Evaluation of the Abbott IMx® Automated Immunoassay of Prostate-Specific Antigen

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We detail the performance characteristics of the new IMx PSA immunoassay developed by Abbott Laboratories, addressing PSA recovery, assay reproducibility, standard curve storage, lower limit of detection, dilution linearity, and correlation with the Hybritech Tandem-R® PSA immunoassay. We analyzed 886 sera for PSA retrospectively, testing 555 of these concurrently with the IMx and the Tandem-R immunoassays. The IMx PSA standard curve was linear from 0 to 100 μg/L, and curve storage was maintained for 4 weeks. The lower limit of detection of the IMx PSA assay was ≤0.03 μg/L; allowing for the assay precision yielded a biological detection limit of 0.06 μg/L. We conservatively set the clinical threshold at 0.1 μg/L. Regression analysis of dilution linearity involving 10 samples (0.44–200 μg/L) resulted in coefficients of correlation ranging from 0.9972 to 1.0000. Reproducibility studies with 18 specimens within the range of 0.39–413.67 μg/L gave intra- and interassay CVs <5.5%. The interassay 95% confidence interval for a specimen containing 0.06 μg of PSA per liter was 0.03–0.09 μg/L. Correlation between IMx and Tandem-R PSA assay results was excellent: r = 0.9909 and slope = 0.95. Overall, the IMx PSA immunoassay, with the conveniences of automation, curve storage, and nonisotopic handling, provided an improved lower limit of PSA detection, which allows for earlier indication of residual or recurrent disease after radical prostatectomy.

Additional Keyphrases: prostate disease · monitoring therapy · radical prostatectomy · lower limits of detection · enzyme immunoassay

Prostate-specific antigen (PSA) is a glycoprotein belonging to the kallikrein family of neutral serine proteases.1 Produced by normal, benign, and malignant prostatic epithelial cells, PSA is present in seminal fluid, serum, and urine. Hara et al. (1) provided the first description of PSA in 1971, referring to a prominent protein in human seminal plasma as γ-seminoprotein. Shortly thereafter, Li and Bening (2) isolated and purified a molecule of M, 31,000, which they referred to as E1, from human seminal plasma. A more detailed biochemical and electrophoretic analysis was subsequently provided in 1978, when Sensabaugh (3) reported that this molecule was glycosylated, was highly immuno-

genic, and had a molecular mass of 30,000 Da, from whence came the name p30. Wang et al. (4) established the unique association of this molecule with the prostate, naming the molecule PSA. Shortly thereafter, Pepsidero et al. (5), from the same research team, detected PSA in serum and proposed that PSA might be used as a tumor marker. Since then, >300 reports have been published on this subject, many of which are discussed in a recent and thorough review by Oesterling (6).

The monitoring of PSA concentrations in serum has become indispensable in the clinical management of patients with primary or recurrent prostate cancer (7–12). PSA, being a constituent of normal prostatic secretions, is found in the sera of normal men at concentrations generally <4 μg/L, as quantified by the Hybritech Tandem-R PSA immunoassay (13). This cited "normal range" is not absolute, being influenced by differences among calibrators in the various PSA immunoassays, by the inclusion criteria used to select the subjects, and by population demographics (14). Also, increases in PSA to 4–10 μg/L are not uncommon among men with benign prostatic hyperplasia (BPH) or prostatitis. Nevertheless, Catalona et al. (15) and our collaborators (16) demonstrated an increase in the cancer detection rate when PSA values were acquired as one aspect of a patient screening protocol.

The overwhelming clinical value of PSA is derived from its apparent organ specificity. This is best appreciated by noting that in the United States a frequent treatment for a tumorous prostate is the complete surgical removal of the organ. Accordingly, after a radical prostatectomy, the serum concentrations of PSA theoretically should be zero, if (a) no residual, PSA-secreting tissue remains and (b) sufficient time has passed for the physiological clearance of pre-operative serum concentrations of PSA (half-life of 2.2–3.2 days; 9, 17). However, PSA assay detection limits do not allow for the reliable measurement of PSA down to zero. For example, with the Hybritech Tandem-R immunoassay, we reported that the lower limit of detection (LLD) was 0.09 μg/L with a biological detection limit of ≥0.4 μg/L (18).

PSA concentration greater than this after a radical prostatectomy, whether immediate or delayed, became a powerful predictor of eventual clinical recurrence (9, 17–19).

We have speculated that decreasing the detection limits of PSA with second-generation immunoassays should provide greater sensitivity in revealing minimal residual disease after radical prostatectomy and allow earlier detection of recurrent disease (20). Abbott Laboratories introduced an automated IMx immunoassay of PSA based on their Microparticle-capture Enzyme Im-

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4 Nonstandard abbreviations: PSA, prostate-specific antigen; LLD, lower limit of detection; MEIA, microparticle-capture enzyme immunoassay; BPH, benign prostatic hyperplasia; and HAMA, human anti-mouse antibodies.

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munoassay (MEIA) technology (21), a technology that, when applied to their α-fetoprotein enzyme immunoas-
say, improved the LLD for this analyte severalfold (22).
This report, expanding on our initial abstract presenta-
tion (23), describes the performance characteristics of the IMx PSA immunoassay, correlates PSA values ob-
tained with this assay to those obtained with the Hy-
britech Tandem-R PSA immunoassay, and makes a preliminarystudy to discern whether the ability to use a lower LLD offersclinicaladvantages, as we had previously speculated.

