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Automation and Analytical Techniques

Comparison of 6 Automated Assays for Total and Free Prostate-Specific Antigen with Special Reference to Their Reactivity toward the WHO 96/670 Reference Preparation

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Background: **Prostate-specific antigen (PSA) assays have historically produced different results. Our aim was to investigate the comparability of assay results of selected commercially available assay methods designed to measure total, free, or complexed PSA (tPSA, fPSA, and cPSA).**

Methods: **We measured tPSA, fPSA, and cPSA in 70 samples and in the WHO PSA 96/670 reference preparation with 6 assays (Beckman-Coulter Access, Abbott ARCHITECT and AxSYM, Bayer Advia Centaur, DPC IMMULITE 2000, and Roche Modular Analytics E170). We also calculated the fPSA/tPSA ratio.**

Results: **The mean deviations from the expected tPSA and fPSA values for the WHO 96/670 reference preparation were 0.37 (range, 0.01–1.32) and 0.19 (range, 0.05–0.49) μg/L, respectively. When plotted against the expected WHO 96/670 reference preparation value, regression slopes varied from 0.99 to 1.22 and r^2 from 0.9996 to 1.000. When total PSA was measured in mixtures of sera with high and low tPSA concentrations, the mean (SD) slope of regression of different assays against an in-house method was 1.04 (0.09). In these specimens, the fPSA/tPSA ratio was 0.11–0.14 with different methods. The tPSA and fPSA values in patient samples measured in different assays and plotted against ARCHITECT gave regression slopes from 0.88 to 0.97. The results of the studied assays for tPSA in serum samples agreed within 15%, from each other, and all results for the WHO 96/670 reference preparation were within 6.8% (confidence interval, 1.7%–15.2%) of the expected value. The results for fPSA were more diverse.**

Conclusions: **Differences among PSA assays appear to have decreased since introduction of the WHO 96/670 reference preparation, but further efforts are needed to harmonize fPSA assays.**

Prostate cancer (PCa)[4] remains an important public health problem *(1, 2)*. Prostate-specific antigen (PSA) testing is used for both early detection and monitoring of PCa *(3)*.

Assays for total PSA (tPSA) are based on the immunochemical detection of 2 major PSA forms: free, or uncomplexed, PSA (fPSA) and complexed, the dominant form of PSA, bound to α_1-antichymotrypsin (PSA-ACT). A third form, PSA bound to α_2-macroglobulin, is not detected by currently available assays *(4)*. Other PSA complexes are considered to be of less significance because of their very low concentrations in blood *(5)*.

Because benign prostate hyperplasia and PCa can both increase tPSA, the ratio fPSA/t PSA is often used *(6)*. Patients with higher fPSA/tPSA ratios are less likely to have PCa than patients with lower ratios *(7, 8)*. Detection of fPSA is based on antibodies specific for at least one PSA epitope masked in the complexed form. Detection of the complexed PSA form is based on at least one epitope specific for the PSA-ACT complex.

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[4] Nonstandard abbreviations: PCa, prostate cancer; tPSA, fPSA, and cPSA, total, free (uncomplexed), and complexed prostate-specific antigen, respectively; and ACT, α_1-antichymotrypsin.

Table 1. Characteristics of tPSA, fPSA, and cPSA assays.

