We compared two recently introduced commercial assays (CanAg and Immulite) for measuring free prostate-specific antigen (f-PSA), total PSA (t-PSA), and the ratio of t-PSA/ f-PSA (f-PSA%) in control materials and sera of 54 healthy men, 50 patients with benign prostatic hyperplasia (BPH), and 45 patients with prostate cancer (PCa). The lower detection limits for f-PSA were 0.038 µg/L and 0.004 µg/L for the CanAg and Immulite assays, respectively. The within-run and between-day precisions of the Immulite assay were <5%; the CanAg assay showed a poorer precision. Whereas f-PSA values differed between controls and patients but not between BPH and PCa patients, the f-PSA% values were lower in PCa patients than in BPH patients and controls. The receiver-operating characteristic (ROC) curve showed an improved diagnostic power of f-PSA% compared with t-PSA to discriminate between BPH and PCa. Discrimination limits of 16% (CanAg assay) and 15% (Immulite assay) are recommended for f-PSA%.

INDEXING TERMS: prostate cancer • benign prostatic hyperplasia • α1-antichymotrypsin • method comparison study • ROC analysis

Serum prostate-specific antigen (PSA) exists in different molecular forms [1]. Most (~80%) of the PSA in healthy men is complexed to the protease inhibitors α1-antichymotrypsin (ACT), α1-macroglobulin, and antitrypsin; a smaller amount is free and noncomplexed (f-PSA). However, patients with prostate cancer (PCa) have a lower proportion of f-PSA than patients with benign prostatic hyperplasia (BPH) [1–6]. It has been suggested that the ratio f-PSA/total (t-) PSA (f-PSA%) allows a better discrimination between BPH and PCa compared with t-PSA [7–10]. However, all the data concerning this problem are essentially based on investigations performed by a few cooperative groups using in-house tests. Commercial test kits for measuring f-PSA are only now being offered by several companies. Commercial kits for the determination of t-PSA include IRMAs (Hybritech; Immunobiological Labs.), enzyme immunometric assays (CanAg Diagnostics), or chemiluminescent enzyme immunometric assays (Diagnostic Products Corp.). t-PSA values determined by different commercial kits are not always compatible [11, 12]. Several factors such as the use of different antibodies, calibrators, and test design have been discussed as reasons for this unsatisfactory situation [11–13]. However, until now, there have been no reports on the analytical and clinical reliability of commercially available tests for measuring f-PSA, and no studies have addressed the issue of whether the results obtained by different tests are comparable. The present study aims to compare a largely manually performed test based on the microtiter plate technique (CanAg® PSA) with a test performed as an automated assay (Immulite® Free PSA) on an analyzer with regard to their analytical and clinical performances.

Materials and Methods

PSA assays. We used the enzyme immunometric assay CanAg PSA (CanAg Diagnostics, Gothenburg, Sweden) and the chemiluminescent enzyme immunoassay Immulite PSA (Diagnostic Products Corp., Los Angeles, CA) for both t-PSA and f-PSA determinations and the AxSym® PSA assay (Abbott Labs., Abbott Park, IL) as a comparative method for determining t-PSA.

The CanAg PSA assay is based on the direct sandwich technique. The microtiter plates are coated with streptavidin, and 25 µL (for determining t-PSA) or 50 µL (for f-PSA) of the samples (calibrators, patients' samples, control materials) are incubated with 100 µL of biotinylated anti-PSA monoclonal antibodies directed against either t-PSA (free and ACT-complexed PSA) or against f-PSA alone for 1 h at room temperature with constant shaking of the plate. By this procedure, t-PSA or only f-PSA in the sample is bound as an antibody sandwich complex. The wells are then emptied, filled and emptied three times...
times with the washing buffer supplied, and incubated as described above with 100 μL of monoclonal anti-PSA antibodies labeled with horseradish peroxidase. After washing and drying the plates, 100 μL of substrate/chromogen reagent (hydrogen peroxide and 3,3′,5,5′-tetramethylbenzidine) is added to each well and incubated with constant shaking for 30 min. The reaction is stopped by adding 100 μL of 120 mmol/L HCl and the absorbance measured within 30 min at 405 nm. We used the Anthos HTII microplate reader (Anthos Labtec Instruments, Salzburg, Austria) and calculated the concentration by the cubic-spline method (EIA/KIN-Star software, version 7.0; WPAH-MED, Berlin, Germany).

