Bayer Immuno 1™ PSA Assay: An Automated, Ultrasensitive Method to Quantitate Total PSA in Serum

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The Bayer Immuno 1™ PSA Assay measures total PSA in human serum and demonstrates excellent performance with an interassay CV $\leq$ 3.4% and a biological detection limit of 0.03 μ g/L. No significant interference from common hormonal and chemotherapeutic drugs, kallikrein, prostatic acid phosphatase, and trypsin, or elevated levels of total bilirubin, hemoglobin, triglycerides, and IgG was observed. The 95th percentile values for healthy individuals increased with age from 3.0 μ g/L for males 50–59 years and 3.3 μ g/L for males 60–69 years, to 4.6 μ g/L for males $\geq$ 70 years. Clinical studies with retrospective samples demonstrated correspondence between serial measurements of PSA and clinical outcome for 98% of 159 prostate cancer patients.

Clinical sensitivity for patients with clinical evidence of disease, untreated at the time of specimen draw, increased with increasing stage from 77.5–100%. Specificity of 60–70% for BPH and other benign urogenital diseases was consistent with previous findings. Bayer Immuno 1 PSA Assay values for 2131 specimens from healthy subjects and patients with prostate cancer, BPH, and other malignant and nonmalignant diseases correlated well with the Abbott IMx® PSA Assay over the range 0.0–6,238 μ g/L ($Y = 1.10 \times + 0.02$). The Bayer Immuno 1 PSA Assay provides automated ultrasensitive, precise, and equimolar measurement of total PSA in human serum. *J. Clin. Lab. Anal.* 12:65–74, 1998. © 1998 Wiley-Liss, Inc.

Key words: prostate-specific antigen; immunoassay; tumor marker

INTRODUCTION

Prostate-specific antigen (PSA) was named and described by Wang et al. (1) in 1979 as a single-chain glycoprotein of 33,000 daltons. Belanger et al. (2) characterized the protein as 8.3% carbohydrate with one occupied N-glycosylation site and a total molecular mass of 28,430 daltons. The cDNA sequence for PSA is now known and codes for a glycoprotein of 237 amino acids with significant homology to human glandular kallikrein (hK2) (3) and other proteases in the kallikrein family (4,5). PSA functions as a serine protease to liquefy the coagulum formed following ejaculation (6–8), and is produced by healthy, benign hyperplastic, and malignant prostate epithelial cells. It is present in serum in three predominant forms: PSA complexed with two different serine protease inhibitors, alpha-2-macroglobulin (PSA-A2M) and alpha-1-antichym-

otrypsin (PSA-ACT), and unbound, free PSA. PSA-ACT, 90 kilodaltons, can be detected along with free PSA (9). Several laboratories have demonstrated that different immunoassays report different PSA values for the same patient specimens

Nonstandard abbreviations: ACT, alpha-1-antichymotrypsin; ALP, alkaline phosphatase; BDL, biological detection limit; Ca, cancer; CaP, prostate cancer; LLD, lower limit of detection; A2M, alpha-2-macroglobulin; BPH, benign prostatic hyperplasia; hK2, human glandular kallikrein; MAbs, monoclonal PSA antibody; PAb, polyclonal PSA antibody; PAGE, polyacrylamide gel electrophoresis; PAP, prostatic acid phosphatase; PSA, prostate-specific antigen; SDS, sodium dodecyl sulfate.

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(10–13). This may result from detection of the free and complexed forms of PSA in different molar ratios. Use of total PSA assays that detect both forms with equal response will eliminate testing bias toward any one form.

Longitudinal measurements of serum PSA values have demonstrated clinical utility for the management of prostate cancer patients. Several clinical studies have reported that serially increasing values of serum PSA in patients following radical prostatectomy, radiation therapy, or antiandrogen therapy can signal recurrence or progression well in advance of alternative diagnostic measures (14–18). Takayama et al. (19) demonstrated that a measurable serum PSA following radical prostatectomy is predictive of disease recurrence with an average lead time of 9–12 months and, conversely, a very low or undetectable level of serum PSA is a strong positive prognostic factor. It is, therefore, important that a clinician know whether there is a detectable level of serum PSA in a patient following treatment for prostate cancer.

A PSA assay's ability to detect very low concentrations of the analyte is characterized by the lower limit of detection (LLD), which is conventionally calculated as a function of the imprecision of the calibrator matrix. It is important, however, that this analytical limit be determined as well from the imprecision profile established with patient sera (20,21). Vessella and Lange (21) introduced the Biological Detection Limit (BDL) calculated as the LLD plus twice the standard deviation of recovery values for patient sera at very low concentrations of PSA. The analytical limit necessary for monitoring patients following radical prostatectomy has been proposed at PSA concentrations $< 0.1 \mu\text{g/L}$ and at concentrations as low as $0.001 \mu\text{g/L}$ (20–22). However, the clinical value of treatment intervention in a postradical prostatectomy patient whose serum PSA has risen from undetectable amounts to 0.001 – $0.005 \mu\text{g/L}$ is still controversial.

