Nucleic Acid Detection Immunoassay for Prostate-Specific Antigen Based on Immuno-PCR Methodology

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BACKGROUND: Serum prostate-specific antigen (PSA) concentrations after radical prostatectomy typically become undetectable with the use of current immunometric assay methods. Despite modern surgical techniques, 15%–30% of prostate cancer patients undergoing radical prostatectomy develop a biochemical recurrence during follow-up. Unfortunately, poor analytical sensitivity of standard PSA assays delays biochemical recurrence detection, and because of day-to-day assay imprecision ultrasensitive PSA assays cannot assess PSA kinetics. We developed an immuno-PCR assay for total PSA that has a limit of quantification >10 times lower than current ultrasensitive assays.

METHODS: The 2-site immunometric assay for total PSA employed 2 monoclonal antibodies, one conjugated to a double-stranded DNA label and the other bound to paramagnetic microparticles. After several washing steps, quantification cycles were determined and values were converted to PSA concentrations. We characterized analytical performance and compared accuracy with a commercially available total PSA assay.

RESULTS: The limit of quantification was 0.65 ng/L and the assay was linear in the range of 0.25–152.0 ng/L. Total imprecision estimates at PSA concentrations of 3.8, 24.1, and 69.1 ng/L were <15.2%, <9.4%, and <10.6%, respectively. Recovery of supplemented PSA ranged from 87.5% to 119.2% (mean 100.3%). Dilution recovery ranged from 96.4% to 115.3% (mean 102.3%). There was no high-dose hook effect up to 50 000 ng/L of PSA. Comparison with the commercial PSA assay showed a regression slope of 1.06 and a correlation coefficient of 0.996.

CONCLUSIONS: The analytical characteristics of the assay support the use of this assay for the accurate and precise measurement of serum PSA, even at sub–nanogram-per-liter concentrations.

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Prostate-specific antigen (PSA)[4] is a 33-kDa glycoprotein with serine protease activity, found in large amounts in the prostate and seminal plasma (1). An abnormally increased serum PSA concentration is one of the hallmarks of prostatic adenocarcinoma. Although the use of PSA as a screening test for prostatic carcinoma is still controversial (2–4), PSA measurement is widely accepted as an excellent tool for managing patients with medically established prostatic cancer (5–8). Serial measurements of PSA to identify increases in serum values in patients following prostatectomy is the most common strategy for detecting recurrent or metastatic cancer (9).

Over the last 10–15 years, research has shown that the effectiveness of PSA for monitoring prostatic carcinoma is enhanced by using ultrasensitive, as opposed to conventional, assays (10). In comparison to conventional assays with detection limits of 100 ng/L or higher, more sensitive assays can detect biochemical recurrence (BCR) several months or years earlier (11–19). Investigational PSA assays with greater sensitivity show that serum PSA in some postprostatectomy patients drops below 1 ng/L, a concentration that could be accurately quantified only by use of more sensitive (fourth- or fifth-generation) PSA assays (20, 21).

The requirement for very sensitive, simple, and practical PSA immunoassays led us to develop a new ultrasensitive assay for total PSA (tPSA) with fourth-generation sensitivity and evaluate its analytical performance. The technology platform, which we call the nucleic acid detection immunoassay (NADiA®), is an immuno-PCR (IPCR) method that can quantify con-

centrations of low-abundance proteins and cells. It is a reengineered version of the first IPCR assay developed and reported by Sano et al. in 1992 (22). Subsequently, several formats of IPCR have been demonstrated (23–25), including quantitative sandwich immunoassays (24, 25). NADiA technology incorporates several modifications that reduce nonspecific binding (NSB) and provide greater precision compared with previous quantitative sandwich IPCR assays.

Initial analytical performance data for the NADiA PSA assay as well as results of pilot clinical studies in prostatectomy patients have been reported at scientific symposia (26–28). This manuscript describes NADiA technology and the design, methodology, and analytical characteristics of the NADiA PSA assay, demonstrating its robust analytical performance. The clinical utility of the linear slope of the NADiA PSA values over time (trade name ProsVue™) in postprostatectomy patients has been reported previously (29).

Materials and Methods

INSTRUMENTATION
The Applied Biosystems (AB) 7500 FastDx quantitative PCR (qPCR) instrument was used according to the directions in the manufacturer's operator manual to perform the NADiA PSA assay.

PSA ANTIBODIES
Two high-affinity murine anti-PSA monoclonal antibodies (MAbs) were used (Medix Biochemica), each recognizing separate epitopes on the PSA molecule.

