Determination of Prostate-Specific Antigen in Serum by Immunoradiometric Assay


A better understanding is needed of the role of pre-analytical factors if prostate-specific antigen (PSA) is to be reliably used as a tumor marker. Reports on the analytical performance of TANDEM-R PSA (Hybritech, Inc., San Diego, CA) differ considerably with respect to detection limit and imprecision, differences that might be due (e.g.) to the use of different control matrices, to between-batch variations in reagent composition, or to nonrobustness of the assay. During nine months we determined PSA in 966 serum samples from 728 male urology patients and 54 samples from women (25 assay runs). As controls we used two serum pools with low PSA concentration and two widely used commercial controls. The within-assay CV for patients' samples was similar to that found with the commercial controls: 2.6% to 3.4% in the upper part of the normal reference interval. Precision was worse at lower concentrations (CVs 5–10% at about 0.5 μg/L). Imprecision tended to be higher at the end of runs. Assay drift for 100-tube runs was −4%. PSA was stable at −20 °C during six months. Neither the polystyrene polymer in SST tubes nor a hemolysate had any detectable effect on PSA values. Clinical analysis of the first 322 patients and all patients with PSA ≤0.20 μg/L highlighted the requirements for strict adherence to sampling instructions and to stringent quality control also at low analyte concentrations (analyte-free sera and sera with PSA concentrations 0.2–0.5 μg/L). Values with TANDEM-R PSA and IRMA-Count PSA (Diagnostic Products Corp., Los Angeles, CA) correlated well with no difference in detection limit or with samples from women. Within-assay precision was better with IRMA-Count PSA in the upper part of the normal reference interval and above. The designs of the two assays were compared in a format that is generally applicable for immunoassay kits (NORDKEM kit group, unpublished), and subjective impressions were recorded.

Additional Keyphrases: prostatic disease · diagnosis and monitoring of treatment · radical prostatectomy · benign prostatic hyperplasia · tumor markers · interfering factors · quality control · "kit" methods · cancer

Prostate-specific antigen (PSA), a 33–34 kDa carbohydrate-containing protease similar to kallikrein, is formed by the prostate and secreted into the seminal plasma. In the normal prostate it is found on the rough endoplasmic reticulum as well as in cytoplasmic vesicles and secretory granules of the epithelial cells. In benign prostatic hyper trophy and in prostatic neoplasms it may also be found in stromal neutrophil granulocytes and macrophages. It is thought that PSA reaches the bloodstream directly from epithelial cells or ducts and, in the case of the abnormal prostate, also from the stromal phagocytic cells (1). PSA biochemistry is considered to be testosterone-dependent during the years preceding adulthood.

PSA is superior to prostatic acid phosphatase (EC 3.1.3.2) for the detection of prostatic cancer and monitoring its therapy (2–10). Before introducing the assay in our laboratory we reviewed recent reports (11–15) on the performance of one widely used commercially available kit for immunoradiometric PSA assay, TANDEM-R PSA (Hybritech, Inc., San Diego, CA). We were concerned by the poor agreement between reported detection limits and the large differences between estimates of imprecision (Table 1), which might be results of changes in kit reagents not revealed to customers (17) or of poor "robustness" of the kit. We were also concerned by the alleged problems with calibrator matrices (18–20). We found no useful information on the performance at low analyte concentration, despite the clinical demands for such assays, nor any information on imprecision for patients' samples in comparison with that for commercially available controls. Our previous experience indicates that the performance of immunoassay kits may be reflected differently by these two types of control materials. Also, there are discrepancies between reported values for the detection limit and ranges of values in women for a seemingly well-established polyclonal radioimmunoassay as well as differences in calibration between different widely used assays (16, and references therein). We therefore conducted a nine-month study of TANDEM-R PSA, using more than 900 samples from more than 700 patients, and we then compared its performance with that of a kit whose design offers some technical advantages for the busy

