Variation in the Quantitation of Prostate-specific Antigen in Reference Material: Differences in Commercial Immunoassays, *Carol D. Cheli,[1*] Martin Marcus,[1] Jack Levine,[1] Zeqi Zhou,[1] Peter H. Anderson,[2] Daniel D. Bankson,[3] Jay Bock,[4] Sharon Bodin,[5] Carlotta Eisen,[6] Marilyn Senior,[7] Morton K. Schwartz,[6] Kwok K. Yeung,[1] and W. Jeffrey Allard[†]*
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Prostate-specific antigen (PSA) is a widely used biochemical marker for prostate cancer *(1)*. PSA, a serine protease, forms complexes with serine protease inhibitors such as α2-macroglobulin and α1-antichymotrypsin (ACT) *(2)*. The major immunoreactive forms of serum PSA are PSA-ACT complex and free PSA. PSA complexed to α2-macroglobulin is not measured by current immunoassays *(3)*. Because the proportion of PSA complexed to ACT is higher in men with prostate cancer than in men with benign prostatic hyperplasia (BPH) *(3, 4)*, the use of a ratio of free-to-total PSA may improve discrimination between men with BPH and prostate cancer *(5, 6)*.

Results obtained with different PSA immunoassays are not interchangeable *(7–10)*. The variation among immunoassays reflects, in large part, the specificity of different PSA antibodies *(7)*. To a lesser extent, the composition of the calibrator, the PSA values assigned to the calibrator, the test diluent, and the physical design of the immunoassays contribute to intermethod differences *(6, 7)*. Assays using a monoclonal-monoclonal sandwich format yield, in general, an equimolar response to free and ACT-complexed PSA, whereas monoclonal-polyclonal formats tend to yield higher values for samples containing greater proportions of free PSA *(8)*. We have recently described the Bayer Immuno 1™ PSA Assay (Bayer Corporation), which uses a monoclonal-polyclonal antibody format that yields an equimolar response to varying proportions of free and complexed PSA *(11)*. This has now been found to be a result of the unique binding properties of the monoclonal antibody used for the capture of PSA (manuscript in submission).

Proficiency testing among clinical laboratories has indicated large intermethod variability in the quantitation of total PSA *(12)*. The aim of this study was to determine the magnitude and cause of this intermethod variation. Here we report our multi-site analysis of three test materials: CAP Basic Ligand Survey Samples, CAP-Certified Reference Serum, and mixtures containing different proportions of free PSA and PSA-ACT complexes, which we tested using five commercially available PSA assays.

The Immuno 1 PSA assay is a magnetic separation sandwich enzyme assay on the Bayer Immuno 1 analyzer. The IMx® PSA Assay (Abbott Laboratories) is a microparticle enzyme immunoassay on the Abbott IMx analyzer. The Tandem®-R PSA immunoradiometric assay (Hybritech, Inc.) is a solid-phase double antibody sandwich assay that utilizes two noncompeting mouse monoclonal antibodies. The PSA-2 assay is a fully automated assay that uses a monoclonal-polyclonal antibody sandwich assay format on the ACS™:180 system (Ciba Corning Diagnostics). The TOSOH AIA (Tosoh Medics) method is a two-site immunoenzymometric assay that uses magnetic bead technology and utilizes two monoclonal antibodies.

Reference materials were obtained from the College of American Pathologists (CAP). CAP Proficiency Survey Samples (K series samples K-06, K-07, and K-08, 1996) are made from semen-supplemented human plasma and contain at least 50% free PSA *(12)*. CAP-Certified Reference Material is made from human serum from donors, which was blended to target values of 0.5, 4.0, and 9.0 μg/L. This material contains ~90% complexed PSA and 10% free PSA *(12)*. To investigate the effect of varying proportions of free and complexed PSA on PSA quantitation by the different assays, test materials containing five mixtures of both free PSA and PSA-ACT (Scripps Laboratories, San Diego, CA) were prepared at Bayer Corporation. Mixtures of seminal purified PSA and PSA-ACT were prepared at 4 μg/L total PSA in several ratios of free PSA to PSA-ACT complex (100:0, 80:20, 50:50, 20:80, and 0:100). The mixtures were prepared with a buffer containing 60 g/L bovine serum albumin, 111 mmol/L sodium chloride, 25 mmol/L Tris, 1 g/L sodium azide, and 2 g/L Proclin-150, pH 7.4. PSA in the free and complexed PSA preparations was measured on the Bayer Immuno 1 analyzer. Measurements of free and complexed PSA, using the Bayer assay calibrators, agree with values obtained with PSA standards measured by biophysical means *(11)*.