Materials and Methods

Selection of Patients and Sera

This study was conducted with retrospective sera (n = 686), processed and frozen at −80°C within a few hours of collection. Informed consent was appropriately ob-
tained, and each patient’s chart was abstracted for pertinent clinical information. Samples from the follow-
ing groups of men were used as controls in this study: apparently healthy subjects (n = 99) and patients with benign diseases (n = 152) or other cancers (n = 46). We
obtained from patients with prostate cancer 173 single-
point serum samples. We also performed three serial
studies: the first involved serial sera (n = 85) from 19
patients who had undergone a radical prostatectomy.
The second was limited to 11 patients (n = 38 sera),
cancer stages A or B, who had a prolonged disease-free clinical course after radical prostatectomy, which was suggestivelofures. The third serial study was from a control set of 26 men (n = 40 sera) with bladder cancer who had undergone a cystoprostatectomy (surgical removal of the tumorous bladder and normal prostate).
The IMx PSA assay evaluation also included 53 sera from normal female volunteers.

Reagents and Instrumentation

PSA determinations with the IMx PSA (Abbott Lab-
atories, Abbott Park, IL) and the Tandem-R PSA (Hybritech, Inc., San Diego, CA) assays were performed concurrently on 555 of the freshly thawed sera. Both
assays have a standard curve for PSA of 0–100 μg/L. Sera with PSA values >100 μg/L were diluted with diluents supplied by the manufacturer. Specimens were
assayed in duplicate during these studies to allow a complete evaluation of the IMx PSA assay. A paired
t-test of the IMx PSA values was used to determine
whether a difference was statistically significant be-
tween the two replicate values in two series: overall (n = 686) [none was observed (P = 0.7017)], and in the PSA
range of 0.0–0.5 μg/L (n = 239) [none was observed (P = 0.7905)]. The IMx assay is designed and FDA-approved
to perform specimen testing in replicates of one. There-
fore, to report results representative of the performance the user can expect to achieve, we used only the first of the replicate IMx PSA determinations to generate the results presented here. Linear-regression analysis was used to correlate the IMx PSA values to the Tandem-R PSA values (mean of duplicate determinations).

The automated IMx instrumentation has been previ-
ously described (21). In the MEIA technology, monoclo-
nal antibody-coated microparticles capture the PSA analyte and are then reacted with a polyclonal goat anti-PSA antibody—alkaline phosphatase immunocon-
jugate, which completes the “sandwich.” The complex
then catalyzes the conversion of the substrate 4-methyl-
lumbelliferyl phosphate to the fluorescent product meth-
ylumbelliferone. The six IMx PSA calibrators (0, 2, 10, 30, 60, and 100 μg/L) are used to generate a calibra-
tion curve for concentration vs. rate (counts/s²) of methylumbelliferone formation. This calibration curve
is stored in the instrument memory for 4 weeks.

The IMx PSA assay uses the Mode 1 calibrator to adjust the stored calibration curve as follows. The Mode 1 calibrator is equivalent to the C calibrator in PSA
concentration (10 μg/L). For each run, a Mode 1 factor is calculated by dividing the rate of the Mode 1 calibrator
in that run by the rate of the C calibrator in the stored
 calibration curve, to provide an adjusted calibration curve. Rates for all patients’ samples and controls as-
 sayed in that carousel are read off the adjusted calibration curve. The performance of the stored calibration curve is monitored by running all of the IMx PSA
controls, i.e., at 4, 15, and 45 μg/L. For an IMx PSA
calibration, all three concentrations of controls must be
processed for evaluating the calibration curve. The
minimum control requirement for each daily IMx PSA
assay is one control on each carousel.

Although the exact chemical composition of the cali-
brators used in the IMx PSA assay and details of PSA
purification are proprietary, the manufacturer does re-
veal that the PSA is purified from human seminal plasma and is diluted in a synthetic matrix buffer containing nonhuman protein components, including
bovine serum albumin, antimicrobials, and sodium
azide. Because there is no international standard for
PSA (14), the IMx PSA calibrators are prepared to give
results equivalent to those obtained with the Hybritech
Tandem-R PSA assay.

Performance Characteristics of the Tandem-R PSA Assay

We previously reported on the performance character-
istics of the Tandem-R PSA immunoassay, including a determination that PSA values >0.4 μg/L were indica-
tive of disease after radical prostatectomy (18). Since
then, the manufacturer has modified some of the assay
reagents (Hybritech, personal communication), and we
updated relevant aspects of the performance profile
during these current studies. The present LLD for PSA
in the Tandem-R assay is 0.06 μg/L, as derived from an
analysis of 20 replicate determinations of the zero cali-
brator (mean ± 2 SD). Assay precision near the LLD
was determined and used to calculate the biological
detection limit (see Discussion for further comments).
Briefly, interassay precision for low concentrations of
serum PSA was calculated for each of three patients’
sera in the range 0.24–0.36 μg/L (10 assays with speci-
mens in triplicate). The overall SD was 0.05 μg/L.
Combining the LLD for PSA of 0.09 μg/L with 2 SD (0.1
μg/L) from the sera precision analysis study yielded an
overall value of 0.19 μg/L, which we rounded up to 0.2 μg/L. Therefore, PSA values ≥0.2 μg/L are, and will be upon reassy, distinguishable from the zero calibrator at a 95% confidence level. We refer to this value as the biological detection limit. Tandem-R PSA results below the biological detection limit are reported hereafter as <0.2 μg/L.

Performance Characteristics of the IMx PSA

Lower limit of detection. Because of the importance of accurately determining the LLD, we used in these studies three sources representing "zero" PSA. The LLD was calculated from 20 replicate intra-assay determinations of (a) the IMx A calibrator (0 μg/L), (b) serum from a patient who had undergone a radical prostatectomy for organ-confined disease and had a prolonged disease-free follow-up period, and (c) serum from a patient who had a cystoprostatectomy for bladder cancer (surgical excision of the bladder and normal prostate). The LLD for each of these samples was calculated as the mean + 2 SD.