Table 1. Estimates of Detection Limit and Imprecision

<table>
<thead>
<tr>
<th>Authors (ref. no.)</th>
<th>Detection limit, μg/L</th>
<th>Within assays</th>
<th>Total between assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chan et al. (11)</td>
<td>0.1^a</td>
<td>3.0 (2.9)^b</td>
<td>4.9 (3.0)</td>
</tr>
<tr>
<td>Rock et al. (12)</td>
<td>2.5 (6.9)</td>
<td>3.6 (7.0)</td>
<td></td>
</tr>
<tr>
<td>Schifman et al. (13)</td>
<td>1.3 (37.2)</td>
<td>3.0 (36.0)</td>
<td></td>
</tr>
<tr>
<td>Devos et al. (14)</td>
<td>0.62^a</td>
<td>16.1 (0.8)^b</td>
<td>20.8 (1.59)</td>
</tr>
<tr>
<td>Dieijen-Visser et al. (15)</td>
<td>10.5 (3.6)</td>
<td>12.9 (2.54)</td>
<td></td>
</tr>
<tr>
<td>Hortin et al. (16)</td>
<td>0.4^a</td>
<td>7 (2.8)^b</td>
<td>15 (2.8)</td>
</tr>
</tbody>
</table>

^a Mean ±3 SD; replicate determinations of zero calibrator.
^b Replicate determinations of commercial controls; no information on single or pooled patients' samples.
^c Mean ±2 SD; replicate determinations of zero calibrator.
^d No information.
^e Mean ±2 SD; replicate determinations of samples from women.
^f Mean ±5 SD; replicate determinations of zero calibrator.
^g Replicate determinations of pooled patient's samples; no information on commercial controls.

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Materials and Methods

Reagents. TANDEM-R PSA was used during a nine-month period (June 1988 to March 1989; 25 assay runs) and IRMA-Count PSA during a four-month period (December 1988 to April 1989, five assay runs). The designs and specifications of the two kits are summarized in tabular form in sheets available from the authors or the Editorial Office of this journal. Information on the lot numbers of the 125I-labeled reagents may be obtained from the authors (expiration dates: for TANDEM-R PSA, 7 July 1988-24 April 1989; for IRMA-Count PSA, 31 Jan 1989-31 May 1989).

Patients' samples, controls. We used patients' serum samples from the Department of Urology (unselected samples from patients with different disorders of unknown etiology at the time of admission and samples from patients being treated for prostatic carcinoma), and samples obtained during post-radiotherapy follow-up of patients attending the Department of Oncology and Radiotherapy. There were only few samples from patients expected to have very high values, so high-dose "hook" effects (21-23) were not of concern in this study.

Except where indicated, venous blood was sampled into 10-mL Vacutainer gel-barrier tubes (SST A 3200, obtained through Becton-Dickinson AB, Stockholm, Sweden), which were sent to the laboratory and centrifuged within 3-4 h. Serum was then transferred to a refrigerator at -20 °C and was stored for up to two weeks before assay, when it was thawed at room temperature. Samples were kept at room temperature for not more than 5 h, and were then again frozen until further assay. For the comparison between results obtained for patients' samples with the two kits we selected samples with values ranging from undetectable to about 60 µg/L as specified for each experiment (the highest concentration found in a patient without prostatic carcinoma was 42 µg/L), and we used samples that had been stored for one to six months. We also sampled blood from 10 patients, both into SST tubes and into regular Vacutainer Tubes (A 3200), to test for any effect of the polyester polymer.

We used four different controls, two pools of human serum with low-normal concentrations and two controls obtained from Hybritech, Inc. The serum used to prepare the pools had been obtained from many patients, and 1-mL aliquots were frozen in sealed glass ampules kept at about -20 °C. New ampules were taken for each assay run.

Assays. Assays were done according to the protocols given by the manufacturers. Our intention was to work under conditions giving optimal precision with TANDEM-R PSA, so incubating and washing solutions were aspirated from the tubes containing the antibody-coated beads. IRMA-Count PSA was used under conditions also allowing rapidity and ease of handling—i.e., all tubes were decanted simultaneously. We did not regularly test for "hook" effects by (e.g.) simultaneous assay of diluted sample, because no patients were included who would be expected to have very high values. Except for the initial runs we analysed samples as singletons, and calibrators and controls in duplicate. Controls were included in the beginning of the runs and, when space allowed, also at the end. Initially we used in-house pooled sera with low PSA concentration; later we included the controls from Hybritech, Inc. The imprecision for patients' samples was calculated from the differences between the duplicate analyses done in the initial runs for each kit. For the controls, the imprecision was calculated from data on 20 replicate assays (both kits) and from the differences between duplicates (TANDEM-R PSA). Detection limits were calculated from assay of 20 replicates of the zero calibrators.