The study was conducted at the following six clinical laboratories: Providence St. Vincent Medical Center (Portland, OR), V.A. Puget Sound Health Care (Seattle, WA), University Hospital (State University of New York, Stony Brook, NY), Memorial Sloan Kettering Cancer Center (New York, NY), Hospital of University of Pennsylvania (Philadelphia, PA), and Huntsville Hospital (Huntsville, AL). Each site measured PSA recoveries from the three test materials, using the Immuno 1 assay. Two of these sites also measured PSA in the test samples, using the Tandem R, IMx, and the TOSOH AIA methods. One of the sites measured PSA with the ACS:180 assay. Test samples were analyzed in duplicate in a single run for each of the immunoassays evaluated in the study. Commercial controls were included in each run to validate method and instrument performance. All assays were performed according to manufacturers' instructions.

The amounts of PSA recovered when test material mixtures containing five different ratios of free PSA to PSA-ACT complex were assayed are presented in Fig. 1. The Bayer Immuno 1 assay was unaffected by changing proportions of PSA complexed to ACT and had an equimolar response to both free and ACT-complexed

Table 1. Mean PSA values (μg/L) for CAP Reference series[a] vs CAP K series[b] using the Bayer Immuno1, Abbott IMx, Hybritech R, Chiron ACS:180, and TOSOH test systems.

	Immuno1 (n = 6)	IMx (n = 2)	Hybritech R (n = 2)	ACS:180 (n = 1)	TOSOH (n = 2)
CAP K-B-07	0.62	0.60	0.68	0.40	0.57
CAP K-B-08	3.93	17.32	10.08	22.75	7.43
CAP K-B-06	7.20	32.75	18.68	47.10	14.12
CAP Reference 1	0.53	0.47	0.55	0.50	0.52
CAP Reference 2	4.13	3.68	3.78	4.40	4.10
CAP Reference 3	9.87	8.53	9.55	9.35	9.84

[a] Blended human serum.
[b] Sera supplemented with semen.

PSA. The TOSOH assay also had an equimolar response to both free and ACT-complexed PSA, but gave slightly lower PSA values compared with the Immuno 1 assay. In contrast, the responses of the IMx and the ACS:180 assays were greater at high proportions of free PSA and decreased markedly as the proportion of complexed PSA increased in the test sample. The Hybritech Tandem-R assay had a lower assay response at high concentrations of free PSA, which increased as the proportion of complexed PSA in the test sample increased. PSA measured by the Hybritech assay was <4 μg/L at all proportions of free and complexed PSA. Agreement between PSA values for the different methods improved as the proportion of complexed PSA increased in the test samples.

The mean PSA values determined for each of the five immunoassays, using the CAP Proficiency Survey K series samples and the CAP-Certified Reference series, are shown in Table 1. Recoveries of PSA from CAP Proficiency Survey samples by the five methods showed large intermethod differences. In contrast to the large intermethod variation in PSA values using the CAP K series, PSA values generated using CAP Certified Reference Material by the five methods produced very similar results.

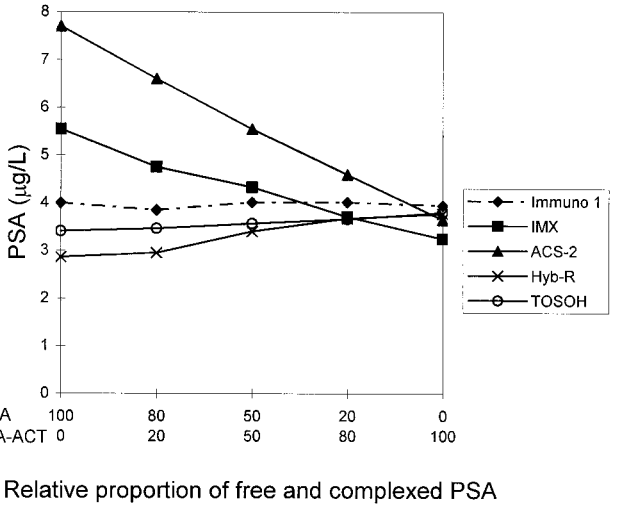

Fig. 1. Variability among manufacturers in PSA quantitation using synthetic mixtures containing five different proportions of free PSA and ACT-complexed PSA.