	Manufacturer	Analyzer	Antibody[a]		Lower detection limit,[b] μg/L	Lot no.	Calibrator
			Capture	Detection			
tPSA	Beckman	Access	Monoclonal	Monoclonal	0.008	318356	Purified PSA[c]
	Abbott	ARCHITECT	Monoclonal	Monoclonal	0.008	06029M200	WHO 96/670
	Abbott	AxSYM[d]	Monoclonal	Monoclonal	0.040	10601Q100	WHO 96/670
	Bayer	Advia Centaur	Monoclonal	Polyclonal	0.010	3254	Purified PSA[e]
	Roche	E170	Monoclonal	Monoclonal	0.003	00166027	WHO 96/670
	DPC	IMMULITE 2000	Polyclonal	Monoclonal	0.04	165	WHO 96/670
fPSA	Beckman	Access	Monoclonal	Monoclonal	0.005	318361	Purified PSA[c]
	Abbott	ARCHITECT	Monoclonal	Monoclonal	0.008	95449m101	WHO 96/670
	Abbott	AxSYM	Monoclonal	Monoclonal	0.020	9269Q100	WHO 96/670
	Roche	E170	Monoclonal	Monoclonal	0.010	00164162	WHO 96/668[f]
	DPC	IMMULITE 2000	Monoclonal	Polyclonal	0.02	125	WHO 96/668[f]
cPSA	Bayer	Advia Centaur[g]	Monoclonal	Polyclonal	0.030	0553	Purified PSA[e]

[a] Monoclonal is mouse monoclonal antibody; polyclonal is polyclonal goat antibody.
[b] Minimum detection limit as claimed by the manufacturer.
[c] Beckman tPSA and fPSA are calibrated with highly purified PSA based on Hybritech assays.
[d] On the AxSYM we used Total PSA (list no. 3C19), available outside the United States since 1997.
[e] Bayer tPSA and cPSA are standardized against highly purified PSA and PSA-ACT respectively.
[f] WHO 96/668 consists of 100% fPSA.
[g] fPSA is prevented from reacting by pretreating the sample with m antibody.

The equimolarity, or lack thereof, of tPSA assays has been a matter of debate *(9, 10)*. An assay for tPSA is called equimolar when it generates the same signal for the same concentrations of fPSA and PSA-ACT complex and thus is not affected by changes in proportions of fPSA and PSA-ACT complex. A lack of equimolarity hampers interchangeability of results of different assays *(11)*. The use of 2 monoclonal antibodies free of steric hindrance and the use of optimal incubation times are ingredients for an assay with equimolar detection.

Traditionally, different assays for PSA have produced different results on the same patient sample. The reasons for this include the differences in epitope recognition by various assays and the use of different reference preparations. To circumvent the latter problem, an international reference preparation has been devised in which 90% of the PSA is complexed to ACT and the remaining 10% is fPSA *(12, 13)*. This proportion is similar to that in the circulation in patients with PCa. Manufacturers of several PSA assays have used this WHO 96/670 reference preparation for calibration, but intermethod differences persist *(14)*. The purpose of the present study was to assess the responsiveness to the WHO preparation of various assays for fPSA and tPSA and to compare the performance of these automated assays on clinical specimens that contain various PSA concentrations.

Materials and Methods

We performed experiments with the WHO 96/670 reference preparation (First International Standard), with patient serum samples, and with serum pools.

The PSA 90:10 WHO 96/670 reference preparation, which contains 500 μg/L tPSA after reconstitution in an fPSA/tPSA ratio of 0.10, was a gift of Abbott Laboratories (Abbott Park, IL). Reconstitution was done at 1 location, and for all solutions we used the recommended 2 mL of 10 g/L bovine serum albumin in 20 mmol/L phosphate-buffered saline (pH 7.4) to create a solution containing 500 μg/L PSA. This stock solution was diluted with 18 mL of

Table 2. Imprecision data of different PSA assays for tPSA and fPSA.

	tPSA				fPSA			
	Low control		High control		Low control		High control	
Assay	μg/L	CV, %	μg/L	CV, %	μg/L	CV, %	μg/L	CV, %
Advia Centaur	0.36	5.0	13.9	3.9	NA[a]	10[a]	NA[a]	2.4[a]
Access	1.00	2.6	89.0	1.7	1.02	1.1	12.9	1.6
ARCHITECT	0.02	5.5	9.9	3.7	0.10	9.2	11.3	6.2
AxSYM	0.50	5.3	25.0	5.6	0.40	4.0	7.0	4.1
E170	4.58	1.6	42.6	1.2	1.14	2.3	10.4	2.0
IMMULITE	3.80	5.8	38.0	6.4	0.19	18	2.6	10

[a] Unknown; these were tPSA controls and we measured cPSA.