The Immulite PSA and Free PSA assays (cat. nos. LKPS1 and LKPF1) are solid-phase, two-site sequential chemiluminescent immunometric tests and are automatically handled on the Immulite Automated Analyzer. The solid phase is a polystyrene bead coated with polyclonal antibody specific for PSA or with a monoclonal anti-PSA antibody specific only for f-PSA. For determining t-PSA, samples are incubated with alkaline phosphatase-conjugated monoclonal anti-PSA antibody and form a PSA-antibody sandwich complex. After removal of unbound conjugate by centrifugal wash, the chemiluminescent substrate, a phosphate ester of adamantyl dioctetane, is added. In the presence of alkaline phosphatase, that substrate is cleaved into an unstable intermediate, emitting photons that are measured by the luminometer of the analyzer. For determining f-PSA, f-PSA is bound to the polystyrene bead during the first incubation period and an alkaline phosphatase-labeled polyclonal goat anti-PSA antibody is bound to that formed complex of f-PSA/anti-f-PSA antibody in a second incubation cycle. The following steps correspond to those described for the determination of t-PSA.

**Analytical evaluation.** The analytical performance was assessed by evaluating the detection limit, within-run and between-day precisions, and accuracy by studying the linearity of dilution and the recovery rate. In addition, we performed comparison-of-methods studies. The detection limits of the two assay systems were determined by two approaches. We performed (a) 20 replicate intraassay determinations of the zero calibrator of the respective assay and (b) 10 replicate intraassay determinations of a female serum pool previously determined to be free of PSA and calculated the lower detection limits on the basis of the mean value of absorbances +2 SD. Precision controls were performed as within-run precision and between-day precision with control materials and human pooled sera. For linearity testing, serum samples were serially diluted with the diluent supplied with the respective test. For measuring the analytical recovery rate, we added known amounts of serum PSA to female serum free of PSA and to pooled serum from healthy subjects. PSA concentrations were measured and the percentage recovery rates were calculated.

**Study materials and blood sampling.** The study was performed with retrospective sera collected within 8 months. Blood samples were taken before digital rectal examination and instrumentation, and centrifuged at 1500g for 10 min after allowing the blood to clot for 1 h at room temperature. The sera were frozen at −80 °C within 2 h after collection and were not thawed (and refrozen) before testing. These procedures of sampling and storage exclude as much as possible such analytical difficulties as the loss of PSA immunoreactivity or the dissociation of the PSA-ACT complex [14, 15]. Samples from a total of 149 men from the following groups were investigated: apparently healthy subjects (n = 54; median age 54, range 20–80 years), patients with BPH (n = 50; median age 71, range 54–89 years), and patients with PCa (n = 45; median age 66, range 49–87 years). The diagnosis of BPH was based on histological analysis of tissue obtained by transurethral resection of the prostate. The diagnosis of PCa was established histopathologically by microscopic examination of prostatic specimens after radical prostatectomy. Clinical staging was made according to the TNM system, including surgical assessment of lymph node status. No patients had metastases and were staged as T1, 2, 3pN0M0. The procedures followed were approved by the ethical standards committee of the hospital.

The assay evaluation of the two tests also included sera from healthy female volunteers and control materials (Lyphochem Immunoassay Controls, levels 1, 2, and 3 from Bio-Rad, Anaheim, CA; TMCO Controls from Diagnostic Products Corp.). The control materials were dissolved according to the manufacturers’ instructions, aliquoted, and stored at −80 °C until use.

**Statistical calculations.** All statistical calculations [Mann–Whitney U-test, Wilcoxon signed rank test of paired data, variance analysis (ANOVA), Spearman rank correlation coefficient (r)] were carried out with the statistical package Statgraphics, version 5.1 (Statistical Graphics Corp., Rockville, MD). Method comparisons were performed according to Passing and Bablok [16]. The diagnostic test accuracy of each indicator was evaluated by receiver-operating characteristic (ROC) curve analysis [17]. The GraphROC for Windows software was used for calculations [18].