The Bayer Immuno 1TM PSA Assay (a trademark of Bayer Corp., Business Group Diagnostics, Tarrytown, NY) has been designed to run on the Bayer Immuno 1TM immunoassay system, a fully automated random access analyzer (23). This study evaluated antibody specificity and assay performance characteristics including: interference, cross-reactivity, reproducibility, analytical detection limit, linearity, parallelism, antigen excess hook effect, and accuracy by comparison to the Abbott IMx[®] PSA Assay (Abbott Laboratories, Abbott Park, IL). The clinical utility of the Bayer Immuno 1 PSA Assay was evaluated by comparing longitudinal measurements of PSA to the clinical status of patients in various stages of prostate cancer. This study demonstrated acceptable clinical and analytical performance. In addition, Zhou et al. (24) reported that the Bayer Immuno 1 PSA Assay is equimolar, or unaffected by varying proportions of free or ACT-complexed PSA in patient samples. This PSA method can therefore provide significant advantages in the clinical laboratory in terms of automation, ease of use, equimolarity, and low end precision and sensitivity.

MATERIALS AND METHODS

Bayer Immuno 1 PSA Assay

Bayer Immuno 1 PSA is a sandwich immunoassay that employs a monoclonal PSA antibody conjugated to fluorescein, a polyclonal PSA antibody conjugated to alkaline phosphatase, and anti fluorescein-coated magnetic particles as the solid phase. The assay format has been previously described by Zhou et al. (24). PSA calibrators are prepared with free PSA at concentrations of 0, 2, 10, 25, 50, and $100 \mu\text{g/L}$. The assay requires $20 \mu\text{L}$ of serum, and results are reported after 38.5 minutes.

Specificity of PSA Antibodies

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting procedures were performed using the Pharmacia Phastgel Electrophoresis System according to the manufacturer's instructions. Preparations of PSA, purified from human seminal fluid, and human source kallikrein, prostatic acid phosphatase (PAP), trypsin, ACT, and A2M were obtained from Scripps Laboratories (San Diego, CA). Each material was diluted into an equal volume of SDS-PAGE sample buffer containing 20 mM Tris (pH 8.0), 2 mM EDTA, 5% SDS, 0.02% bromophenol blue, with and without 10% beta-2-mercaptoethanol. Several antibody preparations were used for detection: Bayer Immuno 1 PSA MAb, Bayer Immuno 1 PSA PAb, mouse IgG (Zymed Laboratories, San Francisco, CA), and goat IgG (Sigma Chemical Co., St. Louis, MO).

Specificity of Bayer Immuno 1 PSA Assay

The potential cross-reactivity of human source PAP, kallikrein, and trypsin in the Bayer Immuno 1 PSA Assay was measured. Each potential cross-reactant was diluted to 100%, 75%, 50%, 25%, and 0% of the maximum concentration in two human serum samples containing different PSA concentrations in the range of 4 – $10 \mu\text{g/L}$. The maximum concentrations in serum were 1 mg/L PAP; 1 mg/L kallikrein; 10 mg/L trypsin. The percent cross-reactivity was calculated with the average of duplicate assays of each sample using one lot of reagent in one analytical run.

Interference

The potential interference of therapeutic drugs, albumin, total bilirubin, hemoglobin, and triglycerides was also tested. Therapeutic drugs were purchased from Sigma, except Lupron (TAP Pharmaceuticals, Deerfield, IL) and Zolodex (Zenec Pharmaceuticals, Wilmington, DE). Biological substances were purchased from Sigma, except albumin, IgG, (Pentex, Kankakee, IL), and triglycerides (KabiVitrum, Clayton, NC). The antineoplastic agents were pooled in a "drug cocktail" at the following maximum concentrations in serum: Amino-

glutethimide (0.398 g/L), Bleomycin (150 units/L), Cis-Platin (0.173 g/L), Cyclophosphamide (0.700 g/L), Diethylstilbestrol (0.005 g/L), Doxorubicin (0.052 g/L), Etoposide (0.415 g/L), 5-Fluorouracil (0.346 g/L), Flutamide (0.010 g/L), Lupron (0.015 g/L), Methotrexate (0.030 g/L), Mitomycin C (0.014 g/L), Vincristine (1.38 mg/L), Vinblastine (1.38 mg/L), Zolodex (7.20 mg/L). Aqueous or relevant organic solvents were used for drug dilution. The biological substances were prepared individually at the following maximum concentrations in serum: albumin (65 g/L), total bilirubin (0.25 g/L), hemoglobin (10 g/L), triglycerides, (9 g/L), and IgG (53 g/L). Each potential interferant was diluted to 100%, 75%, 50%, 25%, and 0% of the maximum concentration in two human serum samples with serum PSA values in the range of 1–3 µg/L and 4–10 µg/L. The percent interference was calculated with the average of duplicate assays of each sample using one reagent lot in one analytical run.