Reproducibility. IMx PSA intra- and interassay reproducibility was determined at multiple points on the calibration curve in two series of studies. Patients' sera and kit controls, assayed in triplicate in 10 separate runs, were used to establish reproducibility. The first series consisted of six serum specimens containing PSA in the range 1.37-413.67 μg/L and three kit controls in the range 3.82-45.09 μg/L. In the second series, reproducibility at very low PSA concentrations was determined with nine test sera in concentrations ranging from 0.04 to 0.58 μg/L. Some of the results from this second series were also used to calculate the biological detection limit (see below).

To assess long-term assay reproducibility at low PSA concentrations, we prepared two ultra-low concentration controls from the kit's B calibrator (2 μg/L) by independent dilutions of 5- and 35-fold in kit diluent to achieve anticipated concentrations of 0.4 and 0.06 μg/L. Aliquots were stored at −80 °C and freshly thawed on the day of use. These low-concentration controls were analyzed in ~30 assays over an 8-week period, during which time there were changes in reagent lots, recalibration of the instrument, and repeat storage of the standard curve. Since performing these low-concentration reproducibility studies, we have realized the importance of running low-concentration controls with each assay in which the PSA concentrations to be measured would be below those of the controls supplied by the manufacturer. Similar to the above-described ultra-low-concentration controls, these are derived from independent dilutions (5- and 35-fold) of the B calibrator in a synthetic matrix buffer (per liter, 0.01 mol of sodium phosphate, 0.15 mol of sodium chloride, 10 g of bovine serum albumin, and 1 g of sodium azide, pH 7.2), which results in controls similar in constitution to the higher-concentration controls supplied by the manufacturer in the IMx PSA assay kit. These ultra-low-concentration controls are not "adjusters" to the stored calibration curve; rather, they are used to assess the reproducible performance of the curve at the ultrasensitive detection region.

The ultra-low-concentration controls are aliquoted, frozen at −80 °C, and freshly thawed for incorporation in the assay. The mean ± 2 SD of 10 interassay determinations set the quality-control limits for each of these ultra-low-concentration control lots. When results for these controls are outside acceptable limits, the assay is repeated, even if the kit controls are within range. If the PSA values are outside the acceptable limits upon reassy, the instrument is recalibrated. Until similar PSA curve controls are supplied by the manufacturer or by a third party, we highly recommend that users incorporate similar in-house quality-control practices.

Linearity of dilution. Ten patients' sera, ranging in PSA concentrations from 0.44 to 585 μg/L, were used for testing linearity after dilution. Six sera having an initial PSA content ≤100 μg/L were diluted two-, four-, and eightfold. Each dilution was made from the original sample, not serially. Four sera having an initial PSA value >100 μg/L were first diluted 10-fold, then further diluted by two-, four-, and eightfold from that first dilution. All dilutions were made in the specimen diluent supplied with the IMx PSA reagents.

Analytical recovery. Two approaches were taken. First, we added known amounts of serum PSA to serum samples from healthy individuals and from patients with prostate cancer. Graves et al. (14) reported that the recovery of seminal fluid PSA added to serum was lower than expected in both the Hybritech and Yang (Procheck®) PSA assays. Our second approach tested this finding in the IMx PSA assay by using the F calibrator (100 μg/L) as the source of purified seminal fluid PSA and performing dilutions in female sera or patients' sera. For both approaches, the enriched samples as well as the endogenous amounts of PSA were determined by the IMx PSA assay and the percentage of recovery was calculated as [(observed PSA value – endogenous PSA value)/PSA added] × 100.

Interfering substances. Extensive interference studies (n = 94) were performed by the manufacturer, who shared the results with us. We did not repeat these studies but summarize them in Results. In one series, samples with known concentrations of hemoglobin, bilirubin, triglycerides, and proteins or immunoglobulins were first assayed by the IMx PSA assay to determine the concentration of endogenous PSA. To aliquots of each sample we added two concentrations of PSA and assayed again. The percentage of recovery was determined as described above. In another series, the following potentially interfering substances were added to pooled normal human serum: hemoglobin, bilirubin, triglycerides, prostatic acid phosphatase, and the therapeutic agents cyclophosphamide, diethylstilbestrol, megestrol acetate, doxorubicin HCl, methotrexate, estramustine phosphate, leuprolide acetate, flutamide, and goserelin acetate. Two concentrations of PSA were added to the samples containing the potentially interfering substances, and PSA determinations were made.
with the IMx PSA assay. Normal human serum enriched with the same amount of PSA was used as a control. The mean PSA values were calculated and the percentage of recovery determined.

Hook effects. In some immunoassays, high antigen concentrations interfere with assay performance, resulting in artificially low readings (the so-called hook effect). The presence of hook effects was evaluated with the IMx PSA assay by analyzing samples (patients’ sera and fortified sera) with PSA concentrations as great as 15 000 µg/L.

**Results**

Analytical Considerations

**Standard curve.** The standard curve is calculated internally by the IMx instrumentation through use of rate constants for the conversion of the nonfluorescent substrate to the fluorescent product. The IMx PSA assay uses a four-parameter logistic curve-fitting method for data reduction. An example of the standard curve (0–100 µg/L) and of count rates (counts/s²) for the six calibrators are presented in Figure 1. For comparison, the rates for the ultra-low-concentration controls of 0.06 and 0.4 µg/L are ~6 and 13 counts/s², respectively. To assess the integrity of the stored curve over a 4-week period, we analyzed the rates for the Mode 1 calibrator (PSA 10 µg/L). This calibrator is used in every assay to adjust the stored curve; our analysis involved 47 assays for which results were read off the stored calibration curve. The Mode 1 calibrator had a mean (SD) rate of 253 (9.8) counts/s² and a CV of 3.9%.

**Lower limit of detection.** The LLD was defined as the mean + 2 SD of the IMx rate values of 20 determinations of a test sample. When the IMx A calibrator (zero PSA in buffer) was used as the test sample, the LLD was calculated as 0.02 µg/L. We also determined the LLD by analyzing two "zero" PSA serum samples, one from a patient after radical prostatectomy and another from a patient after cystoprostatectomy; the LLD for PSA was 0.03 µg/L with each. Therefore, we report the LLD for the IMx PSA as ≥0.03 µg/L. We subsequently repeated this process at ~6 and 14 months into our evaluation of the IMx PSA assay, obtaining identical results.