**RESULTS**

**ANALYTICAL EVALUATION**

**Detection limit.** The lower limits of detection of the CanAg PSA assays calculated from 20 replicate determinations of the zero calibrator were 0.11 μg/L for t-PSA and 0.038 μg/L for f-PSA. Respective values of 0.15 μg/L and 0.08 μg/L were estimated for the PSA-free female serum pool. The corresponding data from the Immulite assays were 0.019 μg/L for t-PSA and 0.004 μg/L for f-PSA with the zero calibrator, and 0.016 μg/L and 0.014 μg/L, respectively, with female serum.

**Precision.** The within-run and between-day precision data were determined with control materials and pooled sera (n = 10). Fig. 1 synoptically illustrates the between-day imprecision profiles of t-PSA and f-PSA determinations obtained with the two assays. The Immulite assays showed a better imprecision profile for both t-PSA and f-PSA. The data for within-run precision were naturally better, but comparable (not shown here).
Method comparison. First, because the two new assays have not been generally used, we correlated the t-PSA concentrations of the Immulite and CanAg tests with the Food and Drug Administration (FDA)-approved AxSym test. The relations calculated as regression lines according to Passing and Bablok [16] were calculated separately for the three study groups. Because the slopes and intercepts were not different between the groups, we calculated the regression lines for all 149 samples combined. The t-PSA concentrations measured by the AxSym test showed the following relations to the other two tests (95% confidence intervals indicated in parentheses): \( y_{\text{AxSym}} = -0.072 (-0.134 \text{ to } -0.011) + 0.869 (0.845-0.869) x_{\text{Immulite}} \), \( r = 0.967 \); and \( y_{\text{AxSym}} = -0.169 (-0.055 \text{ to } -0.256) + 0.952 (0.914-0.997) x_{\text{CanAg}} \), \( r = 0.945 \). Thus, the Immulite t-PSA values and CanAg t-PSA values were about 13% and 5% higher, respectively, than the t-PSA values obtained with the AxSym assay. These results correspond to data frequently reported in PSA assay comparisons [11-13, 19]. In general, the Abbott IMx method, which corresponds to the Abbott AxSym method, has yielded PSA values lower than the Hybritech Tandem methods often used as standard tests for PSA determination [20]. However, lot-to-lot variations of the Abbott reagents explain that the PSA values measured by the Hybritech and Abbott methods may be lower or higher, but also equivalent [20]. This has to be considered when comparing the t-PSA values obtained by the three methods used in our study.

Table 1 shows the characteristic data of the comparison between the Immulite and CanAg assays. The slopes prove that the Immulite assays give both higher t-PSA concentrations and lower f-PSA concentrations than the CanAg assays. Significant differences between the three groups studied are evident. The two assays reveal small correlation coefficients for comparing f-PSA concentrations and f-PSA% values.

### CLINICAL EVALUATION

#### t-PSA, f-PSA, and f-PSA% in the study groups.

The present study included only subjects with t-PSA concentrations up to 20 µg/L, as measured with the FDA-approved AxSym test. Of the 149 subjects studied, 86 had t-PSA values between 0 and 4 µg/L, 44 between 4.1 and 10 µg/L, and 19 between 10.1 and 20 µg/L. The results of t-PSA, f-PSA, and the calculated f-PSA/t-PSA ratios, as f-PSA%, between the groups and measured by the two assay methods, are presented in Table 2. Significant differences between the groups were demonstrated by ANOVA. Although

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**Table 1. Relations of Immulite t-PSA, f-PSA, and f-PSA% values to the corresponding CanAg PSA values calculated by the regression procedure of Passing and Bablok [16].**

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>Slope</th>
<th>y-intercept, µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total PSA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.923</td>
<td>1.11^a</td>
<td>0.24^b</td>
</tr>
<tr>
<td>BPH</td>
<td>0.949</td>
<td>1.10^a</td>
<td>0.16</td>
</tr>
<tr>
<td>PCa</td>
<td>0.953</td>
<td>1.12^a</td>
<td>0.43^a</td>
</tr>
<tr>
<td>Free PSA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.727</td>
<td>0.63^a</td>
<td>0.11^b</td>
</tr>
<tr>
<td>BPH</td>
<td>0.851</td>
<td>0.86^a-c</td>
<td>0.08</td>
</tr>
<tr>
<td>PCa</td>
<td>0.934</td>
<td>0.60^a</td>
<td>0.19^a</td>
</tr>
<tr>
<td>Free PSAs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.761</td>
<td>0.47^a</td>
<td>7.49^b</td>
</tr>
<tr>
<td>BPH</td>
<td>0.707</td>
<td>0.95^a</td>
<td>-0.85</td>
</tr>
<tr>
<td>PCa</td>
<td>0.772</td>
<td>0.65^a</td>
<td>1.80^a</td>
</tr>
</tbody>
</table>

