Performance Evaluation of a New Chemiluminescent Assay for Prostate Specific Antigen

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Prostate specific antigen (PSA) is a glycoprotein found in the epithelial cells of the prostatic duct and acini. PSA is elevated in all four stages of prostate cancer as well as in benign prostatic hypertrophy. We evaluated a new chemiluminescent assay for PSA by comparing this assay with the micro-particle enzyme immunoassay for PSA (MEIA) on the AxSYM analyzer (Abbott Laboratories, Abbott Park, IL) and a Hybritech Tandem R assay for PSA. The new chemiluminescent assay is recently available from Bayer Diagnostics (Tarrytown, NY) and can be run using the ACS: 180 Plus analyzer. Precision of the new chemiluminescent assay was evaluated using commercially available controls (Bayer Diagnostics). The within-run and total CVs were 6.4 and 8.7% for the low control (mean: 0.43 µg/L), 1.6 and 5.2% for the next level control (mean: 1.94 mg/L), 4.3 and 4.9% for the medium control (mean: 2.10 mg/L), 1.2 and 3.9% for the high control 1 (mean: 11.52 mg/L), and finally 3.2 and 6.9% for the high control 2 (mean: 21.52 mg/L). The spike recovery varied from 94.2 to 109.6% for five different specimens we studied. We also observed excellent dilution recoveries. For example, in the speci-

men supplemented with 3.02 mg/L of PSA, the dilution recoveries were 102.1, 104.7, and 103.7% for 1:2, 1:4, and 1:8 dilutions, respectively. We analyzed 113 serum specimens from patients with various concentrations of PSA (range 0.5 mg/L–2040 mg/L) using the new chemiluminescent assay and compared our results with the MEIA and Hybritech (Tandem-R PSA) assays. Using x axis as the PSA concentrations obtained by the Tandem-R assay and the y axis as the PSA values obtained by the new chemiluminescent assay, we observed the following regression equations: $y = 1.04 \times -0.19$ ($r = 0.99$, $n = 112$). One specimen with PSA concentrations of 2040 µg/L by the MEIA and 2156 µg/L by the chemiluminescent assay was not used for regression analysis. Similarly using x axis as the PSA concentrations obtained by the MEIA assay and y axis as the PSA concentrations obtained by the chemiluminescent assay, we observed the following regression equation: $y = 0.88 + 0.02$ ($r = 0.99$, $n = 112$). We conclude that the new chemiluminescent assay has excellent precision and the results compared well with the existing assays. *J. Clin. Lab. Anal.* 14:164–168, 2000.

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Key Words: PSA; chemiluminescent assay; MEIA assay; Tandem-R PSA

INTRODUCTION

Prostate cancer is a common type of cancer in males and is also a leading cause of death. The incidence of prostate cancer also increases with age. It is estimated from autopsy studies that 30% of men in their eighth decade have unsuspected or latent carcinoma of the prostate. The clinical problems associated with the diagnosis and treatment of prostatic cancer are as varied as its presentation. Elevated levels of serum acid phosphatase have long been taken to be indicative of the spread of prostate cancer outside of the gland, but many patients with metastatic disease have normal serum levels of acid phosphatase (1).

Several studies have shown that the measurement of se-

rum prostate specific antigen (PSA) offers several advantages in the early detection of prostate cancer. PSA was first described by Wang et al. in 1979 (2). PSA is a secretion of prostate epithelium and is also produced by prostate cancer cells. PSA is a glycoprotein monomer with a molecular weight of 33,000–34,000 and contains 240 amino acids (2,3).

Elevated serum PSA concentrations can only suggest the presence of prostate cancer, and prostate biopsy is required

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Received 29 November 1999; Accepted 22 March 2000

to confirm the diagnosis. PSA testing also has significant value in detecting metastatic or persistent disease in patients following surgical or medical treatment of prostate cancer (4,5). Persistent elevation of PSA following treatment or increase in a post-treatment PSA level is indicative of recurrent or residual disease (6,7). PSA testing is a widely accepted adjunctive test in the management of prostate cancer patients (8). Egawa et al. demonstrated improved diagnostic value of PSA for differentiating prostate cancer from a benign prostatic condition by using prostate specific antigen-related parameters like PSA density for transition zone and gland volume (9). Recently, Carter and Pearson developed a guideline for prostate specific antigen testing for early diagnosis of prostate cancer (10).

Several commercially available assays measure PSA in human serum. The microparticle enzyme immunoassay (MEIA) of PSA and an immunoradiometric assay of PSA (Tandem-R PSA) are widely used in clinical laboratories. We, as a beta site, evaluated a new chemiluminescent assay for PSA by comparing results with two widely used assays in the market. Here we report our findings.