**Reproducibility.** The inter- and intra-assay precision data from the two reproducibility series are summarized in Table 1. The PSA content in 15 serum specimens ranged from 0.04 to 413.67 µg/L, and each sample was analyzed in triplicate in 10 separate assays. For serum samples with ≥0.40 µg/L, the inter- and intra-assay CVs ranged from 1.8% to 5.3%. At 0.10–0.39 µg/L, these CVs ranged from 6.3% to 11.9%; at ~0.05 µg/L, they ranged from 18.3% to 27.6%. For example, in the interassay study, the mean (SD) PSA value in serum sample no. 468A was 0.06 (0.015) µg/L, with a CV of 25.3%. A graphic representation of the reproducibility studies of the three controls (mean ± 2 SD at ~4, 15, and 45 µg/L) is presented in Figure 2 (top).

To determine the long-term reproducibility of the IMx PSA immunoassay, we included two ultra-low-concentration controls, 0.06 µg/L (control A) and 0.44 µg/L (control B) in defined matrix (see Materials and Methods), in 32 and 34 assay runs, respectively, over a period of 4 weeks under conditions expected in clinical laboratories, including the introduction of new reagents, different reagent lots, IMx recalibration, and repeat generation and storage of the standard curve. The reproducibility and stability of the assay at these low concentrations over this time course are presented in Figure 2 (bottom): for control A, with a mean ± 2 SD range of 0.03–0.09 µg/L, we obtained 32 points, all

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**Table 1. Precision and Reproducibility of IMx PSA Immunoassay**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total n</th>
<th>Grand mean µg/L</th>
<th>Intra-assay</th>
<th>Inter-assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low control</td>
<td>10</td>
<td>3.82</td>
<td>NA</td>
<td>2.5</td>
</tr>
<tr>
<td>Med control</td>
<td>10</td>
<td>14.46</td>
<td>NA</td>
<td>2.6</td>
</tr>
<tr>
<td>High control</td>
<td>10</td>
<td>45.09</td>
<td>NA</td>
<td>2.5</td>
</tr>
<tr>
<td>Sample 1</td>
<td>30</td>
<td>1.37</td>
<td>2.9</td>
<td>4.0</td>
</tr>
<tr>
<td>Sample 2</td>
<td>30</td>
<td>4.62</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>Sample 3</td>
<td>30</td>
<td>16.14</td>
<td>2.7</td>
<td>2.7</td>
</tr>
<tr>
<td>Sample 4</td>
<td>30</td>
<td>36.37</td>
<td>1.8</td>
<td>3.5</td>
</tr>
<tr>
<td>Sample 5</td>
<td>30</td>
<td>62.61</td>
<td>2.6</td>
<td>4.2</td>
</tr>
<tr>
<td>Sample 6</td>
<td>30</td>
<td>413.67</td>
<td>2.3</td>
<td>3.8</td>
</tr>
<tr>
<td>Series II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7526P</td>
<td>30</td>
<td>0.04</td>
<td>23.8</td>
<td>23.8</td>
</tr>
<tr>
<td>6780M</td>
<td>30</td>
<td>0.05</td>
<td>19.7</td>
<td>27.6</td>
</tr>
<tr>
<td>468A</td>
<td>30</td>
<td>0.06</td>
<td>18.3</td>
<td>25.3</td>
</tr>
<tr>
<td>4564P</td>
<td>30</td>
<td>0.10</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>7411B</td>
<td>30</td>
<td>0.10</td>
<td>8.1</td>
<td>11.9</td>
</tr>
<tr>
<td>4564P3</td>
<td>30</td>
<td>0.39</td>
<td>6.3</td>
<td>6.3</td>
</tr>
<tr>
<td>7253H</td>
<td>30</td>
<td>0.42</td>
<td>3.9</td>
<td>5.0</td>
</tr>
<tr>
<td>7763W</td>
<td>30</td>
<td>0.46</td>
<td>3.6</td>
<td>5.3</td>
</tr>
<tr>
<td>5383N</td>
<td>30</td>
<td>0.58</td>
<td>4.6</td>
<td>5.2</td>
</tr>
</tbody>
</table>

*Based on replicates of three.

b Calculated by dividing the SD by the mean.
within 2 SD; for control B, 2 SD range of 0.40–0.50 μg/L, there were 34 points, with 1 assay point outside the 2 SD range. In-house controls at these ultra-low concentrations in defined matrix are now routinely included in each of our IMx PSA assays.

Anecdotally, to further substantiate the stability of the assay in the ultrasensitive range, we determined the reproducibility of PSA values in two human sera controls, using six different IMx PSA assay lots. The mean values and CVs were 0.64 μg/L (7.8%) and 0.04 μg/L (23.5%). These CVs were nearly identical to those observed (Figure 2, bottom) for the ultra-low-concentration defined matrix controls at similar PSA concentrations (7.8% vs 5.7% and 23.5% vs 25%).

**Biological detection limit.** The concept of a biological detection limit for PSA was first approached in our studies to establish the clinical value of PSA determinations after radical prostatectomy (18). As detailed in Materials and Methods, the biological detection limit depends on first calculating the analytical LLD and then determining the precision of the assay at this approximate PSA concentration by using clinical samples. From the LLD studies described above, the LLD for the IMx PSA was ≤0.03 μg/L. Thus a serum sample devoid of PSA could give PSA assay results as great as 0.03 μg/L. The interassay SD of the IMx PSA assay at 0.04, 0.05, and 0.06 μg/L averaged 0.013 μg/L (Table 1). We therefore established the biological detection limit for the IMx PSA assay as <0.06 μg/L, adding 2 SD from the precision study (2 × 0.013 μg/L) to the LLD (0.03 μg/L).