Effects of a hemolysate were tested as follows. A heparinized blood sample was obtained from a healthy woman. The tube was centrifuged, the plasma was aspirated, and the blood cells were resuspended in the same volume of distilled water as the volume of plasma. The tube was frozen in solid CO2 and thawed at room temperature. Of this hemolysate 2, 5, 10, and 20 µL, respectively, were mixed with 200 µL of three different samples: one sample from a woman (undetectable PSA), one sample with 14 µg of PSA per liter, and one sample with 49 µg of PSA per liter. The 4-mL tubes were stopped and incubated at 37 °C for 30 min, then for 2 h at room temperature.

For dilution of patients' samples we used a pooled specimen of serum from women, for which the concentration had been determined with the Diagnostic Products Corp. kit to be 0.00 µg/L.

Radioactivity measurements. These measurements were done in a well-type gamma counter (Selectronik A/S, Denmark), with a 5-min measurement time.

Results

Clinical evaluation of TANDEM-R PSA. We analyzed 986 samples from 728 male patients. Clinical assessment of the first 322 results was done, inter alia, to ascertain that falsely high values had not been obtained, such as those found with heterophilic antibodies and IgM immunoglobulins. Case records were scrutinized, and no cases were found to have concentrations that were inappropriately high in relation to the medical history.

There were 90 cases of prostatic carcinoma. Of 51 patients with stage A or B, including newly diagnosed as well as treated cases, 27 patients had PSA concentrations >4.0 µg/L and five untreated cases had concentrations of less than 4.0 µg/L. Of 26 patients with stage C, 24 had values above that decision limit and one untreated case had a value below it. Of 13 patients with stage D, all had concentrations exceeding the decision limit, 4.0 µg/L. These figures concur with what has been reported for the diagnostic sensitivity of the PSA assay for the different stages of prostatic carcinoma.

Of the 101 patients with benign prostatic hyperplasia, 44 had a concentration >4.0 µg/L. Four patients had grossly increased values (25, 27, 30, and 42 µg/L). Factors of possible importance in the histories of these cases are as follows: urinary retention in two cases with 25 and 27 µg/L, respectively, the latter patient having a urinary-tract infection at the time of sampling and also chronic prostatitis as judged from light-microscopic examination of the resected specimen. The patient for whom the value was 30 µg/L had prostatitis. The patient with a value of 42 µg/L had been subjected to cystoscopy and palpation of the prostate the day before sampling.

The mean concentration in the other 97 patients was 4.4 µg/L (SD 3.3, range 0.46-19 µg/L), i.e., higher than the decision limit, 4.0 µg/L, recommended for users of TANDEM-R PSA. Our findings are similar to what has been reported for the clinical performance of the PSA assay—a high prevalence of increased PSA concentrations in pa-
patients with benign prostatic hyperplasia, as well as marked elevation of PSA concentrations in patients with prostatitis, after rectal and cystoscopic examination (3) and in patients with urinary retention (6).

After the nine-month trial was completed we scrutinized the records of all patients with PSA concentration <0.20 μg/L and all patients who had been treated for prostatic carcinoma by radical prostatectomy. Of 42 cases with serum PSA <0.20 μg/L, only three lacked a medical history with a direct involvement of the prostate gland. The group with the most consistently low values was the group treated for urinary-bladder carcinoma by resection of the prostate gland and radiation (seven of the eight cases having values <0.01 μg/L). This is the expected finding (18). Of interest is one patient with recurrent prostatic enlargement but low PSA concentrations: light-microscopic examination revealed an absence of epithelial cells, and fibromuscular hypertrophy was the cause for the enlargement. Another patient had a long-standing paraplegia; secondarily low gonadotropin secretion might have been of importance for the low PSA concentration when one considers the endocrine regulation of PSA secretion.

