Interchangeability of Measurements of Total and Free Prostate-Specific Antigen in Serum with 5 Frequently Used Assay Combinations: An Update

CARSTEN STEPHAN,† MORITZ KLAAS,† CHRISTIAN MÜLLER, DIETMAR SCHNORR, STEFAN A. LOENING, and KLAUS JUNG

Background: The comparability of total and free prostate-specific antigen (tPSA and fPSA) results among commercial PSA assays has been suggested to be improved by calibration to WHO PSA reference materials and the development of equimolar-response assays. To characterize the current situation, we assessed 5 frequently used commercial assay combinations for tPSA and fPSA regarding the interchangeability of the PSA values and the ratio of fPSA to tPSA (%fPSA), equimolar characteristics, and diagnostic accuracy.

Methods: Sera from 314 patients with prostate cancer (PCa) and 282 men with no evidence of prostate cancer (NPCa) were measured with tPSA and fPSA assays from Abbott (AxSYM), Beckman Coulter (Access), Diagnostic Products Corporation (Immulite 2000), and Roche (Elecsys 2010) and with tPSA and complexed PSA (cPSA) assays from Bayer (ADVIA Centaur).

Results: Method comparisons (Passing and Bablok regressions; Bland–Altman plots) showed assay-dependent results for tPSA, fPSA, and %fPSA. With the Access tPSA values taken as 100%, tPSA concentrations varied from 87% (AxSYM and ADVIA Centaur) to 115% (Immulite), leading to different numbers of patients classified according to the commonly recommended tPSA cutoffs for performing a biopsy. Different %fPSA values also led to assay-dependent ROC analysis results, a finding that shows the importance for the diagnostic accuracy.

Conclusion: Interchangeability of tPSA, fPSA, and %fPSA values obtained by commercial PSA assays remains inadequate, but attention to this issue may minimize the misinterpretation of PSA results obtained by different assays.

Different assays for concentrations of total and free prostate-specific antigen (tPSA and fPSA) provide discordant results [reviewed in Ref. (1)]. These assay-dependent variations could lead to misinterpretation of individual PSA values and erroneous clinical decisions about prostate carcinoma (PCa). Nonuniform assay calibration and nonequimolar detection of the various PSA forms are possible reasons for these discordant results (1–6). PSA reference materials compiled by the WHO and equimolar-response assays were developed to adjust PSA calibration (7–12), but since their introduction, several manufacturers (e.g., Beckman Coulter, Roche Diagnostics, and Diagnostic Products Corp.) have changed their assay platforms, and the few available comparison studies, with limited numbers of assays and small numbers of samples, have not fully characterized the current situation (13, 14). Therefore, focusing on the clinically important tPSA range up to 10 μg/L, we evaluated and characterized 5 frequently used fPSA and tPSA assay combinations with regard to the interchangeability of PSA values among the assays, their equimolar characteristics, and the diagnostic accuracy, estimated by ROC analyses, particularly of the percentage ratios of fPSA to tPSA (%fPSA).

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<table>
<thead>
<tr>
<th>Assay</th>
<th>tPSA, µg/L</th>
<th>fPSA, µg/L</th>
<th>%fPSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Access</td>
<td>Median: 5.14 (4.88–5.54)</td>
<td>0.65 (0.61–0.69)</td>
<td>14.5 (13.7–15.4)</td>
</tr>
<tr>
<td>AxSYM</td>
<td>4.6 (4.26–4.89)</td>
<td>0.70 (0.68–0.74)</td>
<td>17.9 (16.9–18.8)</td>
</tr>
<tr>
<td>Centaur</td>
<td>4.61 (4.30–4.84)</td>
<td>0.68 (0.63–0.71)</td>
<td>17.7 (16.5–18.9)</td>
</tr>
<tr>
<td>Immulite</td>
<td>5.70 (5.40–6.06)</td>
<td>0.58 (0.54–0.60)</td>
<td>11.4 (10.7–12.1)</td>
</tr>
<tr>
<td>Elecsys</td>
<td>5.48 (5.10–5.71)</td>
<td>0.63 (0.61–0.66)</td>
<td>13.8 (12.9–14.5)</td>
</tr>
</tbody>
</table>

### Method Comparison of the Various Assays for tPSA, fPSA, and %fPSA with Reference to the Access Hybritech Assays Charactrized by the Regression Equations According to Passing and Bablok