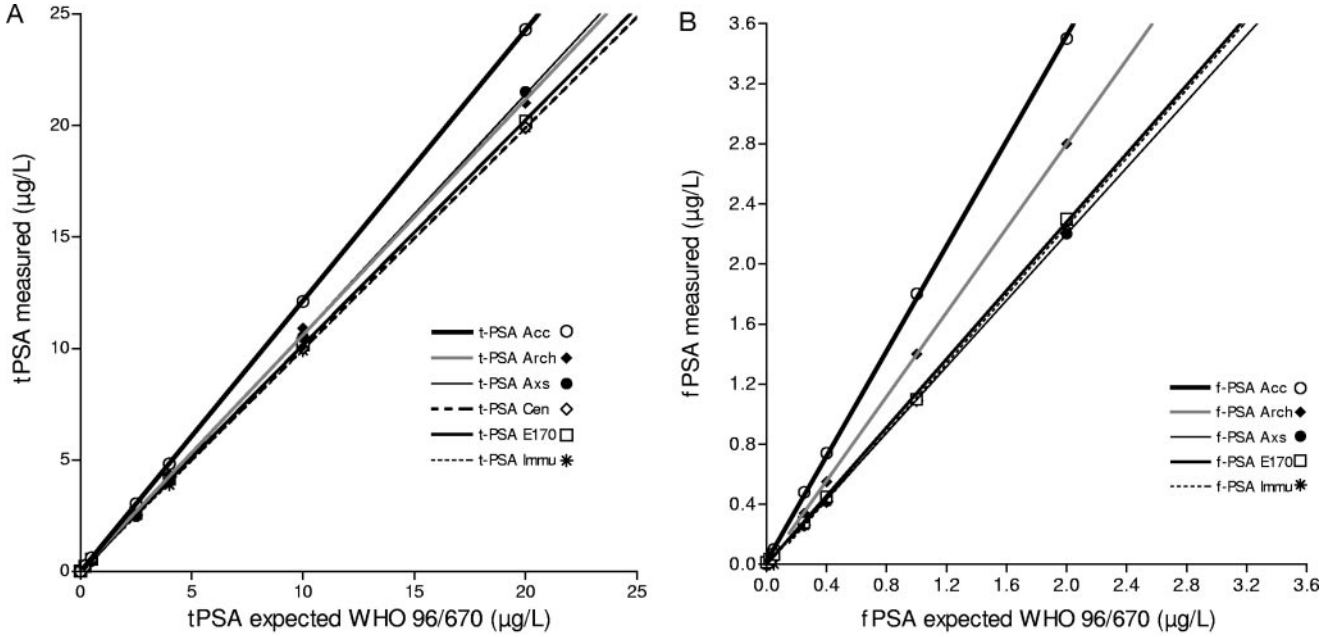

Fig. 1. Comparison of tPSA (*A*) and fPSA (*B*) in the WHO 96/670 reference preparation, measured by 6 different assays.

tPSA and fPSA were measured in buffered solutions of the reference preparation by 6 (tPSA) or 5 (fPSA) methods, and the results were plotted against the expected values. Slopes, intercepts, and correlation coefficients of the different lines are presented in Table 3. *Acc*, Access; *Arch*, ARCHITECT; *Axs*, AxSYM; *Cen*, Centaur; *Immu*, IMMULITE.

buffer to create a 50 µg/L solution. We diluted the 50 µg/L stock solution with buffer to generate 6 concentrations: 20, 10, 4, 2.5, 0.5, and 0.2 µg/L. Each solution, and a portion of buffer, was stored in single-use volumes and frozen at −20 °C.

We obtained serum samples by selecting 70 anonymized surplus samples <6 months old with PSA concentrations (range) of <0.1 to 50 µg/L as measured by one method (ARCHITECT Total PSA; Abbott). From these samples, multiple 500-µL aliquots were made and stored at −20 °C. Finally, we prepared independent serum pools with expected values, as measured by our in-house method (ARCHITECT) for tPSA, by mixing sera with high and low tPSA concentrations in various proportions. Pools and aliquots were analyzed within 3 weeks.