Of the 10 patients subjected to radical prostatectomy, nine are so far reported to be free from recurrence, and the mean concentration in the 18 samples from these nine patients was 0.07 μg/L (range 0.03–0.17 μg/L). One patient with clinical recurrence had a value of 2.2 μg/L.

One patient who had been subjected to prostatectomy for prostatic carcinoma was followed with repetitive sampling. From a preparative concentration of 52 μg/L the concentration decreased exponentially during the next three to seven days, with a half-life of 4.2 days. During days 28–67 the concentration was unchanged at 1.6 μg/L. Light-microscopic examination revealed nonradical resection of the tumor. The patient was then subjected to bilateral orchietomy and obtained radiation treatment, which decreased the PSA concentration to below the detection limit.

Effect of gel-barrier serum separator. Analysis with TANDEM-R PSA of serum samples from 10 patients from whom blood had been taken both into plain Vacutainer Tubes and into SST tubes showed the following: PSA, μg/L (serum from SST tubes) = 1.005 × PSA, μg/L (serum from tube without additive) − 0.020 [r = 1.00, no outliers, range 0.6–15 μg/L (mean 5 μg/L)].

These results show the absence of SST effect with the Hybritech kit. Diagnostic Products Corp. claims absence of interference with SST, and therefore no further studies were done.

Detection limit. The detection limit (mean +2 SD) was found to be <0.05 μg/L for both kits.

Effect of hemolysis. Although Hybritech, Inc. states that up to 2 g of hemoglobin per liter does not interfere, there is a statement in the protocol that blood samples should be collected in such a way that hemolysis is avoided. We therefore tested the effect of adding increasing amounts of a hemolysate to three different serum samples, resulting in a marked red color of the sample (see Materials and Methods). This addition had no effect on the PSA concentration measured in a sample from a woman or in two samples, from men, with PSA concentrations of 14 and 49 μg/L.

Results for women. For 54 samples from women the mean count rate for TANDEM-R PSA in 14 assay runs was 98% of that for the zero calibrator in the respective run. Two SDs for each run corresponded to a concentration range of 0.06 to 0.14 μg/L, figures that may be compared with the manufacturer’s stated detection limit of 0.15 μg/L.

With the Diagnostic Products Corp. kit we assayed 10 serum samples from women in an initial run; the mean count rate was 101% of that for the zero calibrator. In this run two SDs of the mean count rate corresponded to 0.03 μg/L (manufacturer’s stated detection limit = 0.03 μg/L).

Imprecision. Table 2 shows the values for imprecision within assays for the controls as determined from 20 replicate analyses in one run (both kits) as well as from the differences between duplicate analyses during the nine-month period (TANDEM-R PSA only). The results for TANDEM-R PSA were of similar magnitude for these two estimates of within-assay imprecision. Our findings indicate a higher precision with the IRMA-Count PSA at concentrations at or exceeding the upper part of the reference interval.

Table 3 shows the results for the patients’ samples that we assayed in duplicate in the initial evaluation of each kit. These results also indicate a higher precision with the Diagnostic Products Corp. kit at concentrations at or exceeding the upper part of the reference interval.

Total between-assay imprecision, end-of-run effect. The total between-assay imprecision was calculated only for TANDEM-R PSA, because the number of runs with IRMA-Count PSA was too small (Table 4). We obtained a slightly poorer total between-assay precision at the end of these 100-tube runs. As judged from the mean values, there was a downward drift of about 4% in these 100-tube series, i.e., clinically of little importance. With IRMA-Count PSA we found no significant drift but the number of assay runs was small.

### Table 2. Within-Assay Imprecision for Controls

<table>
<thead>
<tr>
<th>Control number</th>
<th>Mean, μg/L</th>
<th>TANDEM</th>
<th>IRMA-COUNT</th>
<th>n&lt;sup&gt;a&lt;/sup&gt;</th>
<th>n&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Mean, μg/L</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.44</td>
<td>5.5</td>
<td>5.4</td>
<td>48</td>
<td>0.43</td>
<td>7.7</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.59</td>
<td>3.7</td>
<td>6.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>48</td>
<td>0.58</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.8&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.4</td>
<td>1.8</td>
<td>25</td>
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<tr>
<td>4</td>
<td>37&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4.5</td>
<td>1.5</td>
<td>24</td>
<td>39</td>
<td>3.1</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Twenty replicate analyses of each control in one assay run. <sup>b</sup> Calculated from the differences between duplicate assays with the Hybritech kit during the nine-month period. <sup>c</sup> Mean value of duplicate assays. <sup>d</sup> No outlier values were observed. <sup>e</sup> Manufacturer’s confidence limits: Control 3: 2.4–3.4 μg/L; Control 4: 36.3–42.8 μg/L.