ASSAY DESIGN
The NADiA platform uses dual-MAb antigen capture and qPCR detection of a nonnative DNA label. Different assay formats have been developed for the quantitative measurement of low-abundance proteins and cells. In the PSA application, the capture MAb (C-MAb) was biotinylated as previously described (13) by using sulfo-NHS-LC-biotin (Pierce), and the excess was removed by size-exclusion chromatography. The degree of biotinylation was determined by the absorbance-based 2-(4′-hydroxyazobenzene) benzoic acid assay (30) and determined to be a mean (SD) of 3.8 (0.7) biotin molecules per C-MAb. C-MAb biotin was reacted with streptavidin-coated paramagnetic microparticles (Seradyn), and the excess was removed by washing (Tris-buffered saline, pH 8.0, 0.05% Tween-20). The labeled MAb (L-MAb) was conjugated to DNA as follows. A synthetic 59-base oligonucleotide containing a C12 modifier was activated with excess disuccidimidyl suberate (Fisher Scientific). The reactive intermediate was isolated by HPLC and combined
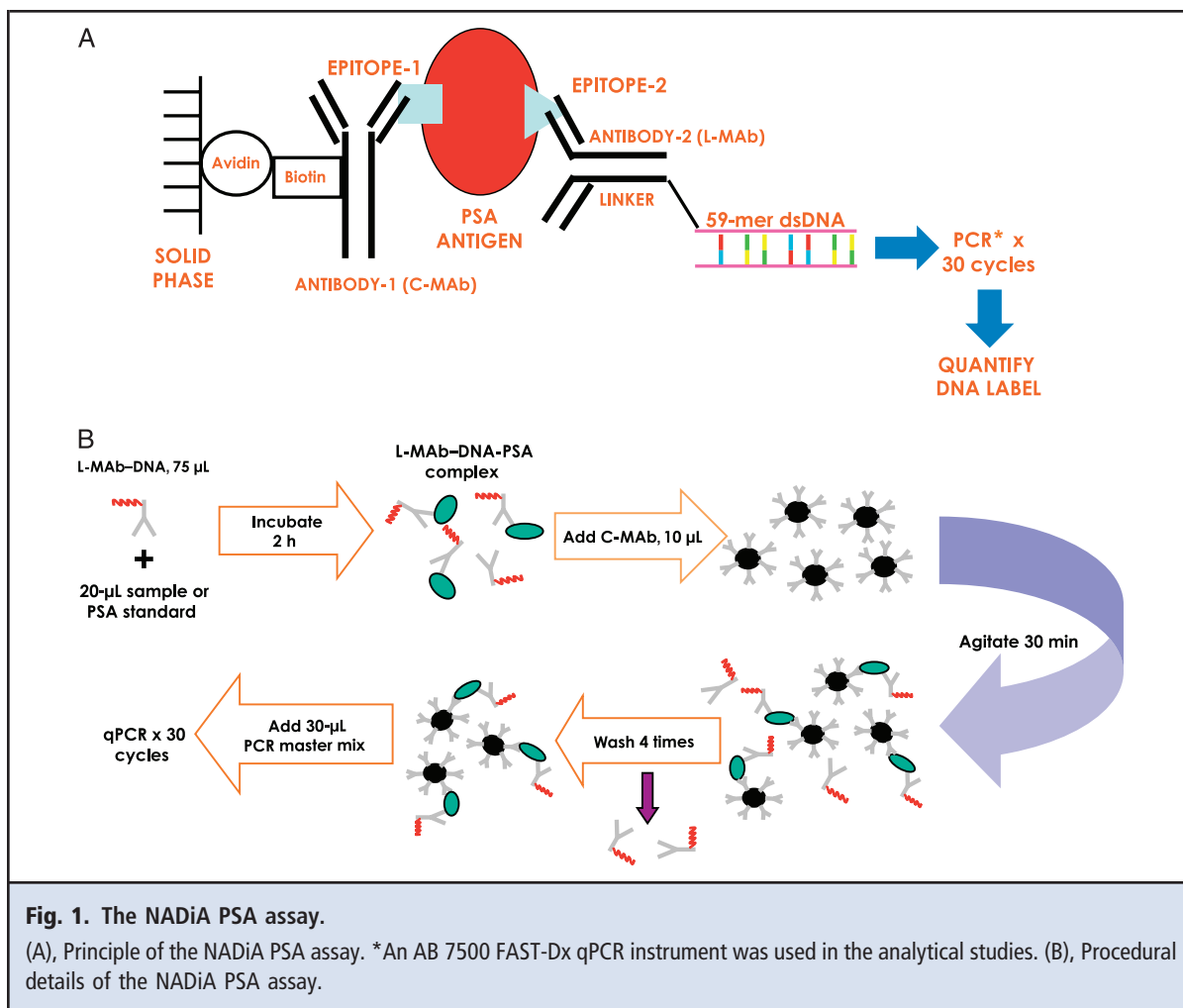
with the L-MAb, and the L-MAb–DNA conjugate was purified by HPLC. Gel electrophoresis demonstrated that the L-MAb was predominantly labeled with 1 or 2 oligonucleotides with some multiple labels present in exponentially decreasing amounts. The conjugate was purified by fast protein liquid chromatography and rendered free of both nonreacted oligonucleotide and MAb by use of a Superdex S-200 10/300 GL column (GE Healthcare). The oligonucleotide was rendered double-stranded (ds) to a perfectly complementary DNA strand. Both the forward and reverse complementary oligonucleotides (see Supplemental Fig. 1, A and B, in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol58/issue4) were chemically synthesized (TriLink BioTechnologies) and purified by reverse-phase chromatography before use. This dsDNA construct was chosen because it exhibited superior detection and reproducibility of manufacture and a much lower degree of NSB to particle supports compared to a single-stranded construct. The principle of the NADiA PSA assay is shown in Fig. 1A and details of the assay procedure in Fig. 1B.