TEST PROCEDURE

Assays were performed according to the instructions of their manufacturers. Only 1 reagent lot and 1 calibration were used. The serum aliquots were kept frozen and thawed only once and analyzed within 4 h of thawing. The methods used were the Access (Beckman-Coulter), ARCHITECT (Abbott), AxSYM (Abbott), Advia Centaur (Bayer), IMMULITE 2000 (DPC), and Modular Analytics

Table 3. Slopes, intercepts, and correlation coefficients for comparison of different assays for tPSA and fPSA in the WHO 96/670 reference preparation.[a]

	Assay	Slope (95% CI)[b]	Intercept (95% CI)	r^2[c]
tPSA	Access	1.22	−0.01	1.000
	ARCHITECT	1.06 (1.03–1.08)	0.05 (−0.15 to 0.25)	0.9996
	AxSYM	1.08 (1.06–1.09)	−0.12 (−0.28 to 0.04)	0.9998
	Advia Centaur	0.99	0.07	1.000
	E170	1.01	0.06	1.000
	IMMULITE 2000	0.99	−0.01	1.000
fPSA	Access	1.75 (1.72–1.78)	0.02 (−0.01 to 0.05)	0.9997
	ARCHITECT	1.40	−0.003	1.000
	AxSYM	1.10	−0.008	1.000
	Advia Centaur	NA	NA	NA
	E170	1.14 (1.11–1.17)	0.00 (−0.02 to 0.02)	0.9994
	IMMULITE 2000	1.14 (1.11–1.17)	−0.03 (−0.06 to −0.00)	0.9995

[a] The data points and regression lines are shown in Fig. 1.

[b] CI, confidence interval; NA, not applicable.

[c] r^2 = 1.000 represents a perfect line; hence, for those cases, there are no confidence limits for slope and intercept to be given.

Table 4. Characteristics of the regression lines representing the relationships between tPSA and fPSA results obtained with different assays in serum pools with arbitrarily assigned PSA values.[a]

		Slope (95% CI)[b]	Intercept (95% CI)	r^2
tPSA	Access	1.12 (1.06–1.18)	0.12 (−0.50 to 0.74)	0.9992
	ARCHITECT	0.99 (0.94–1.04)	0.42 (−0.1 to 0.95)	0.9992
	AxSYM	0.97 (0.95–1.0)	−0.02 (−0.28 to 0.24)	0.9998
	Advia Centaur	0.98 (0.95–1.02)	0.42 (0.05–0.79)	0.9996
	E170	0.97 (0.93–1.01)	0.41 (−0.01 to 0.83)	0.9995
	IMMULITE 2000	1.19 (1.02–1.36)	−0.23 (−1.99 to 1.52)	0.9941
fPSA	Access	0.14 (0.13–0.15)	0.04 (−0.02 to 0.09)	0.9996
	ARCHITECT	0.11 (0.10–0.12)	0.004 (−0.08 to 0.07)	0.9987
	AxSYM	0.13 (0.11–0.14)	−0.06 (−0.21 to 0.08)	0.9964
	Advia Centaur	NA	NA	NA
	E170	0.12 (0.11–0.13)	−0.04 (−0.17 to 0.09)	0.9969
	IMMULITE 2000	0.11 (0.10–0.12)	−0.05 (−0.16 to 0.05)	0.9970

[a] Before the experiment, tPSA and fPSA were arbitrarily assigned with aid of our in-house method.
[b] CI, confidence interval; NA, not applicable.

E170 (Roche). Our in-house method, ARCHITECT, was arbitrarily used to provide a concentration value for comparison of methods. Of the 6 selected methods, 5 measured tPSA and fPSA and 1 measured tPSA and cPSA. For the latter assay, fPSA was calculated as the difference between tPSA and cPSA. Assay characteristics are summarized in Table 1, and the performance characteristics of the assays as run in the participating laboratories are shown in Table 2.