Reproducibility

PSA containing materials, including three levels of BioRad Lyphochek® immunoassay controls (BioRad, Anaheim, CA), six levels of Bayer Immuno 1 PSA calibrators, and three human serum pools with PSA concentrations < 0.1 µg/L, were tested in two runs per day over 6 days at four clinical trial sites. Each run included duplicate assays of each control, calibrator, and human serum pool, and each sample was tested with at least two lots of reagent. BioRad Lyphochek controls were reconstituted and frozen at –20°C or below in aliquots large enough for use within 24 hours. Human serum pool and BioRad control materials were kept frozen until just prior to each run. Total PSA has been shown to remain stable over repeated freeze/thaw cycles and for long periods of time when stored at these conditions (25). The variability for each product, reagent lot, and site was partitioned into within-run, among-run (within-day) and among-day variance components using SAS NESTED (SAS Institute, Cary, NC) procedure. The total standard deviation was taken as the root sum of squares of the within-run, among-run, and among-day standard deviations. The within-run imprecision estimates were pooled across sites and reagent lots using the total number of replicates less the total number of runs for that material, site, and lot as the number of degrees of freedom. Total imprecision estimates were similarly pooled using the number of days less one as the number of degrees of freedom.

Detection Limit

Twenty-four replicates of the Bayer Immuno 1 PSA Level 1 calibrator (0.0 µg/L PSA) and 24 replicates of each of three human serum pools with PSA concentrations < 0.1 µg/L were assayed using multiple lots of reagent in two runs per day for 6 days at each of four clinical trial sites. The variability of the zero calibrator response (absorbance per minute) and the hu-

man serum pool recoveries were estimated using SAS NESTED procedure. The lower limit of detection (LLD) was calculated as the concentration corresponding to the mean zero calibrator rate plus twice the within-run SD. The biological detection limit (BDL) was determined from the LLD plus twice the pooled total SD for the recovery of the human serum pools.

Linearity

Three patient serum samples with PSA concentrations between 90 and 100 µg/L were diluted to three intermediate levels with low PSA patients samples (< 0.5 µg/L). Each neat (100% of sample) and diluted sample was tested in triplicate with two lots of reagent and the results for each reagent lot were averaged. Data was collected in one analytical run. For each high-low pair, the mean observed recoveries for the three lowest concentrations were regressed against coded concentration numbers (–2, –1, 0). The regression line was extrapolated to the higher levels and linearity was assessed based upon the percent deviation of the observed from the predicted concentration.

Parallelism

Four patient serum samples ranging from 86 to 114 µg/L were diluted using the Bayer Immuno 1 PSA Level 1 calibrator (0.0 µg/L PSA) to 10%, 25%, 50%, and 75% of the original sample concentration. Each neat and diluted sample was tested in triplicate with two lots of reagent, and the results for each reagent lot were averaged. Data was collected in two analytical runs; each run assayed dilutions for two of the original four serum samples. Use of the Level 1 calibrator as a sample diluent was evaluated by comparing dilution corrected recoveries to the concentration of the neat sample.

Antigen Excess Hook Effect

A pure preparation of PSA was diluted with the Bayer Immuno 1 PSA Level 1 calibrator (0.0 µg/L PSA) to 13 different concentrations ranging from 100–50,000 µg/L. Each sample was tested in triplicate with two lots of reagent in one analytical run. Average absorbance rates at each concentration level for each reagent lot were compared to the absorbance rate of the highest calibrator.

Clinical Study

The clinical study was performed at four investigational sites under the direction of Dr. Daniel W. Chan at The Johns Hopkins Hospital, Dr. Herbert A. Fritsche at the University of Texas, M.D. Anderson Cancer Center, Dr. Morton K. Schwartz at Memorial Sloan-Kettering Cancer Center, and Dr. Robert L. Vessella at the University of Washington. The study protocol was approved by the Institutional Review Board at each site. Subjects and specimens were randomly

selected from specimen banks categorized by disease. The criteria for inclusion were adequate documentation of diagnostic information, course of therapy, disease status, stage, and grade of tumor at the time of specimen collection, and for longitudinally monitored subjects, the availability of at least four serial serum specimens prior to and/or following the clinical diagnosis of malignant disease. Correspondence of assay results with clinical outcome was evaluated by comparing the clinical history to Bayer Immuno 1 PSA Assay longitudinal values for 159 patients diagnosed with prostate cancer in both early and late stage disease. Clinical sensitivity was determined using cross-sectional (single point) specimens from 458 men with prostate cancer including treated and untreated patients at different stages of disease. Clinical specificity was studied with single point samples from 251 healthy men < 50 years of age, 242 healthy men 50 years or greater, 303 men with BPH, 102 men with other urogenital diseases, 227 men with other benign nonurogenital diseases, and 265 men with other cancers.

