Evaluation of an Automated Chemiluminescent Immunoassay for Complexed PSA on the Bayer ACS:180™ System

Pradip Datta^{1*} and Amitava Dasgupta²

¹*Bayer Diagnostics, Tarrytown, New York*

²*Department of Pathology and Laboratory Medicine, University of Texas–Houston Medical School, Houston, Texas*

Prostate-specific antigen (PSA), the most important tumor marker for the detection of prostate cancer, exists in serum in a free, uncomplexed form (free PSA [fPSA]), and as bound to protease inhibitors (mainly α 1-antichymotrypsin [ACT]). The measurement of complexed PSA (cPSA) concentration in serum has been shown to have better sensitivity and specificity than serum total PSA concentration. A new chemiluminescent immunoassay for cPSA for use on the Bayer ACS:180™ fully automated system (Bayer Corp., Tarrytown, NY) has been developed and evaluated. The precision of the new assay was <3.9% (within-run coefficient of variation [CV]) and <5.0% (total CV). The analytical sensitivity (95% upper limit of noise at zero calibrator) was

<0.03 ng/mL. A comparison of the ACS:180™ cPSA results with the cPSA concentrations calculated from the ACCESS (Beckman-Coulter) PSA and fPSA assays yielded the following regression equation: ACS:180™ cPSA = 0.93* (calculated ACCESS cPSA) + 0.43, $R = 0.993$, $n = 95$. The mean dilution and spike recovery for five samples were both 98%. No interference was observed from hemoglobin, triglyceride, or bilirubin (NCCLS protocol). These results indicate that the ACS:180™ cPSA assay is precise, and compares well with the calculated cPSA from ACCESS total and free-PSA results. *J. Clin. Lab. Anal.* 17:174–178, 2003. © 2003 Wiley-Liss, Inc.

Key words: automated chemiluminescent immunoassay; complexed PSA; ACS:180™ methods; ACCESS methods; evaluation

INTRODUCTION

Prostate cancer is a major cancer and leading cause of death in men. As with any cancer, early detection followed by treatment increases the disease-free survival rate significantly. Prostate-specific antigen (PSA), a single-chain glycoprotein neutral serine protease of 240 amino acids that is secreted by the prostate and is involved in the lysis of seminal coagulum, has recently been recognized as a marker for prostate cancer prognosis, and as an aid to its diagnosis (1–3). Even though serum PSA (“PSA”) levels are known to be increased in men with prostate cancer, PSA is not recommended as the only screening procedure for the diagnosis of cancer, because elevated PSA levels are also observed in patients with benign prostatic hyperplasia. Thus, with a cut-off value of 4 ng/mL (the most commonly used value), PSA has a diagnostic sensitivity and specificity of 88% and 30%, respectively (rendering a positive predictive value [PPV] of 26%) (4). However, previous studies have suggested that the measurement of PSA in conjunction with a digital rectal examination

(DRE) and ultrasound provide a better means of detecting prostate cancer than DRE alone (5).

PSA exists predominantly in the circulation as complexes with various protease inhibitors. The most prevalent form of complexed PSA (cPSA) that can be detected is bound to alpha-1-antichymotrypsin (PSA-ACT complex) (6,7). The relative proportion of the complexed form increases in prostate cancer, and decreases in benign prostatic diseases. However, the percent ratio of fPSA (%fPSA) to total PSA behaves in the opposite manner. A cut-off of 25% fPSA ratio has been used to improve the PPV for a total PSA range of 2–12 ng/mL (where total PSA alone has poor PPV) (8). The use of such a ratio improves the distinction between

*Correspondence to: Pradip Datta, Diagnostics Division, Bayer Corporation, 511 Benedict Ave., Tarrytown, NY 10951. E-mail: pradip.datta.b@bayer.com