MATERIALS AND METHODS

The MEIA assay kits for PSA were purchased from Abbott Laboratories and the assays were run on an AxSYM analyzer. The immunoradiometric assay (Tandem-R PSA) kits were obtained from Hybritech Inc (San Diego, CA). The new chemiluminescent assay kits for PSA were provided by Bayer Diagnostics (Tarrytown, NY) and the assays were run using the ACS:180 Plus analyzer. We analyzed 113 serum specimens with various concentrations of PSA for this study. These specimens, as is routine, were submitted to our clinical laboratory and the study was performed only each specimen was evaluated and after all results were reported, as ordered by physicians. These specimens would have otherwise been discarded.

The new chemiluminescent assay for PSA is a two-site sandwich immunoassay using direct chemiluminometric technology. The first antibody in the "Lite Reagent" is a polyclonal antioat antibody labeled with acridinium ester. The second antibody in the solid phase is a monoclonal antimouse antibody, which is covalently coupled to paramagnetic particles. The assay requires 35- μ l sample for a single determination and this volume does not include the unusable sample volume in the sample cup. The assay also requires a two-point calibration, and this master calibration can be entered directly from the bar code of the reagent. The assay is linear up to a serum PSA concentration of 100 mg/L and the detection limit is 0.06 mg/L.

The MEIA assay is also an automated assay run on the AxSYM analyzer. The assay is linear for a PSA concentration of 100 mg/L. The detection limit of the assay is 0.1 mg/L. However, the Tandem-R PSA assay is a manual assay. The

assay is also linear for a PSA concentration of 100 mg/L and has a detection limit of 0.1 mg/L.

RESULTS AND DISCUSSION

Precision

Precision of the new chemiluminescent PSA assay was evaluated using five different controls (also available commercially) supplied by Bayer Diagnostics. The within-run and total CVs were 6.4 and 8.7% for the low control (mean: 0.43 μ g/L), 1.6 and 5.2% for the next-level control (mean: 1.94 mg/L), 4.3 and 4.9% for the medium control (mean: 2.10 mg/L), 1.2 and 3.9% for the high control 1 (mean: 11.52 mg/L), and finally 3.2 and 6.9% for the high control 2 (mean: 21.52 mg/L). In order to check the precision at the higher end of linearity, we prepared a control containing 80 mg/L of PSA. The within-run and total CV (mean: 79.7 mg/L) were 3.2 and 4.1% respectively.

Recovery Studies

We used five different specimens for spiked recovery study. We supplemented aliquots of each specimen containing no PSA with 0.0 (control), 25.39, 47.68, and 61.31 mg/L of PSA and measured PSA concentrations using the new chemiluminescent assay. The percent recovery varied from 94.2 to 109.6% (Table 1). We also observed excellent dilution recoveries; e.g., in the specimen supplemented with 3.02 mg/L of PSA, the dilution recoveries were 102.1, 104.7, and 103.7% for 1:2, 1:4, and 1:8 dilutions (Table 2).

Detection Limit and Linearity

The detection limit was established by analyzing zero calibrator for twenty times. Then the mean and standard deviations were calculated. The detection limit was set at 0.06 mg/L.

TABLE 1. Spike recoveries of new chemiluminescent PSA assay

Specimen number	Spiked PSA μ g/L	Observed PSA μ g/L	Recovery (%)
1	24.8	25.4	102.4
	43.7	47.7	109.1
	63.4	61.3	96.7
2	24.8	24.7	99.6
	43.7	43.4	99.3
	63.4	59.7	94.2
3	24.8	24.5	98.8
	43.7	42.5	97.3
	63.4	61.3	96.7
4	24.8	26.9	108.4
	43.7	47.9	109.6
	63.4	66.1	104.3
5	24.8	27.1	109.2
	43.7	46.3	105.9
	63.4	64.7	102.0

TABLE 2. Dilution recovery of PSA using the chemiluminescent assay

Specimen	Dilution	Observed value µg/L PSA	Target value µg/L PSA	Recovery (%)
1	Original	3.02		
	1:2	1.54	1.51	102.1
	1:4	0.79	0.76	104.7
	1:8	0.39	0.38	103.7
2	Original	10.87		
	1:2	5.30	5.44	97.5
	1:4	2.76	2.72	101.7
	1:8	1.43	1.36	105.1
3	Original	18.15		
	1:2	9.20	9.07	101.4
	1:4	4.64	4.54	102.2
	1:8	2.31	2.27	101.7
4	Original	19.78		
	1:2	10.30	9.89	104.1
	1:4	5.34	4.94	107.9
	1:8	2.73	2.47	110.3
5	Original	38.45		
	1:2	19.50	19.22	101.4
	1:4	9.89	9.61	102.9
	1:8	5.19	4.81	107.9

L, which was the value of mean $\pm$ 2 S.D. for the zero calibrator. The assay was linear up to a PSA concentration of 100 mg/L. Reportable range as stated by the manufacturer was further verified by adding a known concentration of PSA in specimens already containing some PSA. We observed expected increases throughout the reportable range. Specimens with PSA concentration over that range should be diluted using Multi-Diluent 2 (Bayer Diagnostics) and assayed again.