The reference range was determined as the nonparametric 95th percentile of PSA values from healthy donors who met the criteria for blood bank donation and were free of any known prostate disease or history of prostate disease. Sensitivity and specificity, however, were calculated using 4 µg/L as the upper limit of normal for ease of comparison to historical data. Clinical specimens were assayed by both the Bayer Immuno 1 and Abbott IMx PSA assays following the methods stated in the product insert or method sheet. Samples were kept frozen at -70°C or below until just prior to analysis. With few exceptions, patient samples were run on both instruments on the same day. The longitudinal monitoring environment was simulated by having each serial specimen from an individual patient assayed on the Bayer Immuno 1 instrument on a separate day. In addition, serial specimens from each individual patient were distributed chronologically across three lots of Bayer Immuno 1 PSA Assay reagent so that

the first third of the individual's specimens were assayed using the first lot, the second third of the individual's specimens were assayed using the second lot, and the remaining specimens by the third lot of reagent. One lot of IMx PSA Assay reagent was used at two clinical sites, whereas multiple lots of IMx reagent were used at the other two sites. Specimens demonstrating IMx PSA Assay results ≤ 10 µg/L and Bayer Immuno 1 PSA Assay results different by ≥ 1.5 µg/L; or IMx PSA Assay results > 10 µg/L and Bayer Immuno 1 PSA Assay results different by ≥ 25% were flagged as discordant and re-assayed by both methods using new aliquots of the specimen.

RESULTS

Specificity of PSA Antibodies

Western blotting of the monoclonal antibody under non-reducing conditions is shown in Figure 1 and demonstrates reactivity to PSA at 30 kilodaltons. Reactivity to PSA at 18 kilodaltons corresponds to previous reports of fragments in purified preparations of PSA from human seminal fluid (6). No reactivity was observed with trypsin, PAP, ACT, or A2M. Under reducing conditions, the monoclonal antibody failed to show significant binding to any of the antigens tested including PSA (data not shown). This suggests that the epitope seen by the monoclonal antibody is conformationally dependent and is destroyed by denaturation and disulfide bond reduction. As shown in Figure 2, the polyclonal antibody reacted strongly with PSA and trypsin, weakly with PAP, and did not react with kallikrein. Nonspecific reactivity shown to ACT and A2M, was also observed in the goat IgG control blots (data not shown).

These results demonstrate that the specificity of this assay is determined by the monoclonal antibody used for capture of the antigen. The minimal reactivity of the polyclonal antibody to PAP and trypsin was not seen in performance testing of the Bayer Immuno 1 PSA Assay.

Fig. 1. Western blot analysis of the Bayer Immuno 1 PSA monoclonal antibody under nonreducing conditions. Lane 1: molecular weight standards; Lane 2: kallikrein; Lane 3: prostatic acid phosphatase; Lane 4: trypsin; Lane

5: PSA antigen; Lane 6: blank; Lane 7: molecular weight standards; Lane 8: PSA antigen; Lane 9: alpha-1-antichymotrypsin; Lane 10: alpha-2-macroglobulin; Lane 11: PSA antigen; Lane 12: blank.

Fig. 2. Western blot analysis of the Bayer Immuno 1 PSA polyclonal antibody under reducing conditions. Lane 1: molecular weight standards; Lane 2: PSA antigen; Lane 3: trypsin; Lane 4: prostatic acid phosphatase; Lane 5:

PSA antigen; Lane 6: trypsin; Lane 7: molecular weight standards; Lane 8: PSA antigen; Lane 9: alpha-1-antichymotrypsin; Lane 10: alpha-2-macroglobulin; Lane 11: kallikrein; Lane 12: prostatic acid phosphatase.

Specificity of the Bayer Immuno 1 PSA Assay

Cross-reactivity of kallikrein, trypsin and PAP in the Bayer Immuno 1 PSA assay was tested based upon significant homology in structure to PSA, similar enzymatic reactivity (26), or history as a prostate cancer marker. Both trypsin and PAP were tested at levels well above the normal serum concentration. Normal serum levels of kallikrein have not been established. Observed cross-reactivities among the three substances tested were minimal. The maximum interference, 0.102%, was demonstrated by PAP.