**Linearity of dilution studies.** Ten patients' sera, ranging in PSA concentrations from ~0.4 to 585 μg/L, were used to test for dilution linearity, according to the dilution schemes described earlier. For 8 of the 10 linearity study samples, regression analysis yielded a slope between 0.9998 and 1.0000; the other 2 samples gave slopes between 0.9972 and 0.9993. Representative results from 5 of the 10 studies are presented in Table 2.

**Analytical recovery.** Six recovery studies of serum PSA added to serum from normal men, normal women, or prostate cancer patients showed excellent recoveries: 99–108% (Table 3). However, only 42% to 52% (detailed data not shown) of added seminal fluid PSA was recovered from human female sera or the patients' sera. These results are similar to those observed with the Hybritech Tandem-R and Yang Pros-check assays (14), which suggests that, as in those assays, the detection of PSA with the IMx PSA assay is also highly dependent on the final assay matrix and the source of the PSA. The results further emphasize the need for an international PSA standard.

**Interfering substances.** Ninety-four recovery studies of PSA in the presence of potentially interfering substances were performed by Abbott Laboratories as described in Materials and Methods, with the resulting data being shared with us. In 82 of these studies, the recoveries ranged from 95% to 105%. Of the remaining 12 samples, the recovery for 1 was 106% and recoveries for the remaining 11 ranged from 88% to 94%, but none of the interfering substance was consistently associated with recoveries of <95%. After reviewing these results, we did not believe that repetition of these exhaustive studies was warranted.

However, we had the opportunity to assess the effect of human anti-mouse antibodies (HAMA) on the IMx PSA assay and Tandem-R assays, arising from an unusual clinical situation in which a patient was referred to us for a highly increased Tandem-R PSA result (PSA ~44 μg/L) after radical prostatectomy without any other evidence of disease (manuscript in preparation). At HAMA concentrations ≤7000 μg/L there was negligible interference in the IMx PSA assay (>90% recovery efficiency). However, for HAMA at 13 500 μg/L, recoveries averaged ~40%. Conversely, but as expected from the design of the Tandem-R assay series, HAMA may result in significantly increased false-positive values; the manufacturer states that HAMA concentrations >5000 μg/L can have an adverse impact on assay integrity (detailed in product insert). Cautionary notes are provided by manufacturers of both assays in their product inserts to alert users of potential adverse effects in the presence of HAMA.

Although all of the currently available PSA immunoassays can theoretically be affected by high titers of
Table 2. Dilution Linearity Studies

<table>
<thead>
<tr>
<th>Sample*</th>
<th>Initial value</th>
<th>Mean (SD) of all values</th>
<th>CV, %</th>
<th>Regression analysis: expected vs observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>5916K</td>
<td>0.44</td>
<td>0.45 (0.038)</td>
<td>8.510</td>
<td>r = 0.9972, Slope = 0.98, Intercept = 0.00</td>
</tr>
<tr>
<td>4139J</td>
<td>47.00</td>
<td>47.16 (0.872)</td>
<td>1.849</td>
<td>r = 1.0000, Slope = 1.00, Intercept = 0.02</td>
</tr>
<tr>
<td>4111B</td>
<td>64.61</td>
<td>61.13 (3.570)</td>
<td>5.859</td>
<td>r = 0.9972, Slope = 1.01, Intercept = -1.51</td>
</tr>
<tr>
<td>8177B</td>
<td>81.40</td>
<td>82.40 (3.439)</td>
<td>4.173</td>
<td>r = 0.9998, Slope = 0.99, Intercept = 0.24</td>
</tr>
<tr>
<td>6283D</td>
<td>19.96</td>
<td>20.34 (0.465)</td>
<td>2.285</td>
<td>r = 1.0000, Slope = 0.99, Intercept = 0.15</td>
</tr>
</tbody>
</table>

* Samples 5916K, 4139J, 4111B, and 8177B had undiluted PSA concentrations <100 μg/L; PSA was quantified both from the undiluted sample and after individual dilutions of two-, four-, and eightfold. The patient's sample 6283D had a PSA content >100 μg/L; it was first diluted 10-fold in assay diluent, from which independent dilutions of two-, four-, and eightfold were made. Therefore, the statistical calculations were based on four determinations per sample.

Table 3. Analytical Recovery

<table>
<thead>
<tr>
<th>Serum sample*</th>
<th>Observed</th>
<th>Endogenous</th>
<th>Difference</th>
<th>Added</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal man</td>
<td>11.56</td>
<td>0.34</td>
<td>11.22</td>
<td>11.08</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>28.76</td>
<td>0.32</td>
<td>28.44</td>
<td>26.73</td>
<td>108</td>
</tr>
<tr>
<td>Normal woman</td>
<td>14.04</td>
<td>0.00</td>
<td>14.04</td>
<td>13.32</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>22.64</td>
<td>0.00</td>
<td>22.64</td>
<td>22.77</td>
<td>99</td>
</tr>
<tr>
<td>Prostate cancer patient</td>
<td>26.10</td>
<td>14.16</td>
<td>11.94</td>
<td>11.08</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>39.96</td>
<td>13.56</td>
<td>26.40</td>
<td>26.73</td>
<td>99</td>
</tr>
</tbody>
</table>

* Serum to which defined amounts of PSA were added. The PSA source was serum, not seminal fluid.