Comparison of Methods

We analyzed 113 serum specimens from patients with various concentrations of PSA using the new chemiluminescent assay and compared our results obtained by using the MEIA and Tandem-R assays. Of the specimens we studied, ten were from individuals with no prostate cancer. We studied specimens with a wide range of values in order to ensure that the new method compares well with the existing methods in the market. Most specimens had PSA concentrations of less than 20 µg/L and these are typical specimens analyzed in our hospital laboratory. The specimen with the highest level of PSA had a concentration of 2,040 mg/L by the MEIA assay and 2,156 mg/L by the new chemiluminescent assay. However, we did not use this value for the regression equation because this value is unusually high compared to PSA concentrations in the other 112 specimens.

We observed a very good correlation between the Hybritech Tandem-R PSA assay and the new chemiluminescent assay. Using the concentrations of PSA obtained by the Tandem-R PSA assay as the x axis and the new chemiluminescent assay as the y axis, we observed the following regression equation (Fig. 1):

$$y = 1.04x - 0.19 \quad (r = 0.99, n = 112).$$

The S_y/x value was 0.58.

Similarly, using the x axis to reflect concentrations of PSA obtained by the MEIA assay and the y axis to show PSA concentrations obtained by the chemiluminescent assay, we observed the following regression equation (Fig. 2):

Fig 1. Regression equation comparing PSA concentrations obtained by the Tandem-R PSA assay (x axis) and the new chemiluminescent assay (y axis).

Fig 2. Regression equation comparing PSA concentrations obtained by the MEIA assay (x axis) and the new chemiluminescent assay (y axis).

$$y = 0.88x + 0.02 \quad (r = 0.99, n = 112).$$

The Sy/x value was 0.92.

We also compared PSA concentrations obtained by the MEIA assay and the corresponding values obtained by the Tandem-R PSA assay. Using the x axis to reflect the PSA concentrations obtained by the MEIA assay and y axis to show the values obtained by the Tandem-R PSA assay, we observed the following regression equation (Fig. 3):

$$y = 1.16x - 0.18 \quad (r = 0.99, n = 112).$$

The Sy/x value was 1.03.

Interference Study

Hemolysis (hemoglobin 500 mg/dl), lipemia (up to 1,000 mg/dl of triglycerides), and high bilirubin (up to 40 mg/dl) demonstrated less than 5% changes in PSA values indicating

Fig 3. Regression equation comparing PSA concentrations obtained by the MEIA assay (x axis) and the Tandem-R PSA assay (y axis).

that hemolysis, lipemia, and icteric specimens are suitable for analysis for PSA. When common chemotherapeutic agents used for treatment of prostate cancer were added to serum containing PSA, we did not observe any statistically significant change in PSA values.

We conclude that the new chemiluminescent assay has excellent precision and accuracy. The assay compares well with the existing assays in the market and can be used for accurate determination of serum PSA levels in clinical laboratories.

REFERENCES

1. Fishman MC, Hoffman AR, Klausner RD, Thaler MS. In: *Medicine*, 2nd ed. Philadelphia: J.B. Lippincott; 1985. p 412–414.
2. Wang MC, Valenzuela LA, Murphy GP, et al. Purification of a human prostate specific antigen. *Invest Urol* 1979;17:159–163.
3. Ban Y, Wang MC, Watt KW, et al. The proteolytic activity of prostate specific enzyme. *Biochem Biophys Res Comm* 1984;123:482–488.
4. Lange PH, Brawer MK. Serum prostate specific antigen: its use in diagnosis and management of prostate cancer. *Urology* 1989;33(6 Suppl.):13–17.
5. Killan CS, Yang N, Emrich LJ, et al. Prognosis importance of prostate specific antigen for monitoring patients with stages B2 to D1 prostate cancer. *Cancer Res* 1985;45:886–891.
6. Brawer MK, Lange PH. Prostate specific antigen in management of prostatic carcinoma. *Urology* 1989;33(5 Suppl.):11–16.
7. Stamey TA, Yang N, Hay AR, et al. Prostate specific antigen as a serum marker for adenocarcinoma of the prostate. *N Engl J Med* 1987;317:909–916.
8. Chan DW, Bruzek DJ, Oesterling O, et al. Prostate specific antigen as a marker for prostatic cancer: a monoclonal and a polyclonal assay compared. *Clin Chem* 1987;33:1916–1920.
9. Egawa S, Suyama K, Takashima R, Mizoguchi H, Kuwao S, Baba S. Prospective evaluation of prostate cancer by prostate specific antigen-related parameters. *Int J Urol* 1999;6:493–501.
10. Carter HB, Pearson JD. Prostate specific antigen testing for early diagnosis of prostate cancer: formulation of guidelines. *Urology* 1999;54:780–786.