Interference

Interference in the Bayer Immuno 1 PSA assay from intrinsic biological substances including albumin, bilirubin, hemoglobin, IgG, and triglycerides as well as exogenous antineoplastic pharmaceuticals combined in a drug cocktail was not observed. The highest concentrations of antineoplastic agents tested were greater than the average therapeutic dosages (27), and the highest concentrations of common biological interferants tested were greater than the normal concentrations that would be found in serum (28). The over-

all interference of the drug cocktail after spiking into a base human serum specimen with 1.44 µg/L PSA was < 0.001%. The greatest interferences demonstrated by individual drugs were 0.002% for Diethylstilbestrol, 0.001% for Flutamide, and 0.001% for Zolodex. Percent interferences by elevated levels of albumin, total bilirubin, hemoglobin, IgG, and triglycerides were not distinguishable from zero.

Reproducibility

Table 1 shows assay imprecision for commercial controls, and low PSA (< 0.1 µg/L) human serum pools. The analysis includes 191 or more replicate determinations for each product tested. Imprecision estimates were pooled across reagent lots and clinical trial sites. Interassay imprecision for commercial controls ranged from 2.9–3.4% CV. The difference among individual reagent lot mean concentrations for the commercial controls was minimal, ranging from 2.9–3.7% of the overall mean. These results demonstrate that the Bayer Immuno 1 PSA Assay is highly reproducible over time when tested in different laboratories using different lots of reagent.

TABLE 1. Imprecision of the Bayer Immuno 1 PSA Assay

Product	N	Mean (µg/L)	Within-run		Total		Lot-to-lot Range of means	
			SD	CV (%)	SD	CV (%)	(µg/L)	(%)
Commercial controls ^a								
Level 1	286	0.92	0.017	1.8	0.027	2.9	0.03	3.3
Level 2	284	2.68	0.044	1.6	0.082	3.1	0.10	3.7
Level 3	287	23.02	0.351	1.5	0.778	3.4	0.67	2.9
Human serum pools ^b								
Pool 1	191	0.05	0.005	10.0	0.007	14.0	0.0050	10.0
Pool 2	192	0.07	0.007	10.0	0.008	11.4	0.0025	3.6
Pool 3	192	0.05	0.005	10.0	0.007	14.0	0.0000	–

^aTwo samples were assayed by three lots of reagent in each of two runs per day for 6 days at four clinical sites.

^bTwo samples were assayed by two lots of reagent in each of two runs per day for 6 days at four clinical sites.

Analytical Sensitivity (lower limit of detection)

The analytical detection limit of the Bayer Immuno 1 PSA Assay was calculated considering both intra-assay variation of the calibrator matrix as well as the interassay variation of human serum pools with low levels of PSA ($< 0.1 \mu\text{g/L}$). The imprecision profiles were determined with 861 assays of the Bayer Immuno 1 PSA Level 1 calibrator and 576 replicates of low PSA human serum samples across four clinical trial sites and three PSA reagent lots. The lower limit of detection (LLD), a function of the intra-assay imprecision of the zero calibrator, was determined at $0.02 \mu\text{g/L}$. The biological detection limit, a function of LLD and the interassay imprecision of the patient sera was determined at $0.03 \mu\text{g/L}$. These data support analytical sensitivity of this assay and qualify this method as ultrasensitive with particular utility in the detection of residual disease following prostatectomy.

Linearity

Assay linearity was demonstrated by regression line slopes and correlation coefficients near 1.0. The regression equation: $y = 1.019x - 0.283$, $r = 0.998$, is typical of that obtained in six experiments using three patient serum samples and two lots of assay reagent. Concentrations predicted by the regression lines differed from the observed values by no more than 6.3%.

Parallelism

Dilution of patient samples with the Bayer Immuno 1 PSA zero calibrator had little effect on the measured PSA value. The regression equation, $y = 0.985x - 0.180$, $r = 0.999$, is typical of that obtained in eight experiments using four patient serum samples and two lots of reagent. The dilution corrected recoveries over all experiments ranged from 93.2% to 107.2% of the expected values.

Antigen Excess Hook Effect

High dose hook effect occurs when excess analyte saturates both label and capture antibody and the analyte concentration is erroneously reported within the assay's calibration range. A "hook effect" was not observed in the Bayer Immuno 1 PSA Assay for samples with PSA ranging from $> 100 \mu\text{g/L}$ to $20,000 \mu\text{g/L}$. Patient samples assayed during our clinical studies with concentrations of PSA as high as $6200 \mu\text{g/L}$ were all flagged "out of assay range." These results suggest that it is highly unlikely that patient samples containing up to $20,000 \mu\text{g/L}$ PSA would yield false results due to the "hook effect" with this assay.