Table 4. Correlation of IMx PSA to Tandem-R PSA Values

<table>
<thead>
<tr>
<th>PSA conc in overall specimens* μg/L</th>
<th>Sample size*</th>
<th>r</th>
<th>Slope</th>
<th>y-intercept, μg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–4</td>
<td>300</td>
<td>0.9738</td>
<td>0.97</td>
<td>0.1</td>
</tr>
<tr>
<td>0–10</td>
<td>352</td>
<td>0.9756</td>
<td>0.96</td>
<td>0.1</td>
</tr>
<tr>
<td>0–100</td>
<td>423</td>
<td>0.9928</td>
<td>0.97</td>
<td>0.2</td>
</tr>
<tr>
<td>0–500</td>
<td>441</td>
<td>0.9941</td>
<td>0.95</td>
<td>0.3</td>
</tr>
<tr>
<td>0–6200</td>
<td>470</td>
<td>0.9909</td>
<td>0.95</td>
<td>3.2</td>
</tr>
<tr>
<td>BPH (all)</td>
<td>60</td>
<td>0.9782</td>
<td>1.14</td>
<td>-0.2</td>
</tr>
<tr>
<td>Prostate cancer, 0–2000</td>
<td>164</td>
<td>0.9904</td>
<td>0.99</td>
<td>1.6</td>
</tr>
<tr>
<td>Prostate cancer, 0–6200</td>
<td>173</td>
<td>0.9901</td>
<td>0.94</td>
<td>8.9</td>
</tr>
</tbody>
</table>

* All of the patients' specimens listed in Materials and Methods are included in these calculations except for the serial specimens (n = 85 samples; 19 patients). In the serial study the correlation coefficient between the IMx PSA assay and the Tandem-R PSA assay was 0.9900 with a slope of 1.15 for the 68 sera for which the Tandem-R PSA assay value was ≥0.2 μg/L.

** For each higher concentration range, the sample size for calculation of the linear regression includes all the previously cited samples that were assayed at lower concentrations.

Anti-PSA antibodies or heterophilic antibodies in patients' sera, we did not specifically evaluate this issue here, other than this anecdotal HAMA study. The effects on assay performance by anti-PSA antibodies or heterophilic antibodies will differ among the various immunoassays according to assay design and components. These issues have been further discussed by Boscato and Stuart (24).

Correlation of the IMx PSA assay with the Tandem-R PSA assay. Side by side determinations of PSA were performed with both methods on freshly thawed serum (n = 555) ranging in PSA concentrations from 0 to 6158 μg/L. Within this series of single-point analyses were 60 sera from patients with BPH and 173 sera from patients with prostate carcinoma. The summary of agreement of PSA values reported from these two immunoassays, broken down into concentration subsets and into clinical subsets for BPH and prostate cancer, is provided in Table 4. The correlation coefficients ranged from 0.9738 to 0.9941, with slopes ranging from 0.94 (prostate cancer patients; 0–6200 μg/L) to 1.14 (BPH patients; 0–28 μg/L). Three of the correlation subsets—representing the standard curve for each immunoassay regardless of clinical status (0–100 μg/L), the BPH patients (0–28 μg/L), and the prostate cancer patients (0–6200 μg/L)—are presented in Figure 3. The prostate cancer patient groups in Table 4 and Figure 3 warrant comment. For 164 patients, PSA concentrations were <2000 μg/L, with regression analysis yielding a y-intercept of −1.6, a slope of 0.99, and r = 0.9904. The other nine patients had PSA concentrations >2000 μg/L, but among these two showed substantial discord of PSA values between the two assays (IMx PSA = 3208 μg/L vs Tandem-R PSA 4402 μg/L and 2980 vs 2389 μg/L, respectively).

These discrepant values are noticeable in Figure 3 (right). In contrast to the y-intercept of −1.6 in the 0–2000 μg/L range, these two discordant PSA values >2000 μg/L were sufficient to shift the y-intercept to 8.9.

High-dose hook effects. No high-dose hook effects were observed with the IMx PSA assay for any of the samples tested, ranging up to 15 000 μg/L. Hook effects were observed with the Tandem-R PSA assay when PSA values exceeded 5000 μg/L.

Clinical Studies

Normal range in men. We selected for this correlation study of the two immunoassays 99 retrospective sera from apparently healthy men. We defined normal as a
Tandem-R PSA immunoassay PSA value ≤4.0 µg/L (13, 18), and no history of prostatic disease. We consider this approach justified because of the strong correlation between the IMx PSA and Tandem-R PSA immunoassays at all values studied. We then divided these 99 specimens into two age groups, >40 years (n = 41) and <40 years (n = 58). In the >40 age group, the IMx PSA assay reported that 97.6% of the men (40 of 41) fell within the defined normal range; the current Tandem-R reported 95.1% (39 of 41). The sera in this study were from men with an unremarkable clinical history, and no additional clinical efforts were made to further screen for those men who might have had potential prostate pathology.

Serial correlation studies in men with prostate carcinoma. Serial sera were selected from 19 patients whose clinical course illustrated a response to therapy or recurrence of disease. At least four serum samples per patient were chosen, to reflect changes in PSA concentrations during this course. In all, we assayed 85 sera in parallel with the IMx PSA and Tandem-R PSA assays. Sixty-eight of the 92 sera had PSA values by the Tandem-R PSA of ≥0.2 µg/L, which we established as the current biological detection limit of this assay. The correlation coefficient between the IMx PSA and Tandem-R PSA assays among these 68 sera was 0.9990 (slope = 1.15).

Three representative serial studies along with a brief summary of each patient’s clinical course are illustrated in Figure 4. Both immunoassays tracked the patient’s clinical course extremely well at PSA concentrations ≥0.2 µg/L and, as expected from the correlation coefficient, no appreciable discordance at these concentrations was observed between the IMx PSA and Tandem-R PSA assays.