CAP Proficiency Survey samples are semen-supplemented human plasma containing at least 50% PSA in the uncomplexed form. When we tested synthetic mixtures with similar proportions of uncomplexed PSA, we found large intermethod differences in the quantitation of total PSA. In contrast, with CAP Reference serum, which is composed of blended human serum that contains the majority of PSA in the complexed form (~90% complexed, 10% free PSA), PSA values measured by the different methods were similar. Synthetic mixtures of 80% complexed and 20% free PSA, with proportions of PSA forms similar to those found in CAP reference serum, generated similar PSA values by the different methods (Fig. 1).

Similar to our findings, Garg et al. *(12)* reported similar PSA values with the TOSOH, Abbott IMx, and Hybritech assays for CAP reference serum, but strikingly different PSA values for proficiency survey samples. Similarly, Stamey *(8)*, comparing immunoassays by use of mixtures of free and complexed PSA in which the total concentration of PSA was the same, reported that the Hybritech Tandem-R assay measured low values when the proportion of free PSA was >50%. In addition, the IMx and ACS:180 assays measured high PSA values when mixtures contained 25% free PSA and increased markedly as the proportion of free PSA increased in the test samples. The TOSOH method generated an equimolar response to free and complexed PSA.

In a recent study, Blase et al. *(13)* measured synthetic mixtures with varying proportions of seminal fluid-derived free PSA, using five different immunoassays, and reported findings similar to ours. In a subsequent study, that group showed that the skewed response observed in nonequimolar assays is reflected in patient sera *(14)*. They compared the ratio of PSA values reported with a skewed assay to PSA values reported with an equimolar assay as a function of the mean PSA concentration of patient samples grouped by the proportion of free PSA. The results showed that skewed assays overrecover PSA from serum samples with increased proportions of free PSA. Other studies have demonstrated that commercially available immunoassays vary in their responsiveness to free and complexed PSA in clinical specimens *(7, 8, 10, 15)*. Jacobsen et al. *(15)* measured PSA in serum from healthy men, using the Hybritech Tandem-R and the IMx assay. Although the

two methods correlated well, the IMx assay gave higher PSA values at high ratios and lower PSA values at low ratios of free-to-total PSA. Zhou et al. *(7)* found that the ACS:180 PSA-2 assay measured PSA from patients with prostate cancer and BPH up to three times higher than did the Hybritech Tandem-R method.

It has been suggested that PSA measurements using synthetic mixtures with varying proportions of free and ACT-bound PSA may not reflect results in patient sera *(16)*. However, results from the studies presented suggest that free PSA values can vary in patients' sera, and measurements with skewed assays may compromise the clinical utility of PSA measurements. The proportion of free PSA in serum may reach as high as 55% *(3, 7)*. In the studies reported here and elsewhere *(8, 12, 13)*, at high proportions of free PSA, PSA values do not agree among manufacturers. Because the proportion of free PSA tends to be high in men who do not have prostate cancer, serum from such individuals could give falsely high PSA values in skewed response assays.

The comparison of results for CAP Proficiency samples with the ACS;180, PSA-2, IMx, and Bayer Immuno 1 PSA assays are consistent with the results shown in Fig. 1. An exception, however, is the finding that the Hybritech and TOSOH assay values were higher than the Bayer Immuno 1 assay values for the CAP K series but lower for the synthetic mixtures. This may reflect the fact that different isoforms of free PSA have been reported in seminal fluid *(17)*. Values may differ from assay to assay because antibodies differ in their recognition of the different isoforms of PSA *(17)*. PSA is quite different in serum.

In an effort to standardize PSA, it has been suggested that standard reference material containing 90% ACT-complexed and 10% free PSA be used to bring commercial immunoassays closer to concordance *(8, 12)*. This ratio was chosen because it closely mimics the ratio of free and complexed PSA found in clinical specimens. However, skewed response assays such as the IMx, ACS:180, and Hybritech-Tandem R assay will accurately measure PSA values only when the PSA forms in serum samples approximate a 90:10 ratio. Deviations from this ratio in the PSA forms, as demonstrated in this study, will generate inaccurate PSA values by these assays. The use of reference material containing the 90:10 ratio of complexed-to-free PSA will minimize the intermethod variation among manufacturers but will not solve the underlying problem: Commercial assays report different values for clinical specimens because of differences in responses to free and complexed PSA. The only way to accurately standardize PSA assays is to use two standards, one with low free PSA and one with high free PSA.