CALIBRATORS AND CONTROLS
WHO 96/670 PSA standard was used to prepare calibrators and controls in a serum matrix [processed equine serum, 20 mmol/L HEPES, free acid (Fisher Scientific), NaOH, pH 8.0, and 5 mmol/L EDTA]. Calibrators A, B, and C contained 100, 25, and 5 ng/L of PSA, respectively. Three concentrations of lot-specific controls contained approximately 2, 20, and 80 ng/L of PSA.

PROSVUE ASSAY
In the first step, 20 μL of samples, controls, and calibrators and 75 μL of L-MAb–DNA were added to a MicroAmp® Fast optical 96-well reaction plate (AB), covered with a Corning plate sealer (Corning Life Sciences), and then incubated on a rocker plate for 2 h at ambient temperature. C-MAb (10 μL) was added to each well, then the plate was resealed and agitated for 30 min at room temperature. Excess conjugate and unbound DNA was removed by 4 magnetic separation and wash steps (150 mL; Tris-buffered saline, pH 8.0) using a HydroFlex™ plate washer with a magnetic plate support (Tecan). PCR master mix (30 μL) was added to each well [50 mmol/L KCl, 3 mmol/L $MgCl_2$, 30 000 U/L Taq (Roche Molecular Diagnostics), 0.2 μmol/L primers (Eurofins MWG Operon; see online Supplemental Fig. 1C), 200 μmol/L dNPs (Roche Applied Science), SYBR Green I (Life Technologies), ROX (Affymetrix), 20 mmol/L Tris, pH 8.0, and 0.05% Tween-20]. The plate was sealed (AB MicroAmp® optical

Fig. 1. The NADiA PSA assay.

(A), Principle of the NADiA PSA assay. *An AB 7500 FAST-Dx qPCR instrument was used in the analytical studies. (B), Procedural details of the NADiA PSA assay.

adhesive film) and transferred to an AB 7500 FastDx qPCR. The first thermal cycle (95 °C for 30 s, 62 °C for 30 s) was followed by 30 cycles (95 °C for 15 s, 62 °C for 30 s), and the generations of amplicon were monitored in real time. In each run, mean quantification cycles (Cq) were determined for each assayed calibrator (in triplicate), control (in duplicate) and sample (in duplicate). Any mean sample result having a %CV >20% was repeated.

The initial amount of dsDNA in each PSA–C-MAb complex was inversely proportional to the Cq. Log_{10} PSA concentrations (x) were calculated from the calibration curve: ($y = mx + b$), where y equals the Cq values of the calibrators, m is the slope (Cq/log_{10} ng/L PSA), and b is the y-intercept when log_{10} nanograms per liter PSA is zero. The PSA concentration in each unknown (ng/L) was calculated by using the equation: PSA = $antilog_{10}$ (Cq of unknown/slope) − (intercept/slope).

LIMITS OF PERFORMANCE

Limits of the blank (LOB) and of detection (LOD) were assessed by using CLSI guideline EP17-A *(31)*. Because of the rarity of native PSA-free serum, a serum matrix was used as the blank sample. Four low-concentration PSA pools were prepared in a serum matrix by using WHO 96/670 PSA. Sixty replicates of each pool were assayed in separate runs, and the mean, SD, and %CV were calculated. LOB and LOD were determined by using parametric or nonparametric analyses, depending on the gaussianity of the data distributions *(32)*. The limit of quantification (LOQ) was defined as the lowest PSA sample concentration meeting prespecified requirements for accuracy (80%–120%) and imprecision (<25%).