STATISTICAL EVALUATION

We analyzed results of measurements of the WHO 96/670 reference preparation in buffer by linear regression and Pearson correlation and used Passing and Bablok regression analysis for the serum aliquots to determine slope and intercept. P values ≤0.05 were considered to reflect statistical significance.

Results

MEASURED tPSA AND fPSA IN THE WHO 96/670 REFERENCE PREPARATION

Comparisons of results obtained with the WHO 96/670 reference preparation of each of the several other methods and the arbitrarily selected comparison method (Architect) are shown in Fig. 1 and Table 3.

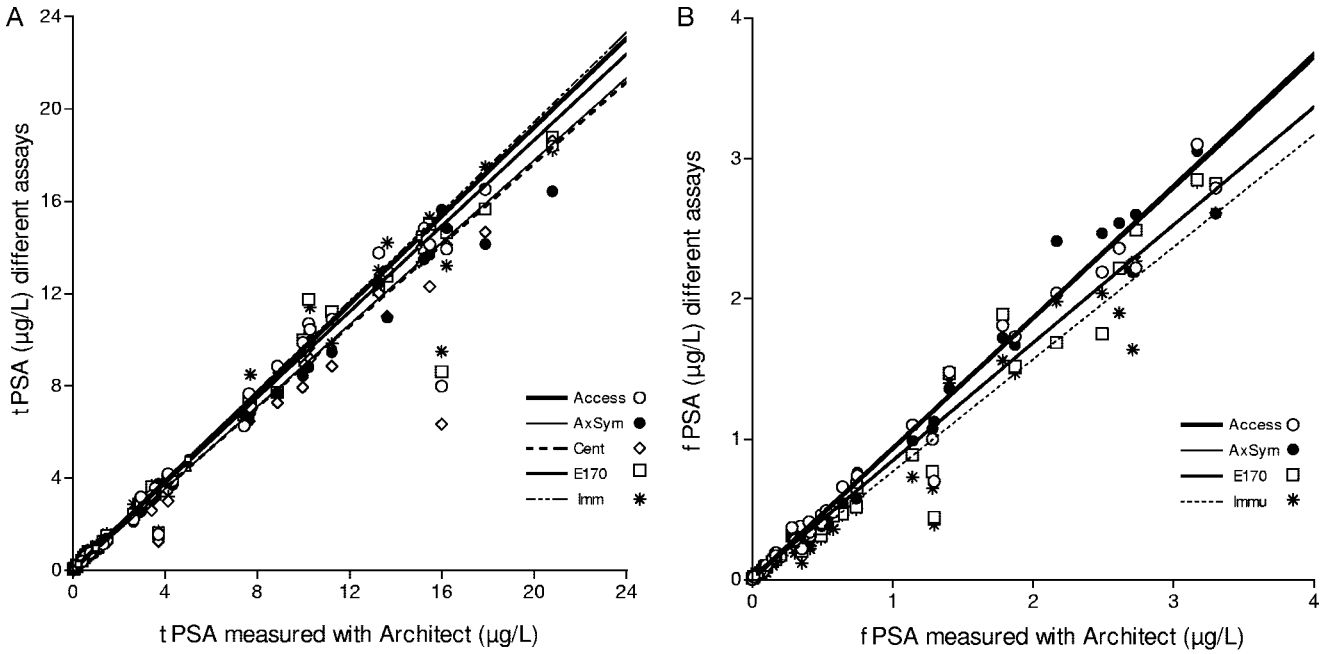

Fig. 2. tPSA (*A*) and fPSA (*B*) in patient sera measured by 6 different assays.

Seventy anonymized surplus samples with tPSA concentrations of <0.1 to 50 µg/L as measured by one method were analyzed for tPSA (5 methods) and fPSA (4 methods). For clarity, the tPSA scale was limited to 24 µg/L. Results >24 µg/L did not deviate from the calculated line. *Cen*, Centaur; *Imm*(*u*), IMMULITE.