Stenman et al. *(18)* suggest that because the proportion of free and complexed PSA differs between benign and malignant diseases, a goal for standardization of total PSA assays should be the detection of free and ACT-complexed PSA on an equimolar basis. However, at present, this may be an unachievable goal because of the varied responses of different immunoassays to the different forms of PSA. As an alternative, manufacturers can develop new generation tests that measure the individual forms of PSA *(6, 7, 12)*. For example, a new generation of automated assay has recently been described, which measures complexed PSA selectively, and has been reported to increase the diagnostic specificity for patients with BPH *(19)*. This test alone could improve the distinction between prostate cancer and BPH without having to measure both free and total PSA. Clinical trials will determine the usefulness of these assays in detecting and monitoring prostate cancer patients.

Portions of this work were previously presented in abstract form [*Clin Chem* 1997;43(Suppl 6):S137]. We gratefully acknowledge Dr. Glen Armstrong for review of the manuscript.

References

1. Oesterling JE. Prostate specific antigen: a critical assessment of the most useful tumor marker for adenocarcinoma of the prostate. J Urol 1993;145: 907–23.
2. Christensson A, Laurell C-B, Lilja H. Enzymatic activity of prostate-specific antigen and its reactions with extracellular serine proteinase inhibitors. Eur J Biochem 1990;194:755–63.
3. Stenman UH, Leinonen J, Alfthan H, Rannikko S, Tuhkanen K, Alfthan O. A complex between prostate-specific antigen and alpha-1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: assay of the complex improves clinical sensitivity for cancer. Cancer Res 1991;51:222–6.
4. Lilja H, Christensson A, Matikainen M-T, Nilsson O, Pettersson K, Lovgren T. Prostate-specific antigen in human serum occurs predominantly in complex with alpha-1-antichymotrypsin. Clin Chem 1991;37:1618–25.
5. Christensson A, Bjork T, Nilsson O, Dahlen U, Matikainen MT, Cockett AT, et al. Serum prostate specific antigen complexed with α1-antichymotrypsin as an indicator of prostate cancer. J Urol 1993;150:100–5.
6. Wu JT. Assay for prostate specific antigen (PSA): problems and possible solutions. J Clin Lab Anal 1994;8:51–62.
7. Zhou AM, Tewari PC, Bluestein BL, Caldwell GW, Larsen FL. Multiple forms of prostate-specific antigen in serum: differences in immunorecognition by monoclonal and polyclonal assays. Clin Chem 1993;39:2483–91.
8. Stamey TA. Second Stanford conference on international standardization of prostate specific antigen immunoassays: September 1 and 2, 1994. Urol 1995;45:173–84.
9. Graves HCB, Wehner N, Stamey TA. Comparison of a polyclonal and monoclonal immunoassay for PSA: need for an international antigen standard. J Urol 1990;144:1516–22.
10. Wener MH, Daum PR, Brawer MK. Variation in measurement of prostate-specific antigen: Importance of method and lot variability. Clin Chem 1995;41:1730–7.
11. Zhou Z, Ng PC, Very DL, Allard WJ, Yeung KK. Technicon Immuno 1® PSA Assay measures both free and alpha-1-antichymotrypsin-complexed prostate-specific antigen on an equimolar basis. J Clin Lab Anal 1996;10:155–9.
12. Garg CG, Howanittz JH, Nakamura RM, Plous RH, Eckfeldt JH. Production, analysis, and characterization of reference materials for prostate specific antigen. Arch Pathol Lab Med 1995;119:1104–8.
13. Blase AB, Sokoloff RL, Smith KM. Five PSA methods compared by assaying samples with defined PSA ratios. Clin Chem 1997;43:843–5.
14. Blase AB, Sokoloff RL. Analytical characteristics of seminal fluid PSA differ from those of serum PSA [Reply]. Clin Chem 1998;44:192–3.
15. Jacobsen SJ, Lilja H, Klee GG, Wright GL, Pettersson K, Oesterling JE. Comparability of the Tandem-R and IMx assays for the measurement of serum prostate-specific antigen. Urology 1994;44:512–8.
16. Tewari PC, Williams JS. Analytical characteristics of seminal fluid PSA differ from those of serum PSA [Letter]. Clin Chem 1998;44:191–2.
17. Zhang W-M, Leinonen J, Kalkkinen N, Dowell B, Stenman U-H. Purification and characterization of different molecular forms of prostate-specific antigen in human seminal fluid. Clin Chem 1995;41:1567–73.
18. Stenman U-H, Leinonen J, Zhang W-M. Problems in the determination of prostate specific antigen. Eur J Clin Chem Clin Biochem 1996;34:735–40.
19. Yeung KK, Zhou Z, Morris D, Dai J, Allard WJ, Brawer MK. Improved specificity in the early detection of prostate cancer using complexed PSA [Abstract]. Tumor Biol 1997;18(Suppl 2):100.