by a leading clinical diagnostic assay (ADVIA Centaur); the green line represents the detection limit of the ADVIA Centaur PSA assay (100 pg/mL or 3 pM). All 30 patient samples were above the detection limit of the PSA digital ELISA, shown by the red line (0.006 pg/mL or ~200 aM), with the lowest patient PSA concentrations measured at 0.014 pg/mL (~400 aM) using digital ELISA.

Conclusions

PSA was successfully detected by AccuPSA™ in sera from all thirty RP patients. No previously reported assay for PSA has successfully detected this protein in all RP patients tested; the most sensitive assays reported previously (LODs in the range 0.3 to 1 pg/mL) would have failed to detect PSA in 30-40% of the samples tested in this study. The lowest concentration detected by AccuPSA™ in the serum of an RP patient was 14 fg/mL (420 aM), and the PSA concentrations ranged from 14 fg/mL to 9.4 pg/mL. The mean concentration of PSA in the sera of these patients was 1.5 pg/mL. These results suggest that digital ELISA using SiMoA has the potential to provide a more favorable prognosis for men with the lowest measurable nadir values, and to detect biochemical recurrence months or years earlier than conventional test methods.

References

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The project described was supported by Award Number R43CA133987 from the National Cancer Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health.