### Table 3. Within-Assay Imprecision for Patients’ Samples

<table>
<thead>
<tr>
<th>Interval, μg/L</th>
<th>n&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mean, μg/L</th>
<th>CV, %</th>
<th>n&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mean, μg/L</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0–0.5</td>
<td>37</td>
<td>0.11</td>
<td>23</td>
<td>12</td>
<td>0.058</td>
<td>21</td>
</tr>
<tr>
<td>0.5–2.0</td>
<td>48</td>
<td>1.1</td>
<td>2.9</td>
<td>6</td>
<td>1.3</td>
<td>3.9</td>
</tr>
<tr>
<td>2.0–4.0</td>
<td>19</td>
<td>2.7</td>
<td>2.6</td>
<td>4</td>
<td>2.7</td>
<td>1.8</td>
</tr>
<tr>
<td>4.0–10</td>
<td>14</td>
<td>6.3</td>
<td>2.7</td>
<td>4</td>
<td>8.0</td>
<td>1.9</td>
</tr>
<tr>
<td>&gt;10</td>
<td>15</td>
<td>40</td>
<td>2.4</td>
<td>16</td>
<td>27</td>
<td>1.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> n = number of samples.
Stability of analyte in patients' samples. For kit comparison we used samples that had been collected during the first six months. Comparison of the original results with those obtained at reassay with TANDEM-R PSA showed the following: (PSA at assay 2, μg/L) = 1.023 × (PSA at assay 1, μg/L) + 0.17 [r = 1.000, n = 36, no outliers, concentration range 0–67 μg/L (mean 11 μg/L)].

We conclude that serum PSA was stable under our storage conditions.

Calibration. We compared results obtained for patients' samples with the two kits on three different occasions separated by about a month. Equations from simple linear regression analyses were as follows:

PSA, μg/L (IRMA-Count PSA) = 1.09 × PSA, μg/L (TANDEM-R PSA) − 0.081 [r = 1.000, n = 36, no outliers, TANDEM-R PSA concentration range 0–67 μg/L (mean 11 μg/L)];

PSA, μg/L (IRMA-Count PSA) = 0.90 × PSA, μg/L (TANDEM-R PSA) − 0.14 [r = 1.000, n = 13, no outlier values, TANDEM-R PSA concentration range 0.53–52 μg/L (mean 12 μg/L)];

and PSA, μg/L (IRMA-Count PSA) = 0.98 × PSA, μg/L (TANDEM-R PSA) + 0.22 [r = 0.998, n = 55, no outlier values, TANDEM-R PSA concentration range 0–61 μg/L (mean 17 μg/L)].

Figure 1 diagrams the differences between results obtained by the two methods in the range 0–4 μg/L and >4 μg/L, i.e., within and above the range expected for benign prostatic hyperplasia. Whereas a plot of absolute differences vs. concentration as originally suggested by Bland and Altman (24) might be advantageous for the physician in charge of the patient, a plot of percentage differences might appear more appropriate for the clinical chemist.

The results indicate the similarity of calibration with these two kits. One source of confusion has been the different calibration of different kits, as pointed out by Hortin et al. (16, and discussion following that report).

Dilution linearity. The dilution linearity was acceptable for two pools of patients' serum with the Diagnostic Products kit (Table 5).

Subjective Assessment (25)

Ease of use. This feature was a selection criterion for the study of an alternative method to TANDEM-R PSA. We confirm that the IRMA-Count PSA is advantageous in this respect, because aspiration of a 100-tube series with the former is time-consuming and tedious. We did not test TANDEM-R PSA for simultaneous decantation, because we regularly assay a series of more than 60 tubes.