Reference Range

The distribution of PSA concentrations measured by the Bayer Immuno 1 PSA Assay for 493 healthy males ranging

in age from 15–85 years is shown in Table 2. The nonparametric 95th percentile over the total population was $2.8 \mu\text{g/L}$. The reference value increased with age from $1.5 \mu\text{g/L}$ for males < 50 years to $3.0 \mu\text{g/L}$ for males 50–59 years, $3.3 \mu\text{g/L}$ for males 60–69 years, and $4.6 \mu\text{g/L}$ for males ≥ 70 years. The mean (SD) PSA value also increased with age from $0.7 \mu\text{g/L}$ (0.4) for males < 50 years to $2.0 \mu\text{g/L}$ (1.8) for males > 70 years.

Clinical Specificity

Distribution of Bayer Immuno 1 PSA Assay values for single point specimens from healthy individuals and patients with nonmalignant and nonprostate malignant diseases is also shown in Table 2. Clinical specificity of 60.1% for 303 males with BPH, and 69.6% for 102 males with other benign urogenital diseases was demonstrated. Specificity for 227 males with benign nonurogenital diseases was 95.2% and consistent with the healthy population. Specificity for males with other types of cancer ranged from 84.6% for 26 males with lung cancer to 93.1% for 58 males with gastrointestinal cancer. Interpretation of these results is difficult as a significant fraction of the patients studied were > 60 years of age. Elevated PSA values due to prostate enlargement is common in men > 60 .

Clinical Sensitivity

Cross-sectional sensitivity is presented in Table 3 by stage and treatment status. All specimens were drawn during an active disease period while there was clinical evidence of prostate cancer. PSA values below the upper limit of normal ($4.0 \mu\text{g/L}$) are demonstrated at all stages of disease and agree with previous findings that prostate cancer patients may exhibit normal to minimally increased levels of PSA. Palken et al. (29) reported a study where 10% of the patients had normal PSA concentrations in spite of the presence of malignancy. Overall sensitivity for 458 treated and untreated patients across

TABLE 2. Clinical Specificity

		0.0–4.0 $\mu\text{g/L}$ N (%)	>4.0–10.0 $\mu\text{g/L}$ N (%)	>10.0–40.0 $\mu\text{g/L}$ N (%)
Healthy subjects				
Males < 50	251	251 (100.00)	0 (0.0)	0 (0.0)
Males ≥ 50	242	237 (97.7)	4 (1.7)	1 (0.4)
Total healthy males	493	488 (99.0)	4 (0.8)	1 (0.2)
Nonmalignant patients				
BPH	303	182 (60.1)	86 (28.4)	35 (11.6)
Other urogenital	102	71 (69.6)	22 (21.6)	9 (8.8)
Nonurogenital	227	216 (95.2)	8 (3.5)	3 (1.3)
Nonprostate malignant				
Gastrointestinal	58	54 (93.1)	3 (5.2)	1 (1.7)
Liver/pancreas	44	40 (90.9)	2 (4.5)	2 (4.5)
Lung	26	22 (84.6)	3 (11.5)	1 (3.8)
Urogenital	125	113 (90.4)	10 (8.0)	2 (1.5)

TABLE 3. Clinical Sensitivity

	N	0.0–4.0 (µg/L) (N (%))	>4.0–10.0 (µg/L) (N (%))	>10.0–40.0 (µg/L) (N (%))	>40.0 (µg/L) (N (%))	Sensitivity (%)
Stages A,B treated	60	46 (76.7)	10 (16.7)	4 (6.7)	0 (0.0)	23.3
Stages A,B, untreated	89	20 (22.5)	35 (39.3)	29 (32.6)	5 (5.6)	77.5
Total stages A,B	149	66 (44.3)	45 (30.2)	33 (22.1)	5 (3.4)	55.7
Stage C treated	49	32 (65.3)	4 (8.2)	6 (12.2)	7 (14.3)	34.7
Stage C untreated	72	13 (18.1)	15 (20.8)	34 (47.2)	10 (13.9)	81.9
Total stage C	121	45 (37.2)	19 (15.7)	40 (33.1)	17 (14.0)	62.8
Stages D0,D1 treated	76	23 (30.3)	12 (15.8)	11 (14.5)	30 (39.5)	69.7
Stages D0,D1 untreated	38	4 (10.5)	9 (23.7)	13 (34.2)	12 (31.6)	89.5
Total stages D0,D1	114	27 (23.7)	21 (18.4)	24 (21.0)	42 (36.8)	76.3
Stage D2 treated	62	15 (24.2)	5 (8.1)	12 (19.4)	30 (48.4)	75.8
Stage D2 untreated	12	0 (0.0)	1 (8.3)	2 (16.7)	9 (75.0)	100.0
Total stage D2	74	15 (20.3)	6 (8.1)	14 (18.9)	39 (52.7)	79.7

four stages of disease was 66.6%. Sensitivity increased by stage from 55.7% at Stages A,B to 79.7% at Stage D2, regardless of therapy activity. When considering only untreated patients, sensitivity increased from 77.5% at Stages A,B to 100% at Stage D2. These results correspond with previous reports demonstrating PSA values proportional to clinical stage of disease (30), but do not confirm the use of PSA values for preoperative staging.