* Controls, n = 54; BPH, n = 50; PCa, n = 45.
* Significantly different by at least \( P < 0.05 \) from the slope 1 or the intercept 0.
* Significantly different by at least \( P < 0.05 \) from the slopes in the controls and PCa patients.
* Significantly different by at least \( P < 0.05 \) from the intercepts in the BPH and PCa patients.

---

**Fig. 1.** Between-day precision profile of t-PSA (A) and f-PSA (B) measurement with the CanAg assays (C) and Immulite assays (D). Study materials were either pooled human serum or control materials (n = 10).
PCa, as expected, had higher t-PSA concentrations than controls and BPH patients, the f-PSA concentrations were not different in PCa patients and BPH patients. The essential result obtained by both assays was that f-PSA% values were significantly lower in PCa patients than in both controls and BPH patients, whereas the f-PSA% values obtained by both assays were not significantly different (Mann–Whitney U-test) between controls and BPH patients. Despite these differences, the individual f-PSA% values partly overlap between the groups studied (Fig. 2). However, the comparison of the two assays clearly showed that the Immulite assay reported higher t-PSA values and lower f-PSA values than the CanAg assay. These results corresponded to the data obtained by the regression analysis presented above.

The f-PSA values were closely correlated to the t-PSA values (corresponding $r_2$ values: 0.601, 0.858, and 0.686). The f-PSA% values did not depend on age or f-PSA concentrations but were inversely correlated to the t-PSA values ($r_2$ for controls –0.754, for BPH patients –0.470, for PCa patients –0.428).

**ROC analysis and diagnostic validity.** Fig. 3 demonstrates ROC curves of t-PSA, f-PSA, and f-PSA% obtained with the two assays to discriminate between PCa patients and BPH patients. The curves for f-PSA% values run above the curves for t-PSA and f-PSA concentrations. The area under the f-PSA% curve of the Immulite assay (Fig. 3B) is significantly higher than the area under the t-PSA curve, demonstrating a higher discriminating power of f-PSA% than t-PSA ($P < 0.05$). It is obvious that the measurement of f-PSA concentrations alone is without diagnostic significance. The corresponding significance level between the areas under f-PSA% and t-PSA measured by the CanAg assays (Fig. 3A) is $P = 0.087$. If we select the point with the highest diagnostic efficiency (83% for the Immulite assay, 79% for the CanAg assay) as the optimal decision limit, the threshold value is 15% for the Immulite and 16% for the CanAg assay. Table 3 lists the sensitivity, specificity, and predictive values of these two assays at these points comparing the data with t-PSA alone. We used the generally accepted t-PSA threshold value of 4 µg/L for these calculations and additionally applied the indicator combination of t-PSA and f-PSA% (Table 3). The diagnostic specificity and the positive predictive value in detecting PCa patients were essentially improved.


**Fig. 3.** ROC curves of t-PSA (O), f-PSA (A), and f-PSA% (■) obtained with the CanAg assays (A) and Immulite assays (B).

The calculations are based on 45 subjects with PCa and 50 men with BPH. In A, the areas under the curve (and SE) for f-PSA, t-PSA, and f-PSA% were 0.498 (0.059), 0.709 (0.053), and 0.819 (0.043); in B, the corresponding data were 0.528 (0.059), 0.721 (0.053), and 0.872 (0.036).

**Table 3.** Diagnostic validity of t-PSA, f-PSA%, and their combination to discriminate between PCa and BPH patients by using the two test kits.a

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Negative predictive value</th>
<th>Positive predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>t-PSAb</td>
<td>84</td>
<td>89</td>
<td>50</td>
<td>46</td>
</tr>
<tr>
<td>f-PSA%c</td>
<td>76</td>
<td>87</td>
<td>82</td>
<td>78</td>
</tr>
<tr>
<td>t-PSA + f-PSA%d</td>
<td>96</td>
<td>96</td>
<td>90</td>
<td>88</td>
</tr>
</tbody>
</table>

*a The first columns of the respective parameter represent data of the CanAg assay; the second columns the data of the Immulite assay. Data were calculated by using the 50 BPH and 45 PCa patients investigated in our study. All values are in %.

b Value of 4 μg/L was selected as the conventional upper reference limit for both tests.
c f-PSA% limits (16% for the CanAg assay and 15% for the Immulite assay) showing the highest diagnostic efficiency were selected.
d t-PSA (4 μg/L) as first decision criterion and additionally combined with the f-PSA% limits (16% for the CanAg assay and 15% for the Immulite assay) were used.