The results from patient 6737 B (Figure 4) warrant special mention. This patient had an increased PSA (~5.8 µg/L) with subsequent needle biopsy confirmation of prostate cancer. A radical prostatectomy revealed stage B disease of Gleason grade 2/2. The surgical margins and lymph nodes were negative for tumor. The retrospective serum obtained 4 months after surgery revealed an IMx PSA value of 0.07 µg/L (increased) with a historical Tandem-R PSA report of <0.4 µg/L and a current Tandem-R PSA assay reading of <0.2 µg/L. Four subsequent serum samples obtained during the next three years of follow-up revealed slowly increasing concentrations of PSA by the IMx PSA assay. Only at 3.5 years after surgery did his PSA concentration exceed 0.4 µg/L, which at the time of clinical consideration was the first indication of an “abnormal” post-surgical PSA, based on the historical Tandem-R biological threshold. This increase resulted in additional clinical follow-up, including a needle biopsy of the anastomosis. The biopsy showed recurrent disease and the patient received a course of radiotherapy. The high precision of the IMx PSA assay at low PSA concentrations (≥0.06 µg/L) also affords reliable tracking of responses to therapy, and in this particular case, revealed the slow decline in PSA from 0.54 to 0.10 µg/L over the next 6 months as a consequence of radiotherapy.

PSA quantification in women, cystoprostatectomy patients, and “cured” patients after radical prostatectomy. None of the subjects in this series should have PSA-producing tissues (normal or malignant); typically their sera would yield a Tandem-R PSA value <0.2 µg/L. Accordingly, simultaneous quantification by the Tandem-R PSA immunoassay was performed on some but not all of these sera; unless noted, only the IMx PSA results are presented for this series. Among the 53 samples from women, 50 (94%) had PSA ≤0.06 µg/L; the other 3 sera had PSA concentrations of 0.22, 0.22, and 0.50 µg/L. Repeat testing of these three sera yielded similar results. An additional serum from a different blood sampling more than 6 months later was available from two of these women, and both of these were still above normal (0.31 and 0.45 µg/L; data not included in series).

The cystoprostatectomy sera (n = 40) were from 26 men diagnosed as having transitional cell carcinoma of the bladder, who had undergone surgery removing the tumorous bladder and adjacent normal prostate. The sera in this series were acquired ≥8 weeks after surgery. All but one of these 40 sera had PSA <0.06 µg/L. The single sera with an above-normal PSA value had a concentration of 0.08 µg/L.

In the patients in whom a radical prostatectomy should have removed all normal and cancerous tissue,
given that the cancer was confined to the organ (pathological stages A and B), we evaluated the most recent serum samples from 11 patients whose clinical course during follow-up (range 24–97 months; median 38 months) was consistent with disease-free status. None of these sera had PSA concentrations ≥0.06 μg/L. To establish the consistency of PSA concentrations <0.06 μg/L in this group of patients, we determined PSA in an additional 27 sera obtained during follow-up. In 25 of the 27 sera, the results were consistently <0.06 μg/L. One patient had a single increased value of 0.10 μg/L, with two preceding and one subsequent value <0.06 μg/L. Another patient with stage B2 disease had two PSA readings of 0.09 μg/L within 8 weeks of each other 1 year after surgery; 19 months later, his PSA was <0.06 μg/L. Therefore, of 38 total determinations, 35 showed PSA concentrations <0.06 μg/L, with three transient increases of unknown etiology in the range 0.06–0.10 μg/L during follow-up.

**Discussion**

During the past few years numerous reports and extensive reviews have discussed the clinical merit of determining PSA values in patients with prostate carcinoma. The preponderance of these reports have relied on PSA values obtained from assays developed by two companies, the Tandem-R and Tandem-E double monoclonal sandwich-type assays of Hybritech and the standard RIA-type assay produced by Yang Laboratories. These assays have been well received by the medical community, although differences in the PSA calibrators have complicated the comparison of results obtained from the Hybritech and Yang PSA assays (14, 19, 25).

The results presented here, obtained with the IMx...
PSA immunoassay from Abbott Laboratories, provide substantial verification of acceptable performance of a PSA assay that differs from the other available assays in several respects, including automation, the proprietary MEIA technology, and a monoclonal–polyclonal antibody assay design. The purpose of this study was fourfold: to evaluate assay performance; to document the correlation in PSA readings between the IMx PSA immunoassay and the Tandem-R assay, currently the most widely used PSA immunoassay; to determine whether the IMx PSA assay afforded an improved LLD and lower biological limit of detection; and, if so, to initiate studies of the clinical benefits of an improved LLD.

The first two goals have been fully addressed in Results, and only a brief discussion of these data is necessary. In each of the studies of assay performance, including reproducibility, dilution linearity, curve storage, and "hook" effects, the IMx PSA assay provided highly acceptable results. Many of these tests were performed at PSA concentrations <0.2 μg/L and, even at concentrations as low as 0.04 μg/L, the reproducibility and linearity of dilutional results were impressive. In addition, there was an exceptional equivalence in PSA readings between the IMx PSA assay and the Hybritech Tandem-R from 0.2 to 6200 μg/L. Although not tested, this implies that a correlation, not equivalence, in PSA values also exists between the Yang Pros-check RIA and the IMx PSA immunoassay. Such comparisons would likely depend on use of the conversion table prepared by Graves et al. (14) for comparing Hybritech Tandem-R and Yang Pros-check sample values.

The issues of LLD, biological limit of detection, and clinical relevance involve distinct areas of investigation. In our initial abstract of this work, we used the term analytical sensitivity as being equivalent to the LLD (23). Rudy (26) and Anderson (27) suggest that the term LLD is more precise and should be used to describe the detection limits of an assay; we concur. Unfortunately, the method of determining the LLD is not standardized; however, we used nearly identical methods to establish these values for the Tandem-R assay (18) and the IMx PSA as reported herein. In brief, we use the mean ± 2 SD of a zero calibrator (or a control sera with "0" antigen), similar to that reported or suggested by others (28, 29). By this approach, the LLD for the IMx PSA assay was 0.02 μg/L with the zero calibrator and 0.03 μg/L with the negative control sera.