Serial Monitoring

We conducted studies to demonstrate the value of the Bayer Immuno 1 PSA Assay for monitoring the course of disease and therapy in prostate cancer patients. A total of 159 patients were monitored with serial measurements of serum PSA. In this group, 156 patients were observed to have increasing and/or decreasing, or stable trends of PSA values, corresponding to their clinical status. Figure 3A, B represents longitudinal profiles of sequential PSA values that correspond to the patient's disease course and therapy. The 62-year-old patient described in Figure 3A underwent radical prostatectomy within 3 months of the diagnosis of prostate cancer. Serum PSA decreased to near zero at 4 months postsurgery and remained relatively stable for 2 years. Persistent disease was suggested by elevated PSA beginning at 2.8 years postsurgery, but was not corroborated by any other diagnostic test until 1 year later when increasing PSA values were consistent with a bone scan confirming metastatic disease. Bilateral orchiectomy was performed at 3.7 years postprostatectomy after which PSA values decreased again to near zero levels. This patient, however, expired with metastatic prostate cancer 2.8 years after the illustrated monitoring period. The 66-year-old patient depicted in Figure 3B demonstrated a similar pattern of decreasing, then increasing PSA values postprostatectomy. This patient received radiation therapy approximately 4 years postsurgery. A very low PSA value was obtained 1 year after radiation treatment, and the patient continued to exhibit no evidence of disease at 2 years postradiation.

Overall, longitudinal PSA measurements agreed with the clinical course of disease for 98% of the patients studied. This level of longitudinal sensitivity is in good agreement with other studies described in the current literature (14,19,31) and demonstrates clinical utility of the Bayer Immuno 1 PSA Assay for management of prostate cancer patients.

Fig. 3. Serial measurements of PSA for a 62-year-old prostate cancer patient demonstrating disease progression following prostatectomy and subsequent response to orchiectomy (A) and for a 66-year-old prostate cancer patient demonstrating progression and subsequent response to radiation (B).

Method Comparison

The Bayer Immuno 1 PSA Assay was compared to the Abbott IMx PSA microparticle enzyme immunoassay using 2,131 specimens obtained from healthy subjects, patients with nonmalignant diseases, and patients with prostate cancer and other malignant diseases. The Abbott assay was selected as the comparative device because it was the most widely used automated enzyme immunoassay for PSA at the time of the study. The relationship between the two assays was analyzed using the Passing-Bablok (32) regression technique. Separate regressions were performed with specimen results from healthy subjects, patients with BPH, and patients with prostate cancer.

The Bayer Immuno 1 PSA Assay is correlated to the Abbott IMx PSA Assay in Table 4. Passing-Bablok regressions using 2,131 specimens in the range 0 to 6,238 $\mu\text{g/L}$ ($y = 1.1 \times + 0.02$) and 2,060 specimens in the range 0 to 100 $\mu\text{g/L}$ ($y = 1.09 \times + 0.02$) demonstrate overall general agreement between the two assays. Regression line slopes are 1.05–1.07 using specimen results from healthy males and patients with BPH; and 1.15 using specimen results from prostate cancer patients.

The frequency of discordance between Bayer Immuno 1 and Abbott IMx PSA Assay results was also evaluated. Among 2,787 patient specimens (including dilutions) assayed at four clinical sites, 103 (3.7%), were confirmed by repeat testing to be discordant according to our discrepancy criteria. Further analysis of discordant samples was not pursued.

DISCUSSION

PSA is currently the most valuable serum marker for carcinoma of the prostate. Current literature has demonstrated this tumor marker's effectiveness for aiding in detection, monitoring therapeutic efficacy, evaluating tumor volume, detecting recurrent disease, and prognosis.

The Bayer Immuno 1 PSA Assay demonstrates acceptable performance and meets expectations for an ultrasensitive PSA assay (detection limit $< 0.1 \mu\text{g/L}$). We determined a lower limit of detection of $0.02 \mu\text{g/L}$ and a biological detection limit of $0.03 \mu\text{g/L}$ from multiple determinations of the Bayer Immuno 1 PSA Level 1 calibrator ($0.0 \mu\text{g/L}$) and low PSA human serum samples ($< 0.1 \mu\text{g/L}$) across four clinical trial sites and three PSA reagent lots. We calculated the biological

detection limit according to Vessella and Lange (21) taking into consideration both intra-assay imprecision of the zero calibrator and interassay imprecision of measuring PSA in patient serum.