**Discussion**

PSA has proved to be an invaluable marker in early detection of PCa and in monitoring patients treated either by radical prostatectomy, radiation, or hormones [21, 22]. Since increased PSA concentrations are also found in BPH and inflammatory prostatic diseases, considerable efforts have been undertaken to improve the clinical validity of PSA as a cancer marker. Various concepts have been used to eliminate the interference factors that reduce the diagnostic power of PSA, e.g., the use of age-related reference values, PSA density, and PSA velocity, as well as the quantitative differentiation of the different PSA isoforms [22, 23].

The occurrence of various PSA forms (complexed or free) in BPH and PCa patients was briefly outlined in the introduction. The reasons for the phenomenon of decreased f-PSA/t-PSA have not been clarified until now. It is not yet clear why the PSA occurs as f-PSA despite the high excesses of ACT in serum. On the basis of immunohistochemical results and in situ hybridizations, Bjerrell et al. postulated that PCa cells, in contrast to prostate cells of BPH, synthesize increased amounts of ACT and thus PSA-ACT complexes preferably form early in the cancer cells [24, 25]. They [24, 25] suggested that these cellular differences would also be reflected when PSA is released into the serum and that this could explain the lower ratio of f-PSA/t-PSA in the serum of PCa patients than in patients with BPH.

Recent data suggest that the determination of PSA-ACT complex may be considerably impaired by preanalytical conditions (e.g., storage of samples) [14, 15]. Although PSA-ACT is the major portion of serum PSA apparently directly related to PCa, and experimental data provide additional support for replacing t-PSA assays with assays specific for PSA-ACT [26], those preanalytical problems have led to the argument to abandon the PSA-ACT measurement and to prefer the determination of f-PSA/t-PSA ratio [14]. However, the determination of f-PSA% needs an assay of high analytical sensitivity to f-PSA [27], because f-PSA is the minor portion of serum PSA. Our results show that this requirement is fulfilled by both assays, since the lower detection limits were 0.004 μg/L for the Immulite assay and 0.038 μg/L for the CanAg assay. The corresponding lower detection limits of 0.014 and 0.08 μg/L obtained with the female serum pool can be considered biological detection limits. We believe that the low limits obtained with the zero calibrator solution allow the use of female serum as samples to estimate the biological detection limit. Vessella et
al. [28, 29] introduced the concept of biological detection limit by measuring PSA concentrations at the level of the lower detection limit ± 2 SD. These data, together with the other analytical performance characteristics such as precision, linearity, and analytical recovery, prove that both tests are sufficiently reliable. However, the automated Immulite assays showed significantly better precision than the CanAg test.

Comparison of the two tests by regression analysis (Table 2) demonstrates that values produced by the two systems are not very well compatible. Wu [12], in his review on the nonresolved analytical problems of PSA determination, pointed out that the generally known pitfalls in t-PSA determination must also be considered when measuring f-PSA. It is likely that the insufficient compatibility of PSA values between different commercial assays will be still more distinct if f-PSA% is to be determined, since f-PSA% values result from two different PSA determinations. For example, an accurate determination presupposes an equimolar recording of PSA irrespective of its occurring in the serum in a free or complexed form [30]. Since both assays claim to be equimolar tests, the different slopes of the regression lines of f-PSA and t-PSA suggest that these differences are due to calibration. It is understandable that the compatibility of f-PSA values also needs standardization, as is being discussed for t-PSA measurement [31–34]. Whereas t-PSA values measured by the Immulite assay were higher than the corresponding values of the CanAg assay, f-PSA values showed an inverse behavior. These differences affect the ratio f-PSA/t-PSA. Whatever the definitive reason for these differences may be, they have to be taken into account and, we believe, underline the importance of standardization of PSA determination. From the practical point of view, two essential conclusions have to be drawn: (a) One should use only t-PSA and f-PSA assays from the same manufacturer and not apply f-PSA and t-PSA assays from different manufacturers; (b) cutoff limits of f-PSA% are assay-specific and cannot be transferred from one assay to another.