<table>
<thead>
<tr>
<th>Assay</th>
<th>Median</th>
<th>Slope</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Access</td>
<td>5.14</td>
<td>1.00</td>
<td>0.00</td>
</tr>
<tr>
<td>AxSYM</td>
<td>4.6</td>
<td>0.87</td>
<td>-0.04</td>
</tr>
<tr>
<td>Centaur</td>
<td>4.61</td>
<td>0.87</td>
<td>0.11</td>
</tr>
<tr>
<td>Immulite</td>
<td>5.70</td>
<td>1.15</td>
<td>-0.04</td>
</tr>
<tr>
<td>Elecsys</td>
<td>5.48</td>
<td>1.01</td>
<td>0.15</td>
</tr>
</tbody>
</table>

### Notes
- Values in parentheses are 95% confidence intervals.
- Median values for all assays (tPSA, fPSA, %fPSA) were significantly different from the Access Hybritech assay values (Wilcoxon test of paired samples, \( P < 0.0001 \)) except for fPSA measured by ADVIA Centaur for all patients and for PCa patients \( P < 0.020 \) and \( < 0.0287 \), respectively, whereas fPSA values for the NPCa patients measured with the ADVIA Centaur and with the Elecsys was not significantly different \( P = 0.320 \) and 0.130, respectively.
- The tPSA values were transformed into fPSA concentrations by use of the equation fPSA = tPSA - cPSA.
- %fPSA values were calculated as percentage ratios of fPSA to tPSA.
Materials and Methods

STUDY GROUPS AND SAMPLES

We investigated archival sera collected between February 2001 and June 2004 from 596 untreated white men with tPSA concentrations of 0.49–10 μg/L (median, 5.14 μg/L) as determined with the Access Hybritech PSA system (Beckman Coulter). Only patients with at least 3 unthawed serum specimens were included in this retrospective study. The patients were classified into 2 groups: those with histologically confirmed PCs (314 men; median age, 66 years; range, 38–85 years) and those with no evidence of PCs on prostate biopsy (NPCa; 282 men; median age, 63 years; range, 43–79 years). Blood samples were collected in evacuated tubes (Sarstedt GmbH) in the Department of Urology or its outpatient division at the University Hospital Charité. The samples were taken before any diagnostic or therapeutic procedures involving the prostate and at least 4 weeks after digital rectal examination, prostatic biopsy, or transrectal ultrasound. Blood samples were allowed to clot for 1 h at room temperature and then were centrifuged at 1600 g for 15 min at 4 °C. Sera were stored at −80 °C until analyzed. After thawing at room temperature, samples were processed within 3 h. The study was carried out in accordance with the standards of the local ethics board and the Helsinki Declaration of 1975 as revised in 1996, including informed consent obtained from all participants.

PSA ASSAYS AND MEASUREMENTS

We performed measurements in June to August 2004 in series of up to 30 samples, according to the manufacturers’ instructions on the following analyzers: AxSYM (Abbott; tPSA, cat. no. 3C19-20; fPSA, cat. no. 3C 20-20), Elecsys 2010 (Roche Diagnostics; tPSA, cat. no. 11731262; fPSA, cat. no. 03289788), and ADVIA Centaur [Bayer Diagnostics; tPSA, cat. no. 118157; complexed PSA (cPSA), cat. no. 124830] on the same day as well as on the Access (Beckman Coulter; Hybritech PSA, cat. no. 37200; Hybritech fPSA, cat. no. 37210) and Immulite 2000 systems (Diagnostic Products Corp.; PSA, cat. no. L2KPS6; fPSA, cat. no. L2KPF2) on another day. The cPSA values were transformed into fPSA concentrations by use of the equation fPSA = tPSA − cPSA, and the %fPSA values were calculated as percentage ratios of fPSA to tPSA. The between-run imprecision profiles of the measurements were estimated by use of control materials supplied by the manufacturers, commercial control materials, and in-house serum pools; all interassay CVs were <8% (n = 17–20 days).

STATISTICAL ANALYSIS

Data were analyzed with MedCalc (Ver. 8.1) and GraphPad Prism (Ver. 4.03 for Windows). Molar response plots to characterize the equimolarity of the assays were prepared according to Semjonow et al. (11). The Access Hybritech assay was used as the comparison method because its equimolarity is well characterized (11, 15). Significance was defined as P < 0.05.