Advantageous with TANDEM-R PSA is the larger sample volume (50 μL compared with the 10 μL used with IRMA-Count PSA). The 50-μL volume is pipetted more easily in the busy routine with different operators; also, with the former assay, an 11-fold dilution to check for hook effect is most simply done by taking 5 μL of sample and 50 μL of zero calibrator or pool serum from women, whereas a separate dilution must be performed with IRMA-Count PSA.

Design and presentation of components. Both kits are satisfactory.

Clarity of instructions. IRMA-Count PSA is superior.

Labeling of components. Both kits are satisfactory.

Time for a 100-tube run. Total time required from start of assay to completion of the washing steps was 5 h with the TANDEM-R PSA, 3 h with the IRMA-Count PSA. However, with TANDEM-R PSA there is a free interval of 2 h, compared with the two 30-min intervals with IRMA-Count PSA.

In conclusion, the separation and washing steps with IRMA-Count PSA were less demanding than working with

<table>
<thead>
<tr>
<th>Control number</th>
<th>Position</th>
<th>n*</th>
<th>Mean, μg/L</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Beginning</td>
<td>25</td>
<td>0.43</td>
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<td>0.59</td>
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<tr>
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<tr>
<td></td>
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<td>End</td>
<td>6</td>
<td>37</td>
<td>7.8</td>
</tr>
</tbody>
</table>

* n = number of assay runs. No outlier values were observed.
TANDEM-R PSA, whereas the pipetting step was slightly more demanding. IRMA-Count PSA was less time-consuming and slightly better suited to our routine with large assay series.

Discussion

Method evaluation. In our hands, the performance of TANDEM-R PSA was acceptable in terms of detection limit, matrix effects (as judged from results for women), repeatability, and reproducibility. We found no evidence for inconsistency of reagents during nine months of study. The repeatability may be improved, however, by using a slight modification of the methodology, as shown by our results with the IRMA-Count PSA.

Our estimated detection limit for TANDEM-R PSA is lower than that stated in the product insert (for tracer antibody at expiration of the kit), slightly lower than given in references 11-13 and 16, and considerably lower than that reported by still others (14, 15). Our figure for IRMA-Count PSA is similar to that given by Diagnostic Products Corp. We did not, however, study the possible variation of the detection limit with time after labeling. The validity of the formal detection limit as a decision limit for (e.g.) post-prostatectomy patients may be questioned in light of the considerably higher values for upper reference limit reported for women with some assays and the different results for zero calibrators when different kits are compared (16). One confounding factor may be endogenous anti-PSA antibodies (18), but there may also be other matrix differences between different samples and zero calibrators in kits.

Our values for imprecision were acceptable in relation to those found by other laboratories (Table 1). Furthermore, we found no differences between the figures for the Hybritcall controls and our pooled sera or single patients' sera. Our precision was less favorable at lower analyte concentrations but not as poor as that found in the single report so far on imprecision at low PSA concentrations (9). This carefully conducted study, which was published after the present study was completed, shows that considerable caution should be exercised in the interpretation of low PSA concentrations with the current TANDEM-R PSA, even if imprecision at conventionally used control concentrations is good by current standards. Obviously the internal quality-assurance program must include pooled sera from individuals lacking circulating PSA (sera from women lacking anti-PSA antibodies, post-cystoprostatectomy patients) as well as pooled sera from patients with PSA concentrations of the order of 0.2-0.5 \( \mu g/L \).

Homogeneity of circulating PSA, elimination rate. The possibility of circulating PSA fragments or PSA complexes in addition to intact PSA is poorly explored, but it seems plausible in the face of the light-microscopic findings of PSA in phagocytic cells in patients with prostatic disease (1). Endoproteolytic cleavage of PSA may yield up to four large fragments (28). Alfhani and Stenman (21) identified a 16-kDa PSA-immunoreactive material comprising 9% of the total immunoreactive material in one patient. A half-life of 3.15 (SE? SD? 0.08) days for the elimination of PSA from blood was reported by Oesterling et al. (7) from analysis of data from patients subjected to radical prostatectomy and pelvic lymphadenectomy whose cases had been followed during the first 10 postoperative days. In the study by Stamey et al. (3) patients were followed for four days postoperatively, and an initial rapid elimination phase (1.28 about 0.5 days) was found to be followed by a slower elimination phase with a half-life of 2.2 (SD 0.8) days, the expected finding if immunoreactive PSA were heterogeneous in size. Our figure of 4.2 days during days 3-7 in one patient—a moderately increased value—is in good agreement with finding of residual prostatic tissue by histomorphological examination of the material removed at operation. The same explanation may be given to the finding of concentrations >1.0 \( \mu g/L \) at postoperative days 28 and 67. The results indicate the value of repeated PSA determinations in the postoperative phase.