ASSAY LINEARITY

A single study spanning PSA concentrations 20%–30% wider than the ProsVue measuring range was done ac-

Fig. 2. Linearity of the NADiA PSA assay.
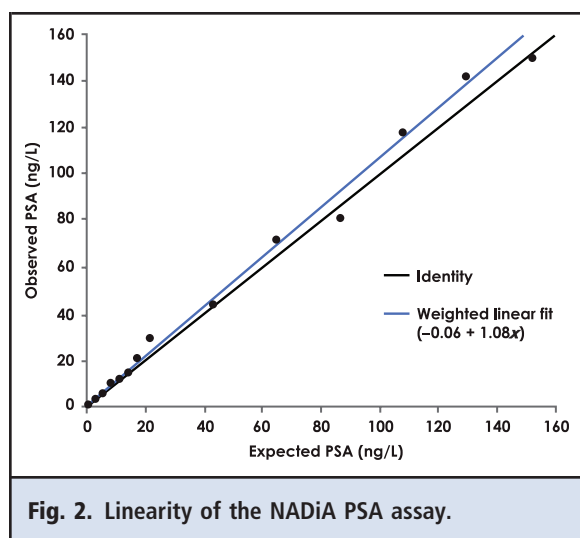
cording to CLSI guideline EP06-A (33). Varying amounts of a high-PSA pool of male serum and a low pool consisting of serum matrix were mixed to create a 15-member panel. Each mixture was assayed in triplicate in a single run and mean, SD, and %CV of the observed values were calculated. Expected PSA values were calculated from the dilution mixtures (see online Supplemental Table 1A), and ordinary linear-fit weighted regression was performed and plotted (Fig. 2). Linear-fit PSA values were calculated by using the equation of the best fitted line. Percentage deviation from the linearity of each pool was calculated by using the equation: $100 - (observed\ PSA/linear\ fit\ PSA) \times 100$ (see online Supplemental Table 1B).

RECOVERY STUDIES
WHO 96/670 PSA was supplemented into serum samples from 6 prostatectomy patients with PSA concentrations ranging from 2.7 to 37.3 ng/L. Neat and supplemented samples were assayed in triplicate in a single run, and mean, SD, %CV, and percentage recovery were calculated. Serum samples from 8 prostate cancer patients with PSA values ranging from 249.4 to 1269.2 ng/L determined by a commercial assay were diluted 1:10, 1:20, and 1:40 with a serum matrix. Neat and diluted samples were assayed in duplicate in a single run and mean, SD, %CV, and percentage recovery were calculated.

HOOK EFFECT STUDY
Serum matrix containing 50 000 ng/L of WHO 96/670 PSA was assayed neat and after a series of 1:2 dilutions with a serum matrix. Samples were assayed in duplicate in a single run, and the mean, SD, and %CV of the observed concentrations were calculated (see online Supplemental Table 2). Mean observed vs expected

PSA concentrations were plotted graphically (see online Supplemental Fig. 2).

IMPRECISION STUDY
Low, intermediate, and high male serum PSA pools were analyzed following CLSI guideline EP5-A2 (34). Samples from each pool were assayed in duplicate at 2 sites by 3 operators (2 at site 1) using 2 AB 7500 Fast Dx instruments and 2 reagent lots. At site 1, each operator performed 1 run per day, alternating reagent lots, over 20 nonconsecutive days (40 assays). At site 2, a single operator performed 2 runs a day with alternating reagent lots for 5 days (10 assays). The mean, SD, and %CV of each PSA pool were calculated. Two statistical analyses were performed to determine the contribution of components of variation to total variation using Analyse-it software, v.2.07 (Analyse-it Software). The first estimated within-run, between-run, and between-day components of variation; the second estimated within-run, between-run, and between-lot components of variation (Table 1). In addition, site-specific results were calculated (see online Supplemental Table 3). Multivariate ANOVA was performed to analyze factor effects by use of NCSS 2004 software (NCSS).

EQUIMOLARITY
Molar response was evaluated at tPSA concentrations of 5, 30, 70, and 100 ng/L. Five mixtures of free PSA (Scripps Laboratories) and PSA complexed to α_1-antichymotrypsin (PSA-ACT) (Scripps Laboratories) in a serum matrix were prepared at each level at molar ratios of 100%/0%, 75%/25%, 50%/50%, 25%/75%, and 0%/100%. Each sample was assayed in triplicate in a single run and mean, SD, %CV, and percentage recovery were calculated by using the 50%/50% molar ratio as the reference (see online Supplemental Table 4).