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prostate cancer and benign prostatic disease, and helps avoid costly and painful prostate biopsies (9,10). A new method was recently developed to measure the complex PSA directly. Serum cPSA concentration is used in the monitoring of prostate cancer, to determine residual disease and early recurrence after therapy when used in conjunction with other diagnostic indices. cPSA levels increase in men with cancer of the prostate, and after radical prostatectomy cPSA levels routinely fall to the undetectable range. If prostatic tissue remains after surgery, or metastasis has occurred, cPSA levels can be observed. Serum cPSA has been shown to be at least as good as the %fPSA ratio in detecting prostate cancer as well. In a multicenter study (4), the PPVs of prostate cancer screening for the serum markers PSA (cut-off: 4.0 ng/mL), cPSA (cut-off: 3.75 ng/mL), and free/total PSA ratio (cut-off: 25%, for PSA 4.0–10.0 ng/mL) were found to be, respectively: site 1: 31%, 40%, and 37% ($n = 75$); site 2: 18%, 25%, and 23% ($n = 167$); and site 3: 23%, 29%, and 42% ($n = 78$). The overall PPVs, for all three sites, were 26%, 33%, and 31%, respectively. However, it is important to note that the PPV is dependent on the prevalence of disease in the population tested.

In the current study we evaluated the precision, detection limit, linearity, accuracy, prozone effect, and interference of a new chemiluminescent immunoassay for cPSA for use on the Bayer ACS:180™ fully automated immunoanalysis system (Bayer Corp., Tarrytown, NY).

MATERIALS AND METHODS

ACCESS PSA and fPSA kits were purchased from Beckman (Fullerton, CA). The assays were run on the ACCESS automated immunoassay system (Beckman). The new cPSA assay, ACS:180™ cPSA (Bayer Diagnostics, Tarrytown, NY), was run on the ACS:180™ automated continuous random access immunoassay system (Bayer Diagnostics). The system automatically pipettes the sample into a disposable cuvette, adds the assay reagents to incubate for a total of 7.5 min at 37°C, separates the particles bound magnetically, and “flashes” the chemiluminescent label to generate signal (Relative Light Unit, or “RLU”). Three reagents are used in the ACS:180™ cPSA method. The sample is first incubated with a pretreatment reagent that contains an antibody to bind to the fPSA in the sample. The bound fPSA then cannot react with the solid-phase reagent, which contains PSA-specific antibodies immobilized on paramagnetic particles. The rest of the detectable PSA in the sample (the cPSA) is then measured by forming a “sandwich” complex among the cPSA, the solid phase, and the third reagent – the “Lite Reagent.” The signal is

directly related to the cPSA concentration in the sample. Standardization of the assay is performed by a nine-point master curve, generated by the manufacturer and supplied with the reagent kit. Two lyophilized calibrators (low and high) are used to calibrate the assay in routine use. Standardization is traceable to an International Reference PSA (90:10) preparation (11). The range of the assay is 0.03–100 ng/mL.

The ACCESS PSA (“PSA”) and fPSA assays use monoclonal capture and detection antibodies. The detection antibodies are conjugated to alkaline phosphatase enzyme, and the capture antibodies are immobilized on paramagnetic particles. After the particles are magnetically separated and washed, the bound antigen-antibody complexes are incubated with a chemiluminescent substrate to generate signal. Both assays use a six-point calibration, and are reported as traceable to the international PSA reference standards. The cPSA concentration was calculated on this system by subtracting fPSA from total PSA.

The performance of the new ACS:180™ cPSA assay was evaluated by the following experiments:

1. Imprecision. Three commercial controls (Bayer Diagnostics) were analyzed in three replicates per run over four runs ($n = 12$ for each sample).
2. Limit of Detection. The assay’s zero calibrator was analyzed in 20 replicates in a single run.
3. Linearity. Linearity was verified by diluting five sera containing ACS:180™ cPSA concentrations of 1.89–43.22 ng/mL with the manufacturer’s recommended assay diluent (Multidil2) and assaying them by ACS:180™ cPSA.
4. Accuracy–Spike Recovery. Five different sera, each containing no cPSA, were spiked with a PSA-ACT complex of 0.0 (control), 8.91, 16.22, and 24.66 ng/mL concentration. The resulting 20 samples were assayed with the ACS:180™ cPSA method. The PSA-ACT complex was purchased from Scripps Laboratory (La Jolla, CA). It has been reported to be >99% pure by SDS-PAGE (denaturing electrophoresis).
5. Accuracy – Method Comparison. Serum specimens ($n = 95$) from patients with various concentrations of cPSA were analyzed with ACS:180™ cPSA, ACCESS fPSA, and ACCESS total PSA methods.
6. Prozone Effect. The prozone effect was measured by spiking pure PSA-ACT antigen in the assay’s zero calibrator, and was then tested in the assay.
7. Interference. To determine the interference of hemolysis, icterus, and lipids in the new chemiluminescent assay, serum pools were spiked with hemolysate (prepared from lysed red blood cells), bilirubin (total and conjugated; Sigma, St. Louis, MO), and triglyceride (Intralipid; Sigma), and analyzed on the