Table 5. Characteristics for fPSA/tPSA ratio in patient serum samples between the ARCHITECT and other assays: Passing and Bablok regression.

	Slope (95% CI)[a]	Intercept (95% CI)
Access	0.87 (0.79–0.96)[b]	0.01 (0.00–0.03)
Advia	1.08 (0.93–1.23)	0.06 (0.03–0.09)[c]
AxSYM	1.03 (0.97–1.12)	0.00 (−0.01 to 0.01)
E170	0.79 (0.72–0.85)[b]	0.01 (0.00–0.03)
IMMULITE 2000	0.73 (0.64–0.84)[b]	0.00 (−0.02 to 0.01)

[a] CI, confidence interval.
[b] $P < 0.05$ for difference from 1.00.
[c] $P < 0.05$ for difference from 0.00

measured tPSA and fPSA in serum pools

The values for serum pools measured by various methods plotted against the arbitrarily assigned PSA values by the in-house method are shown in Table 4. Slopes of the regression lines varied between 0.97 and 1.19, and the intercepts ranged from 0.23 to 0.12.

patient serum samples

The relationships between the assays for tPSA and fPSA in patient serum samples are shown in Fig. 2. Seventy patient specimens were tested, those with tPSA <24 μg/L and fPSA <20 μg/L are shown in Fig. 2. When the results were plotted against those obtained with the ARCHITECT assay, the slopes varied between 0.88 and 0.97, whereas all r^2 values were >0.983. Results (Bland–Altman plots) obtained by various assays showed substantial differences; i.e., 0.5 to 1.0 μg/L for tPSA and 0.12 to 0.40 μg/L for fPSA. The data for tPSA are illustrated in Fig. S1 and the data for fPSA in Fig. S2 of the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol52/issue8/.

Passing and Bablok regression characteristics for fPSA/tPSA ratios in patient serum samples, comparing the ARCHITECT with the other 5 assays, are shown in Table 5. The values obtained with the original calibrator were recalculated against the WHO 96/670 reference preparation.

Passing and Bablok regression of the recalculated values against those obtained with the original calibration showed >20% deviation from the ideal slope for the Access assay, whereas for all other methods the deviation was <7%.

fPSA/tPSA ratio in serum and buffer

The values for the fPSA/tPSA ratio measured in the WHO 96/670 reference preparation and the serum pools with arbitrarily assigned values are presented in Table 6.

Discussion

This study demonstrates that overall the results of the tested assays agreed well and that all gave PSA results close to the WHO 96/670 reference preparation value. Lot-to-lot differences were not accounted for, however, as all measurements were made with a single lot of each reagent.

Results for tPSA appears to be less diverse than those for fPSA (Figs. 1 and 2). This obviously has consequences for the intermethod differences in the fPSA/tPSA ratio.

The differences between assays were larger when the WHO reference preparation was analyzed than when the assays were used for clinical samples. This may indicate that the assays are fine-tuned to yield comparable results on actual specimens. In addition it demonstrates that standardizing against an international standard does not necessarily mean that the results will agree for specimens.

Table 6. fPSA/tPSA ratios for different assays measured in WHO 96/670 reference preparation buffered solution and in independent patient serum pools with assigned expected tPSA values.

	fPSA/tPSA ratio in WHO 96/670 reference preparation buffered solution				
	<0.2 μg/L PSA	2.5 μg/L PSA	4 μg/L PSA	10 μg/L PSA	20 μg/L PSA
Access	0.16	0.16	0.15	0.15	0.14
ARCHITECT	0.14	0.13	0.13	0.13	0.13
AxSYM	0.11	0.11	0.10	0.10	0.10
Centaur[a]	0.14	0.12	0.15	0.14	0.15
E170	0.10	0.10	0.11	0.11	0.11
IMMULITE 2000	NA[b]	0.10	0.11	0.11	0.11
	fPSA/tPSA ratio in patient serum pools				
	<0.2 μg/L PSA	3 μg/L PSA	5.7 μg/L PSA	10.5 μg/L PSA	21 μg/L PSA
Access	0.09	0.13	0.13	0.13	0.13
ARCHITECT	0.07	0.09	0.10	0.11	0.11
AxSYM	0.12	0.11	0.12	0.12	0.13
Centaur[a]	NA	0.13	0.20	0.17	0.13
E170	0.10	0.11	0.10	0.11	0.12
IMMULITE 2000	NA	0.08	0.08	0.09	0.09

[a] Calculated from tPSA and cPSA.
[b] NA, not applicable.