ACCURACY
We assessed accuracy by comparison to a commercially available equimolar tPSA method standardized to WHO 96/670 PSA (35) following CLSI guideline EP9-A2 (36). We diluted as required 106 male serum samples with tPSA values >100 ng/L, assayed with the ADVIA Centaur tPSA assay, and assayed them in duplicate with the NADiA PSA assay. Descriptive statistics were calculated for results from both methods and the gaussianity of the data distributions was assessed. Both least-squares linear and orthogonal regression techniques with bootstrapping were performed on the mean observed vs expected tPSA values (Table 2) and plotted graphically (Fig. 3). Point estimates and 95% CIs were determined for R^2, Pearson R, slope, and y-intercept. The nonparametric Spearman rank correlation coefficient ρ and associated P value were determined, as was the P value for the Pearson R analysis. Agreement between methods was evaluated by using a

Table 1. Imprecision study results in the NADiA PSA assay.[a]

	Sample		
	Low	Intermediate	High
NADiA PSA analysis 1			
Mean, ng/L	3.8	24.1	69.1
Within-run variation			
SD	0.3	1.7	6.0
%CV	9.0	7.2	8.6
Between-run variation			
SD	0.3	0.8	3.2
%CV	9.0	3.4	4.7
Between-day variation			
SD	0.3	1.2	2.8
%CV	8.3	5.1	4.1
Total variation			
SD	0.6	2.3	7.4
%CV	15.2	9.4	10.6
NADiA PSA analysis 2			
Mean, ng/L	3.8	24.1	69.1
Within-run variation			
SD	0.2	0.4	1.4
%CV	5.6	1.7	2.0
Between-run variation			
SD	0.5	1.5	4.4
%CV	12.5	6.1	6.3
Between-lot variation			
SD	0.2	0.3	0.0[b]
%CV	4.0	1.1	0.0[b]
Total variation			
SD	0.8	1.7	4.6
%CV	14.0	6.5	6.6

[a] Within-run, between-run, and between-day variation (analysis 1), and within-run, between-run, and between-lot variation (analysis 2) of NADiA PSA with low (3–5 ng/L), intermediate (20–30 ng/L), and high (60–80 ng/L) PSA pools. Fifty assays were run in duplicate on 25 nonconsecutive days.
[b] 0.0% CV may be a result of the partial-factorial design of the analysis or colinearity.

Table 2. NADiA tPSA vs ADVIA Centaur tPSA assay methods.[a]

Descriptive statistics		
Parameter	NADiA tPSA	Centaur tPSA
Mean PSA, μg/L	19.6	19.0
SD	87.7	82.4
Median	0.88	1.16
Minimum–maximum	0.11–543.8	0.11–547.6
Non-parametric p-value	<0.0001	<0.0001

Regression parameters	
Standard least squares	Result
Intercept (95% CI)	−0.54 (−0.92 to 0.12)
Slope (95% CI)	1.06 (0.96 to 1.13)
R^2	0.996 (0.991 to 0.998)
Pearson R (P)	0.998 (<0.0001)
Pearson R (95% CI)	0.996 to 0.999
Spearman ρ (P)	0.9817 (<0.0001)
Orthogonal	
Intercept (95% CI)	−0.57 (−0.98 to 0.06)
Slope (95% CI)	1.07 (0.97 to 1.14)
R^2	0.996 (0.991 to 0.998)