A few authors were not able to prove the mentioned differences between BPH and PCa patients [35, 36]. Other authors doubt that the concept of complexed and free PSA is a useful diagnostic tool [37]. Assumed reasons for these negative results are methodological difficulties (e.g., quality of antibodies for f-PSA or PSA-ACT complex; detrimental effect of storage on samples) and inadequate consideration of assay-specific cutoff limits of f-PSA/t-PSA or PSA-ACT/t-PSA ratios. Conclusions drawn on the basis of PSA-ACT determinations should be interpreted with caution because PSA-ACT complexes dissociate during storage [14]. This may be one reason why the f-PSA/t-PSA ratio is more sensitive for differentiating PCa and BPH than the PSA-ACT/t-PSA ratio [5]. However, since we froze serum samples at −80 °C within 2 h after collection of blood samples and since we used only samples stored no longer than 8 months, we were able to exclude potential pitfalls such as the loss of PSA immunoreactivity or the dissociation of PSA-ACT complex to the greatest possible extent [14]. We show here for the first time that results described by numerous working teams [1–4, 7, 9, 13, 34, 38] using in-house f-PSA tests were confirmed by the two commercial tests used in our study. Our data prove that PCa patients show a significantly lower ratio of f-PSA/t-PSA compared with BPH patients and healthy men.

In contrast to the early studies on the molecular PSA forms that included patients with a wide spectrum of t-PSA [5, 14], we studied only men with PSA concentrations to 20 µg/L. We believe that such a limitation is well-founded because of known epidemiological data. t-PSA values of >4 µg/L have been generally accepted as a general guideline for biopsy [39]. However, age-dependent reference ranges will be increasingly used [40]. Studies on >6000 men for >50 years showed a positive predictive value of 22.4–26.5% for t-PSA values ranging between 4.1 and 10 µg/L. At PSA values >10 µg/L, positive predictive values were 50–67% [22]. On the other hand, ~20% of patients with positive biopsy results of PCa had t-PSA concentrations <4 µg/L [22]. Upon finishing our experiments, two papers came to our attention in which again results of two studies with noncommercial tests were summarized [8, 9]. These studies included patients with PSA concentrations ≤10 µg/L. The analytical and clinical results reported were similar to those described by us. However, the cutoff limits given in the two papers differ. The data presented in those papers draw attention to the fact that, in addition to the obvious assay-specific dependence of cutoff limits, the respective approach to define the cutoff limits must be taken into account. Whereas Oesterling et al. [8] recommended a normal f-PSA/t-PSA of >0.15 based on the concept of the 95th percentiles, Catalona et al. [9] selected the f-PSA% cutoff to maintain at least 90% sensitivity for PCa detection. The value depended on the size of the gland, such that in men with an enlarged gland, a f-PSA% cutoff value of 23.4% was recommended [9]. We used another approach and selected as cutoff limits the points with the highest diagnostic efficiency to obtain both high diagnostic sensitivity and high diagnostic specificity. These limits were 15% for the Immulite assay and 16% for the CanAg assay. When we used these limits alone or in combination with t-PSA, all diagnostic indicators improved compared with t-PSA alone. Thus, whatever approach was selected for establishing cutoff limits for f-PSA%, numerous recent results have shown that the determination of f-PSA% improves the specificity for PCa detection. Our results demonstrate that the specificity and the positive predictive value increased by ~20–40% (Table 3). Measurement of f-PSA% may reduce the number of prostate biopsies required and may be useful in further improving the evaluation of patients at risk for PCa [8, 9].

In conclusion, our data have proven that the two commercial test kits for measuring f-PSA—the largely manually performed CanAg assay and the automated Immulite assay—warrant comparable analytical performance and diagnostic validity. Both are suited to improve the specific detection of PCa and support incorporating the f-PSA% measurement into the diagnostic program for the detection of PCa. Various other commercial assays for the measurement of f-PSA have been already introduced into the market or will be available in the near future. However, clinicians and clinical chemists should be aware that these tests need to be carefully tested, and that assay-specific
cutoff limits must be used, because the standardization of f-PSA and t-PSA measurements has not yet been done.

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