Reproducibility, or precision, is an important performance criteria for a PSA assay. Increases or decreases in a patient's PSA result due to assay imprecision can lead to unnecessary diagnostic evaluations or failure to diagnose cases of cancer. Imprecision of the Bayer Immuno 1 PSA Assay is less than Vessella's and Lange's (21) recommended imprecision requirements of $< 10\%$ CV at PSA concentrations between $0.1 \mu\text{g/L}$ and $1.0 \mu\text{g/L}$, and $< 5\%$ CV at concentrations $> 1 \mu\text{g/L}$. This assay demonstrates interassay imprecision $< 3.5\%$ CV for three levels of commercial controls.

An important component of the imprecision estimate is reagent lot-to-lot variation. The range of variation among Bayer Immuno 1 PSA Assay reagent lots was notably small. The difference in reagent lot means expressed as a percent of the overall mean for three levels of commercial controls was not $> 3.7\%$. The overall precision demonstrated by this assay ensures accurate detection of true changes in serum levels of PSA.

The Bayer Immuno 1 PSA Assay is a sandwich assay employing a monoclonal capture antibody and a polyclonal detection antibody. The assay demonstrates antigenic specificity determined by significant and specific reactivity of the monoclonal antibody to PSA. Much attention is currently focused on the molar ratio of detecting free versus complexed PSA and the make-up of the antigen in the calibrator (11). As reported by Zhou et al. (24), this assay is equimolar and standardized to the 10% free PSA, 90% PSA-ACT calibrator material prepared at Stanford University.

In our method comparison study to Abbott IMx, 3.7% of the paired specimen results demonstrated differences that met our discordant criteria. The observed discordance may be explained by the established equimolarity of the Bayer Immuno 1 PSA Assay versus the Abbott assay's preference for the free form. In addition, a large positive slope (15%) was observed when correlating Bayer Immuno 1 versus Abbott IMx results for prostate cancer patient specimens. This also may reflect the difference in ability of the two assays to detect complexed versus free PSA. The proportion of PSA complexed to ACT may be increased in prostate cancer patients (9,33,34). If the Abbott IMx assay detects free PSA preferentially, then the observed positive slope would be expected for the prostate cancer population.

During our clinical studies of healthy individuals, asymptomatic for prostate disease, the mean PSA value increased with age from $0.7 \mu\text{g/L}$ for males < 50 years of age to $2.0 \mu\text{g/L}$ for males > 70 years. The nonparametric 95th percentile value also increased with age from $1.5 \mu\text{g/L}$ for males < 50 years to $4.6 \mu\text{g/L}$ for males ≥ 70 years. Oesterling et al. (35) observed that increasing the upper limit of normal to $4.5 \mu\text{g/L}$ for men 60–69 years and $6.5 \mu\text{g/L}$ for men 70 years or older

TABLE 4. Patient Sample Correlation: Bayer Immuno 1 PSA (Y) vs. Abbott IMx PSA (X)

Range ($\mu\text{g/L}$)	Subject category	N	Passing-Bablok		
			Slope	Intercept	$S_{y,x}$
0–100	All	2,060	1.09	0.02	1.69
0–6,238	All	2,131	1.10	0.02	48.70
0–11	Healthy	492	1.07	0.06	0.21
0–26	BPH	303	1.05	0.04	1.66
0–6,238	CaP	564	1.15	0.01	90.25

would have avoided 5.5% of biopsies performed but missed 0.6% of the prostate cancers. Catalona et al. (36) demonstrated that lowering the cutoff to 3.5 µg/L for men 50–59 years would have resulted in a 45% increase in the number of biopsies, but also a 15% increase in cancer detection. Therefore, the use of age adjusted reference ranges may improve either sensitivity or specificity, but not both concurrently.

Bayer Immuno 1 PSA Assay longitudinal measurements demonstrated correlation with changes in clinical status for 98% of the 159 monitored patients. Sensitivity of longitudinal PSA measurements has previously been reported at 100% for detection of recurrence of disease following prostatectomy (14,19,30), 93% for correspondence to disease status following androgen ablation therapy, and 63% for detection of residual prostate cancer following radiation therapy (31).

Clinical sensitivity corresponding to stage of disease and treatment status was also demonstrated for single point specimens from prostate cancer patients. Sensitivity before treatment, increased from 77.5% at Stages A,B to 100% at Stage D2. Sensitivity was particularly striking for untreated early stage disease. Given the very small size of Stage A and Stage B tumors, the increase in PSA levels for these patients demonstrates a high level of clinical utility for patient management even in early stage cancers. This sensitivity to small tumors, along with a biological detection limit of 0.03 µg/L, supports this assay's ability to detect recurrence of prostate cancer early, at a point where therapeutic intervention may improve patient outcome.

In conclusion, these data demonstrate analytical performance and clinical effectiveness of the Bayer Immuno 1 PSA Assay. This automated method provides the clinical laboratory with ultrasensitive, precise, and equimolar measurement of total PSA.

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