Results and Discussion

Calculations were made for all patients together and for the 2 groups (PCa and NPCa) separately to show potential differences depending on the clinical situation. The data are summarized in Tables 1 and 2. The results of the method regression analyses according to Passing and Bablok (16) and the median values obtained with the various assays showed considerable differences among the assays (Table 1). These findings are illustrated in Figs. S1 and S2 [with the regression analyses and the Bland–Altman plots (17)], respectively, of the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol52/issue1/). Characteristic examples of the percentage difference plots of the %fPSA results of the various PSA assays are shown in Fig. 1. When we set tPSA values obtained with the Access

Table 2. Diagnostic performance data given as numbers of patients in relation to the conventional tPSA threshold of 4 μg/L, cutoffs at 90% sensitivity and 90% specificity, and areas under the ROC curves for the various tPSA and %fPSA assays. 

<table>
<thead>
<tr>
<th>tPSA</th>
<th>Access</th>
<th>AxSYM</th>
<th>Centaur</th>
<th>Immulite</th>
<th>Elecsys</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPCa &gt;4 μg/L</td>
<td>139</td>
<td>117</td>
<td>120</td>
<td>144</td>
<td>139</td>
</tr>
<tr>
<td>PCa &lt;4 μg/L</td>
<td>68</td>
<td>86</td>
<td>93</td>
<td>46</td>
<td>39</td>
</tr>
<tr>
<td>Cutoff at 90% sensitivity, μg/L</td>
<td>2.82 (2.40–3.40)</td>
<td>2.60 (2.12–3.04)</td>
<td>2.52 (2.12–3.07)</td>
<td>3.12 (2.68–3.75)</td>
<td>3.02 (2.48–3.54)</td>
</tr>
<tr>
<td>Cutoff at 90% specificity, μg/L</td>
<td>7.68 (7.21–8.21)</td>
<td>6.71 (6.20–7.05)</td>
<td>6.67 (6.26–7.32)</td>
<td>8.70 (8.33–9.15)</td>
<td>8.04 (7.38–8.60)</td>
</tr>
<tr>
<td>Area under the ROC curve</td>
<td>0.70 (0.66–0.74)</td>
<td>0.72 (0.68–0.75)</td>
<td>0.71 (0.67–0.75)</td>
<td>0.71 (0.68–0.75)</td>
<td>0.70 (0.66–0.74)</td>
</tr>
<tr>
<td>%fPSA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutoff at 90% sensitivity, %</td>
<td>18.7 (17.2–20.0)</td>
<td>24.2 (22.0–25.7)</td>
<td>24.1 (21.8–25.8)</td>
<td>15.4 (14.5–17.1)</td>
<td>17.8 (17.0–19.1)</td>
</tr>
<tr>
<td>Cutoff at 90% specificity, %</td>
<td>10.2 (9.39–11.7)</td>
<td>12.8 (11.5–14.2)</td>
<td>9.94 (8.73–12.9)</td>
<td>8.50 (7.53–9.35)</td>
<td>9.94 (8.80–10.7)</td>
</tr>
<tr>
<td>Area under the ROC curve</td>
<td>0.81 (0.78–0.84)</td>
<td>0.80 (0.77–0.83)</td>
<td>0.77 (0.74–0.81)</td>
<td>0.81 (0.77–0.84)</td>
<td>0.79 (0.75–0.82)</td>
</tr>
</tbody>
</table>

* Values in parentheses are 95% confidence intervals.

** Number of the 314 PCa patients with tPSA values below the conventional threshold of 4 μg/L.

*** The cPSA values transformed into fPSA concentrations by use of the equation fPSA = tPSA − cPSA were used to calculate %fPSA values.

**** Only the area obtained with the ADVIA Centaur assay was significantly different from the Access Hybritech value (pairwise comparison, P = 0.006).
Hybritech as 100%, tPSA concentrations varied from 87% (AxSYM and ADVIA Centaur) to 115% (Immulite).