[a] Descriptive statistics and bootstrapped standard least squares linear regression and correlation and bootstrapped orthogonal regression statistics for the comparison between NADiA and Siemens ADVIA Centaur tPSA assays (N = 106).
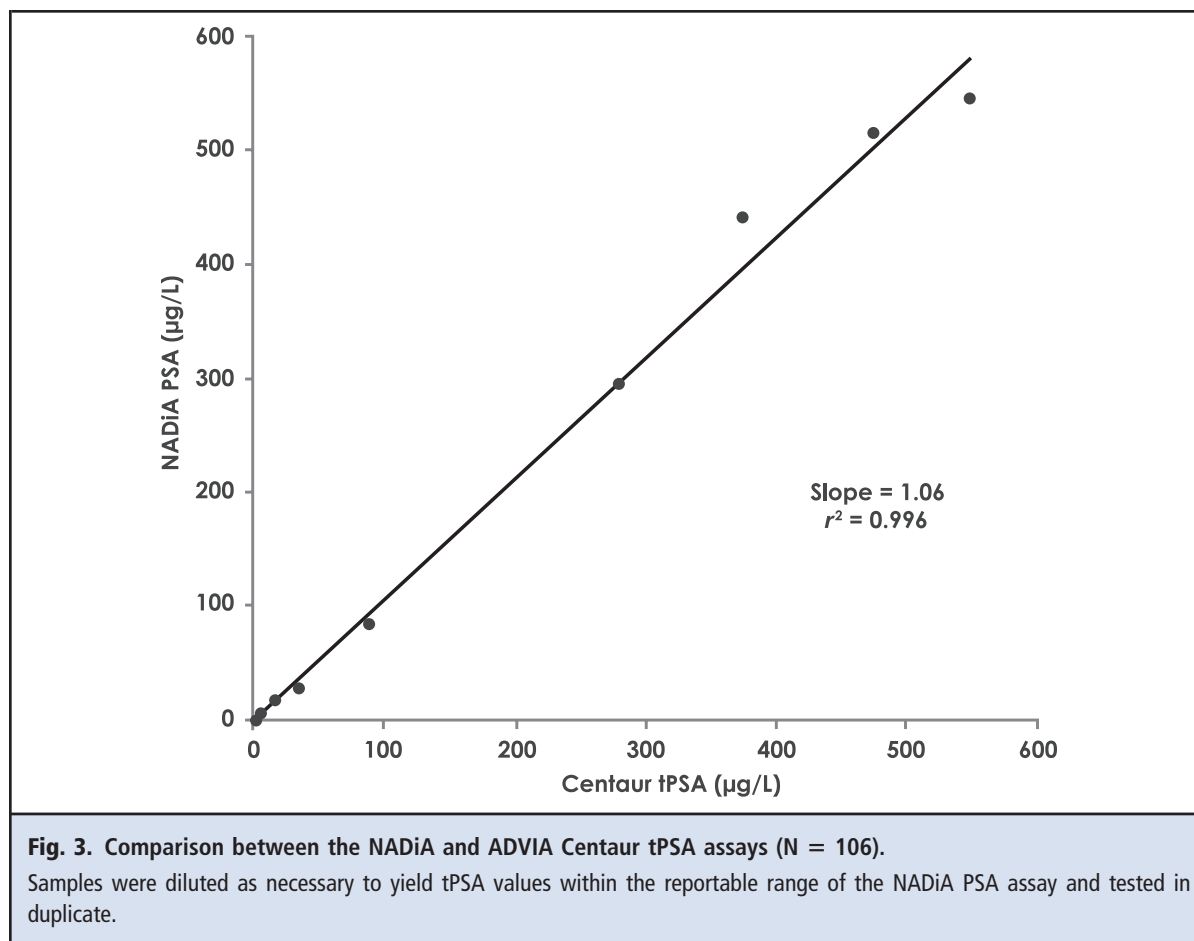
(n = 60). The lowest amount of PSA in the samples detected with type I and II error rates set to 5% (LOD) was 0.27 ng/L (n = 60). The PSA sample meeting the prespecified requirements for accuracy and imprecision (LOQ) was 0.65 ng/L.

ASSAY LINEARITY
Observed and expected values of the 15 PSA pools are shown in online Supplemental Table 1A. Ordinary weighted least-squares linear regression demonstrated a slope of 1.08 and a weighted R of 0.995 (Fig. 2). Predicted linear fit PSA values and the percentage differences between observed and expected values demonstrated the linear range of the assay as 0.25–152 ng/L with deviation from linearity <24% (see online Supplemental Table 1B). The reportable range was established as 0.65 ng/L (the LOQ) to 100 ng/L.

RECOVERY STUDIES
Mean percentage recovery of neat and supplemented samples was 100.3%, range 87.5%–119.2% (data not shown). Mean percentage recovery of neat and diluted

2-tailed matched pairs *t*-test (see online Supplemental Fig. 3).

Results

LIMITS OF PERFORMANCE
The highest NADiA PSA result observed with an α of 0.05 (5%) for the blank sample (LOB) was 0.17 ng/L

Fig. 3. Comparison between the NADiA and ADVIA Centaur tPSA assays (N = 106).
Samples were diluted as necessary to yield tPSA values within the reportable range of the NADiA PSA assay and tested in duplicate.

samples was 102.3%, range 92.5%–115.3% (data not shown). These results demonstrated that supplemented and diluted serum samples appropriately recover in the assay.

HOOK EFFECT STUDY

There was no evidence of a high-dose hook effect up to 50 000 ng/L of PSA (see online Supplemental Table 2 and online Supplemental Fig. 2).

IMPRECISION STUDY

Total %CVs of the low, intermediate, and high PSA pools were 15.2%, 9.4%, and 10.6%, respectively. Components of variation were within acceptable limits for within-run, between-run, and between-day and for within-run, between-run, and between-lot (Table 1) and also between sites (see online Supplemental Table 3). By multivariate ANOVA, there was no difference in imprecision according to site of testing, day of testing, or operator and instrument use, except the factor of lot reached significance ($P = 0.0423$) for the low imprecision sample (data not shown). This difference was not

observed in the other 2 pools and may represent a type 1 error.

EQUIMOLARITY

Percentage recovery of all tPSA samples at all free-PSA/PSA-ACT ratios ranged from 88.0% to 113.2%, with a mean of 99.5%, demonstrating that the assay is equimolar throughout its linear range (see online Supplemental Table 4).