ACS:180™ cPSA assay. A *t*-test was performed on the results from the spiked samples and their unspiked controls to determine significance.

RESULTS AND DISCUSSION

Precision

The within-run and total coefficients of variation (CVs) were 3.9% and 5.0% for the low control (mean 3.65 ng/mL), 2.4% and 3.8% for the medium control (mean 25.13 ng/mL), and 1.9% and 3.3% for the high control (mean 65.71 ng/mL).

Detection Limit and Linearity

The analytical detection limit of the ACS:180™ cPSA assay was calculated as the cPSA concentration equivalent to two times the standard deviation plus the mean of the observed signal (RLU) for the zero calibration. The detection limit was determined to be 0.03 ng/mL. The linearity results are presented in Table 1. The recovery for the samples, calculated as the observed over the theoretical cPSA concentration,

ranged from 70% to 113%. The mean recoveries for the five samples were 86–102%, with a grand mean recovery of 98%.

Spike Recovery

The recoveries for the spiked samples, calculated as observed over expected ACS:180™ cPSA concentrations, ranged from 89.2–106.0% (Table 2). The mean spike recovery for each of the five samples ranged from 92.1–102.8%, and the grand mean recovery for all five samples was 96.8%.

Comparison of Methods

We calculated the ACCESS cPSA by subtracting the ACCESS fPSA concentration of a sample from its ACCESS total PSA concentration. We studied specimens with a wide range of values across the analytical range of the new assay. Eighty-two percent of the specimens (78 of 95) had ACCESS PSA concentrations of <20 ng/mL. Using linear regression analysis between ACS:180™ cPSA (*y*) and the calculated ACCESS cPSA for all 95 samples (with ACS:180™ cPSA concentrations of 0.05–68.36 ng/mL), we observed

TABLE 1. Dilution recovery of cPSA using the chemiluminescent assay

Sample	Dilution	ACS:180 cPSA		% Recovery
		Observed (ng/mL)	Expected (ng/mL)	
1	–	5.52		
	1:02	3.05	2.76	110.5
	1:04	1.18	1.38	85.5
	1:08	0.72	0.69	104.4
	Mean			100.1
2	–	1.89		
	1:02	1.02	0.95	107.9
	1:04	0.33	0.47	69.8
	1:08	0.19	0.24	80.4
	Mean			86.1
3	–	33.27		
	1:02	17.56	16.64	105.6
	1:04	7.32	8.32	88.0
	1:08	4.44	4.16	106.8
	Mean			100.1
4	–	43.22		
	1:02	22.79	21.61	105.5
	1:04	9.52	10.81	88.1
	1:08	6.07	5.40	112.4
	Mean			102.0
5	–	34.16		
	1:02	19.25	17.08	112.7
	1:04	7.43	8.54	87.0
	1:08	4.54	4.27	106.3
	Mean			102.0
Grand mean				98.1

TABLE 2. Spike recoveries in the new chemiluminescent cPSA assay

Sample	Complexed PSA added (ng/mL)	ACS:180 cPSA observed (ng/mL)	% Recovery
1	–	<0.03	
	8.91	8.64	97.0
	16.22	15.64	96.4
	24.66	23.82	96.6
	Mean		96.7
2	–	<0.03	
	8.91	8.44	94.6
	16.22	15.01	92.5
	24.66	21.98	89.2
	Mean		92.1
3	–	<0.03	
	8.91	8.75	98.2
	16.22	15.67	96.6
	24.66	22.78	92.4
	Mean		95.7
4	–	0.04	
	8.91	9.48	106.0
	16.22	16.77	103.2
	24.66	24.52	99.3
	Mean		102.8
5	–	<0.03	
	8.91	8.65	97.1
	16.22	16.16	99.6
	24.66	23.19	94.0
	Mean		96.9
Grand mean			98.4

Fig. 1. Bias plot comparing the difference between ACS:180TM cPSA and the calculated ACCESS cPSA (y-axis) vs. the mean of the two methods (x-axis).