When we used as cutoff a tPSA value of 4 μg/L, a limit commonly used for making the clinical decision to perform a prostate biopsy, the different assays classified distinctly different numbers of PCA or NPCA patients as either false negative or false positive (Table 2). For example, the Immulite and ADVIA Centaur systems did not give positive results for 46 and 93 of the 314 PCA patients, respectively, and falsely classified 144 and 120, respectively, of the 282 NPCA men as positive. If we had chosen threshold values lower than 4 μg/L, as recommended recently (18–20), similar differences would have occurred. ROC analyses for tPSA measured with the various assays (areas under the curves given in Table 2) did not reveal these clinically significant differences, suggesting that the differences were related to calibration differences that affect the cut point.

fPSA and tPSA values differed inversely from the comparison method in some pairs of assays. For example, fPSA values obtained with the AxSYM were higher and Immulite fPSA values were lower than the Access Hybritech fPSA values, compared with the tPSA values. Thus, %fPSA values were clearly assay dependent, as indicated by the nonoverlapping 95% confidence intervals of the medians (Table 1), by the cutoffs at 90% sensitivity and specificity, and by the different ROC analysis results (Table 2). This finding is of particular clinical significance because the %fPSA values are generally used as tools to differentiate between patients with malignant and benign prostate diseases in the tPSA concentration gray zone of 2–10 μg/L. In addition, the differences in slopes between...
the PCa and NPCa patients for the calculated fPSA values and \%fPSA obtained with the ADVIA Centaur assay (Table 1) are considerable.

The molar response plots in Fig. 2 indicate that the AxSYM and ADVIA Centaur tPSA assays measured free and bound PSA in an equimolar fashion (Fig. 2, A and B). The tPSA assays of Immulite and Elecsys showed non-equimolar characteristics with a slope significantly different from zero (Fig. 2, C and D). These differences were not shown in previous studies with fewer serum samples (21) or with experiments using the WHO reference materials (13). For samples with \%fPSA values <25%, the responses obtained with the Immulite and Elecsys tPSA assays were equimolar, as demonstrated by the slopes of the regression lines, which did not differ significantly from zero (\(P = 0.105\) and 0.099 for Immulite and Elecsys).

Our study documents the comparability among PSA assays from several manufacturers. The manufacturers generally claim that their PSA assays are calibrated against the WHO PSA reference material and that equimolar assays have been applied, procedures that should decrease the differences between assays (8, 10–12). Nevertheless, we found that the interassay variability could not be eliminated and that there was inadequate interchangeability of results. Although the study group was not representative for the prevalence of PCa in the population, the abbreviated diagnostic evaluation of the results also shows that this analytical limitation continues.

Fig. 2. Molar response plots for total PSA assays.
Percentage ratios of the total PSA concentrations obtained with the respective assays to the corresponding concentrations measured with the comparison method (Access Hybritech) are plotted against the \%fPSA values obtained with the Access Hybritech assay. Assays with equimolar characteristics (A and B) show horizontal regression lines with slopes not significantly different from zero. Lines with positive slopes (C and D) indicate assays that overestimate fPSA. The equations of the regression lines (95% confidence intervals of the intercepts and slopes in parentheses) and the deviations of the slopes from zero with the corresponding \(P\) values are as follows: AxSYM (A), \(y = -0.04 (-0.08 to 0.15)x + 88.7 (86.5–90.9\%) (P = 0.55)\); ADVIA Centaur (B), \(y = 0.11 (-0.01 to 0.24)x + 88.0 (85.7–90.2\%) (P = 0.08)\); Immulite (C), \(y = 0.33 (0.16–0.51)x + 108 (105–111\%) (P = 0.0002)\); Elecsys (D), \(y = 0.40 (0.26–0.53)x + 98.4 (95.9–100\%) (P < 0.0001)\).
to have a severe impact on the clinical decision of whether to perform a prostate biopsy. Similar results were demonstrated in 2 recent studies (14, 22) and a population-based simulation study (23). To show the situation as it is, we did not recalibrate the assays using a common calibrant. According to our findings, the goal of assay-independent, interchangeable fPSA and tPSA results has not been achieved and may be unrealizable because of PSA heterogeneity, including structural diversity depending on malignant or benign origin, different analytical conditions based on the use of numerous antibodies with different epitope specificities and affinities (24), and the different technical principles underlying the various analyzers. To minimize the misinterpretation of PSA results obtained by different assays, clinical chemists should alert clinicians to the variation in assay results as well as the biological variation of PSA (25).

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