ACCURACY

There was strong agreement between methods and non-gaussian distributions of patient values (Table 2). Both least squares linear and orthogonal regression lines were nearly identical, with a linear slope of 1.06, 95% CI 0.96–1.13 (Fig. 3). Correlation was good ($R^2 = 0.996$, 95% CI 0.991–0.998), and both Pearson and Spearman Rank correlations were highly significant ($P < 0.0001$). A 2-tailed matched pairs t test testing the equivalence of both methods demonstrated insignificant differences in means between the NADiA and Centaur tPSA assay methods ($P = 0.38$; see online Supplemental Fig. 3).
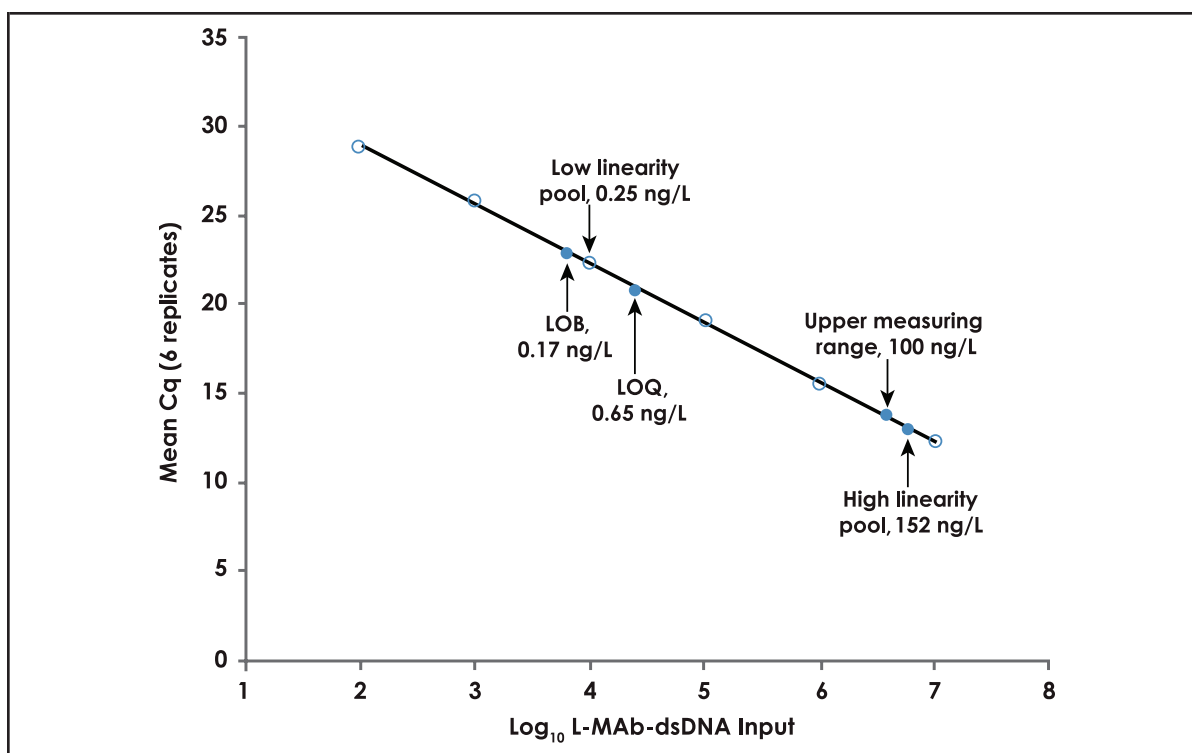
Fig. 4. Calibration curve constructed from the mean Cq of 6 sample replicates of L-MAb–dsDNA input (open circles) ranging from 10^2 to 10^7 molecules assayed by qPCR in a single run.

The solid circles represent Cq values that relate with the PSA concentrations for the LOB and LOQ determined in the imprecision study, the PSA concentrations of the low and high serum pools used in the linearity study and the upper measuring range of the assay.

Discussion

The NADiA PSA assay was carefully designed and optimized for detecting sub–nanogram-per-liter concentrations of several analytes and cells. We studied the NADiA PSA assay and have demonstrated its fourth-generation sensitivity with an LOD and LOQ of 0.27 and 0.65 ng/L PSA, respectively. Linearity extends above and below the reportable range, recovery is appropriate in supplemented and diluted samples, and the assay is equimolar. No hook effect was observed up to 50 000 ng/L of PSA, and the response in this study was linear up to 3125 ng/L. Imprecision of the low PSA pool (3.8 ng/L), which was prepared from native serum obtained from men after successful prostatectomy, was excellent.