Fig. 2. Subset of the correlation data for the range used in prostate cancer diagnostics (cPSA concentration up to 20 ng/mL).

a good correlation: $y = 0.92x + 0.43$ ng/mL ($r = 0.993$). Most of the data points ranging up to ACCESS cPSA concentration of 26.1 ng/mL (93 samples) in the bias plot (Fig. 1) were equally distributed by the no-bias line. The two remaining samples, both at ~ 73 ng/mL of ACCESS cPSA, had negative biases of 5 and 8 ng/mL. However, the mean bias was only -0.10 ng/mL. Most of the samples (90 of 95) had a cPSA concentration of < 20 ng/mL. Since most routine hospital samples submitted for cPSA analysis have a similar concentration, we also looked at the regression equation for this subset. The regression equation in this subset of the samples was: $y = 1.02x - 0.02$ ng/mL ($r = 0.98$) (Fig. 2).

We also performed a concordance analysis among ACCESS calculated cPSA, and the ACS:180TM cPSA results, using the respective methods' cut-off concentrations for the diagnosis of prostate cancer. The cut-off value was 3.6 ng/mL for ACS:180TM cPSA. Patients with serum ACS:180TM cPSA < 3.6 ng/mL are less likely to have prostate cancer compared to those with serum cPSA > 3.6 ng/mL. No cut-off value was recommended for ACCESS-calculated cPSA. We used the ACS:180TM

cPSA cut-off for the ACCESS-calculated cPSA as well. There was agreement between ACS:180TM cPSA and ACCESS-calculated cPSA in 93 of 95 samples (97.8% concordance). For the two samples in which there was no agreement with the cut-off, the cPSA concentrations were near the cut-off (ACS:180TM cPSA followed by ACCESS-calculated cPSA, ng/mL): 3.76 vs. 3.46, and 3.36 vs. 3.91.

We compared the performance of the new cPSA assay with cPSA indirectly calculated from total and fPSA assays on the ACCESS system. The reason we chose ACCESS as the reference method is that there are few commercial, direct cPSA methods available, and the ACCESS total and fPSA assays use antibodies that are utilized in clinically established PSA assays (e.g., Hybritech, Beckman, Fullerton, CA).

Hook Effect

Immunometric assays often reveal a "hook," or prozone effect, such that a high concentration of the analyte in a sample may give false low-recovery results. The prozone studies with the new chemiluminescent cPSA assay showed that samples containing up to 12,000 ng/mL of PSA-ACT show no hook effect ACS:180TM cPSA assay.

Interference

When serum pools containing 1.2 and 4.6 ng/mL ACS:180TM cPSA were spiked with up to 500 mg/dL hemolysate, or 1,000 mg/dL of triglycerides, or 40 mg/dL of conjugated or unconjugated bilirubin, no statistically significant interference was observed (Table 3).

From the above results, we conclude that the new Bayer ACS:180TM cPSA assay is a precise, accurate, linear, and reliable tool for measuring serum cPSA in routine clinical laboratory practice. The assay correlates well with ACCESS-calculated cPSA (= total – fPSA),

TABLE 3. Interference in the new chemiluminescent cPSA assay

Interferent	ACS:180 cPSA, blank sample, ng/mL	ACS:180 cPSA, interference sample, ng/mL	Statistically significant?
Hemoglobin	1.19	1.24	No
(500 mg/dL)	4.73	4.70	No
Triglyceride	1.23	1.20	No
(1000 mg/dL)	4.65	4.74	No
Conjugated bilirubin	1.24	1.23	No
(40 mg/dL)	4.71	4.77	No
Unconjugated bilirubin	1.20	1.14	No
(40 mg/dL)	4.53	4.58	No

and is free from interference from hemolysis, icterus, and triglycerides. The new assay requires only a single measurement to determine the cPSA concentration in a sample, whereas the ACCESS system requires two measurements (total and fPSA).

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