Pre-analytical factors. Possible pre-analytical factors affecting the PSA assay are poorly studied, a fact that may seem surprising when one considers the many reports on PSA concentrations in patients with prostatic hyperplasia and neoplasia. PSA concentrations reportedly decrease by 18% (with large variations) during 24 h after hospitalization (3); the cause is unknown. Normally, PSA concentrations seem to vary only little during the day (27). If one considers the molecular mass of 33-34 kDa, PSA would be expected to be eliminated in part by filtration through the renal glomeruli if it is not complex-bound to plasma proteins. Thus, values may be higher in patients with diminished glomerular filtration rate. Urinary retention was recently reported to affect PSA concentration (6), which indicates that sampling for PSA assay should be deferred until serum creatinine concentrations stabilize after acute urinary retention is treated. The relationship between circulating PSA concentration and prostatic volume, therefore, might be different in old men as compared with middle-aged men, but, to our knowledge, no such studies have been performed. PSA fragments would be expected to be more rapidly eliminated from the blood than intact PSA in patients with normal glomerular filtration rate, whereas the difference might be less for patients with decreased glomerular function, e.g., in uremia and in patients with urinary retention. Depending on the degree of cross-reactivity of PSA fragments in the PSA assay, changes in the glomerular filtration rate may have a more or less pronounced effect on PSA concentrations.

Prostatic palpation has a time-honored reputation as a cause for increased acid-phosphatase concentrations after physical examination of the patient. Whether PSA concentrations are affected is a matter of dispute (3, 28). To our knowledge, there is little useful information on this matter from studies of normal men and men with prostatic hyperplasia or prostatic inflammatory disease. The possible effect by bladder distention and manipulation of the prostate during cystoscopy is unknown. Large increases in PSA concentration have been observed after needle biopsy and transurethral resection (3). The possible effect of urinary tract infection has not been explored.

One or more of these factors was present in the four of our 101 cases of benign prostatic hyperplasia with PSA >20 \( \mu g/L \) and may well have contributed to the skewness observed in many reports of PSA concentrations in patients with benign prostatic hyperplasia.

**Decision limits.** The upper health-associated reference limit for the serum PSA concentration is usually reported to be 2-3 \( \mu g/L \), but some studies on reference limits seemingly have failed to exclude individuals with prostatic hyperplasia. The latter factor may explain as high a decision limit as 4.0 \( \mu g/L \) suggested by Hybritcall Inc. for TANDEM-R PSA despite the fact that this kit reportedly gives considerably lower values than some other assays.
(16). To increase the diagnostic specificity of PSA as a tumor marker, the use of higher values as decision limits—e.g., 10 μg/L (4)—has been recommended. Possibly, the relationship between circulating PSA and prostatic volume has higher diagnostic specificity for prostatic carcinoma than do PSA concentrations alone (3).

Diagnostic importance of low PSA concentrations. Concentrations ≤0.20 μg/L are uncommon in healthy men, particularly those younger than 40 years (17). This indicates that the finding of a value lower than that limit might be medically important. There is an increasing interest in measuring low PSA concentrations during treatment of prostatic cancer. Different therapeutic modalities are available such as radical prostatectomy (see above), irradiation, and different forms of endocrine therapy. A pronounced decrease in concentrations of circulating PSA during such therapy appears to be prognostically favorable, but to what extent the concentration change reflects a change in neoplasia mass and growth potential is largely unknown. From analytical considerations given above it is difficult to assess the prognostic value of these low PSA concentrations unless the laboratory also runs a quality-assurance program in this concentration range—and, judging from reports published so far, this is not commonly done.


References