The NADiA PSA assay incorporates several improvements to the original qualitative IPCR assays (22, 23). As with more recent sandwich ICPR assays (24, 25) the DNA label is covalently attached to the L-MAb and can dissociate only under qPCR conditions. In addition, the 59-mer dsDNA label is of suffi-

cient length to prevent loss of the duplex during typical wash procedures. The net result is highly efficient qPCR amplification. Fig. 4 displays a calibration curve created from 10^2–10^7 molecules of L-MAb–dsDNA input. The −3.3363 slope is nearly identical to the ideal slope of −1/log 2 (−3.3333, i.e., exponent = 2), proving that the L-MAb does not degrade dsDNA amplification efficiency.

The background is represented by the LOB, which in turn limits the assay's LOD. Fig. 4 overlays the equivalent amounts of dsDNA for the NADiA PSA values determined in the imprecision and linearity studies and the assay's reportable range. All of these PSA values were within the range of mean Cq results determined for 10^2–10^7 dsDNA molecules. This background signal limits the molecular LOB to approximately 6.8×10^3 dsDNA molecules per 20 μL sample. NSB significantly affected the sensitivity and reliability of the first qualitative IPCR assay (22, 23) but has been less problematic in IPCR assays that employ microtiter plate-based, 2-antibody sandwich assay formats similar to NADiA (24, 25). We have further reduced NSB in the NADiA

assay by optimizing the L-MAb concentration in the ProsVue conjugate.

Other improvements in the design of the NADiA PSA assay provide greater accuracy compared with other assays that use DNA amplification. Because reactants are thoroughly removed by a series of magnetic separation and wash steps, NADiA does not transfer inhibition factors from the sample to qPCR. Because the dsDNA sequence and primers are noncomplementary to the open reading frame of human DNA, the assay can amplify only the dsDNA label and not potential contamination from human DNA. Importantly, the dsDNA label in the assay is 59 bases long, not the hundreds or thousands typically amplified in genomic analysis; this ensures complete and consistent replication efficiency. L-MAb reagents, PCR master mix components, primers, and template sequences are identical for the analysis of standards and samples. Lastly, the number of cycles is limited to 30, which reduces the possibility of any progressive error as well as primer–dimer formation, typically an issue with SYBR Green after 30 cycles.

A fourth-generation PSA assay mentioned earlier employed a gold nanoparticle biobarcode, exhibited a detection limit of approximately 0.3 ng/L *(20)*, and was preliminarily evaluated in serum of 18 prostate cancer patients following prostatectomy. Although the LOQ was not reported, the data demonstrated stable PSA concentrations over time in patients who did not develop BCR and PSA increases in patients who eventually relapsed. A fifth-generation, single–PSA molecule ELISA (SiMoA™) has also been developed that uses nanotechnology principles *(21)*. This is the most analytically sensitive PSA assay reported, capable of detecting PSA at concentrations as low as 14 pg/L, albeit with high imprecision (%CV = 286%). The enhanced sensitivity is attributed to the assay's single-molecule detection capability and lower labeling reagent concentration requirements, which reduces NSB and enables high signal-to-background ratios. Nonetheless, NSB of the detection antibody and enzyme conjugate to the capture bead surface was reported in this assay system *(21)*.

Third-generation PSA assays that detect serum PSA concentrations ≥ 10 ng/L (≥ 0.01 μg/L) have proved useful for predicting subgroups of patients more and less likely to exhibit BCR several months or years earlier *(11–19)*. At these low levels, however, day-to-day reproducibility in the laboratory appears to limit their usefulness for accurately quantitating changes in PSA concentrations over time. Thus, discriminating between men who are stable postprostatectomy from those who may be manifesting early BCR

has proven difficult *(37, 38)*. Also, recent studies have shown that PSA doubling-time calculations performed with PSA values at low concentrations correlate poorly with those calculated with values measureable with standard PSA assays, particularly for patients manifesting aggressive BCR *(39, 40)*.

Our assay demonstrates analytically acceptable day-to-day assay reproducibility in the sub–nanogram-per-liter PSA range and an LOQ that can reliably quantify serial PSA measurements in prostatectomy patients for calculation of the rate of PSA change over time. Fifth-generation PSA assays would appear to possess few clinical advantages compared to fourth-generation PSA assays because higher PSA concentrations are found in most prostatectomy patients, usually around 1–3 ng/L.

In conclusion, we described and validated a new fourth-generation IPCR assay for quantifying PSA in serum, which possesses optimized performance compared to previous IPCR assay versions. The measuring range of the assay is well suited for its proposed intended use in the population of postprostatectomy cancer patients. Studies investigating the prognostic utility of ProsVue PSA slope in prostatectomy patients have been completed and will be reported separately.

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