This is illustrated by the 2 assays from the same manufacturer, which not only are calibrated against the WHO preparation but also use the same monoclonal antibodies. They differ with respect to detection system (chemiluminescence for the ARCHITECT and fluorescence for the AxSYM), microparticle sizes, and incubation times. The differences between Figs. 1 and 2 may also be related, in part, to matrix differences (buffer vs serum) *(15)*. Obviously, correct measurement of patient samples is preferred over correct measurement of a standardized buffer solution.

As shown in Table 6, in the tPSA range from 2.5 to 10 μg/L in the WHO 96/670 reference preparation buffered solution, the fPSA/tPSA ratios for the AxSYM, E170, and IMMULITE 2000 remained within 10% of the expected ratio for the 90:10 WHO reference preparation. Agreement between results for the fPSA/tPSA ratio appears to be dependent on the matrix, however. This confirms that results obtained for fPSA or tPSA from different assays or manufacturers are still not interchangeable.

Because the detection of tPSA in serum is less prone to differences among assays, the effects on the fPSA/tPSA ratio are caused mainly by variation in fPSA. This once again emphasizes the principle and need for assay equimolarity *(11, 14, 16)*. It also demonstrates that the effect of specimen matrix should be taken into account when developing assays.

The Advia Centaur fPSA/tPSA ratio appeared to be higher in buffer than in serum. This may be attributable to either a higher (calculated) fPSA or a lower tPSA. Because the results in Fig. 1A and Fig. 2A show that Advia Centaur tPSA values are comparable to those of other assays, it appears that not all cPSA is detected.

Higher fPSA/tPSA ratios were obtained with the Access. According to the Beckman Access package insert, the Access calibrators for tPSA and fPSA are composed of human PSA with no reference to the WHO 96/670 reference preparation. Further information from Beckman stated that the original Hybritech Tandem R calibration was based on an internal reference preparation of purified human PSA, which yielded 16% higher PSA results than the WHO 96/670 calibration. This may also explain the difference from an ideal slope in the Passing and Bablok regression when the original values were plotted against the results obtained by recalculation to the WHO reference preparation.

The fPSA/tPSA results obtained in serum with the IMMULITE 2000 were lower than those obtained with other methods. In serum this method gave the highest tPSA results (Fig. 2A) and the lowest fPSA results (Fig. 2B). Because this is in contrast to the results obtained with the WHO reference preparation in buffer, this method also appears to show a matrix effect. Assay design may also play a role because the IMMULITE total PSA has a polyclonal-monoclonal design and the free PSA is a monoclonal-polyclonal assay.

Both the ARCHITECT and AxSYM yielded higher values for 2 samples. After we diluted the samples, the differences remained, which led us to conclude that the Abbott assays probably bind to a different site on PSA, which may not be recognized equally well by other antibodies. Alternatively, these specimens may contain aberrant PSA forms *(17)*.

In conclusion, the differences among PSA assays appear to have decreased since introduction of the WHO 96/670 reference preparation, but the results obtained with different assays are not yet interchangeable *(18, 19)*. Taking into account the clinical use of the fPSA/tPSA ratio, however, it is imperative that further efforts are undertaken to optimize assays to yield more comparable results for fPSA. Future steps toward PSA harmonization may include, for example, development of a reference method. Until then, monitoring of patients should be done with one method only. When a new assay must be used during monitoring, the results of the 2 assays may need to be compared on the same sample from each patient.

We thank Abbott Diagnostics for providing us with the WHO PSA 96/670 reference preparation and reagents with no further obligations.

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