To avoid false-positive serum PSA readings after radical prostatectomy because of improved assay precision, we established a higher value, the biological detection limit, above which an assay result indicates a 95% probability of PSA presence even upon repeat testing of the sample. For the IMx PSA assay the biological detection limit was 0.06 μg/L. In comparison, we and others reported a corresponding value of 0.3–0.5 μg/L for the Tandem-R and Yang PSA assays (14, 18, 30). During the present study, we observed a smaller standard deviation in the precision of the Tandem-R assay than previously noted (18), in the range 0.2–0.4 μg/L; therefore, for data presented here, we used a revised biological detection limit for the Tandem-R, 0.2 μg/L.

The issues of LLD and biological threshold are clinically relevant only for measuring PSA after therapy, especially after radical prostatectomy, when theoretically the PSA content of serum should be zero. Improving the limits of detection as afforded by the IMx PSA assay provides no advantages in the diagnosis or staging of prostate cancer. Even for the post-therapy patients, concern has been voiced that having an earlier indicator of disease recurrence for a cancer for which no effective curative therapies exist only complicates patient management. We do not agree with this argument, believing instead that the accurate quantification of PSA in patients with minimal disease may eventually reveal previously unappreciated therapeutic opportunities.

Measuring PSA concentrations down to the ultra-low concentration will certainly require a new learning curve as such values are applied clinically. For those who will monitor post-radical prostatectomy concentrations of serum PSA with the IMx PSA assay, the clinical relevance of PSA values in the ultra-low range becomes important. We therefore refer to a clinical threshold, defined as the PSA value above which there is confidence of disease presence. With previous PSA assays, the clinical and biological detection limits were synonymous. However, with a biological detection limit of 0.06 μg/L (or even less, as future PSA assays are developed), conceivably a PSA result at or slightly above this value, even though accurately revealing PSA presence in the serum, does not indicate recurrent prostatic cancer. For example, occasionally during surgery, minute remnants of benign prostate tissue are inadvertently left intact (31). In addition, recent reports suggest that cells in the periurethral glands—microscopic clusters of cells along the urethra—can express PSA (32, 33). Although we observed similar indications of expression by immunohistology in some of these cells and have other supporting, preliminary results of periurethral gland production of PSA (34), it is uncertain whether the PSA from these microscopic structures, in the absence of recurrent disease, would result in detectable concentrations of serum PSA.

To explore the clinical threshold issue, we attempted to detect PSA in the sera of patients who had a cystoprostatectomy or who were long-term survivors of radical prostatectomy for organ-confined disease and presumed to be free of disease. Combining the data from these two series, we found that only 5% (4 of 80 sera) from 38 patients had PSA >0.06 μg/L, and none of these 4 exceeded 0.10 μg/L. The source of the transient PSA in these sera remains undefined. Although only 5% of these 80 sera revealed detectable PSA, a tentative and conservative setting of the IMx PSA clinical threshold at 0.1 μg/L seemed appropriate while we performed additional evaluations. For example, in a retrospective serial study of post-prostatectomy patients that was
independent of the study discussed in Results, the PSA concentration in all of the 25 patients who reached this conservative clinical threshold (often months to years after apparently disease-free status) not only remained above normal but continued to climb (unpublished data).

Of the 19 serial studies, the three selected for presentation (Figure 4) not only show the excellent concordance between the IMx PSA and Tandem-R PSA assays but also give insight into the complex clinical management of prostate cancer. In the top panel, the presence of residual disease after a radical prostatectomy is predicted from the pathological involvement of the lymph nodes and confirmed by a PSA concentration of 1.6 μg/L. Increasing PSA concentrations over the next 5 years was followed by a course of radiotherapy; this resulted in an 11-month slow decrease in PSA concentrations, from 22 to 0.3 μg/L.

The PSA profiles in the other two panels of Figure 4 are important because they reflect the potential opportunities and importance of detecting recurrent disease earlier than heretofore possible. For example, the PSA profile in the middle panel exemplifies the fact that PSA concentrations <0.06 μg/L do not guarantee an absence of disease. This should not be surprising: 0.06 μg of PSA per liter corresponds to several trillion molecules of PSA in the total circulation. In fact, we are currently engaged in an investigative prospective study of patients considered at risk of recurrence but whose PSA concentrations are undetectable, to determine whether a brief administration of androgens will result in a spur to the production to values that are detectable with the IMx PSA assay. The early detection and tracking of slowly progressing recurrent disease was clearly reflected by the IMx PSA profile of patient 6737B (Figure 4, bottom), in whom PSA increased from 0.07 to 0.60 μg/L over a period of 42 months. Furthermore, in our just completed study of 26 patients after radical prostatectomy, sufficient stored sera was available from 18 patients to expand upon this initial finding. Using 0.1 μg/L rather than 0.4 or even 0.2 μg/L to signify disease recurrence provided lead time advantages of months to years (unpublished data).

In conclusion, the new IMx PSA immunoassay will contribute well to the clinical management of patients with prostate cancer. PSA values >0.2 μg/L correlate exceedingly well with the Hybritech Tandem-R immunoassay. The 0.03 μg/L lower limit of detection provided by the IMx PSA immunoassay is unsurpassed by other current commercial assays, although custom modifications of the Hybritech and Yang assays (35) can result in improved lower limits of detection. We join Graves et al. (35) in urging users of ultrasensitive PSA assays to include at least two controls in the ultra-low-concentration range. For the IMx PSA assay, one of these should be close to the biological detection limit (~0.06 μg/L). We include two ultra-low-concentration controls prepared in a defined matrix in every IMx PSA assay, and make periodic performance checks with ultra-low-concentration human serum curve controls. Other users may elect to use ultra-low human serum controls as their primary form of quality assurance. Regardless of the method selected, quality assurance of acceptable assay performance in the ultrasensitive range is critically important. Finally, although the clinical value of applying lower limits of detection to the management of patients with prostate cancer has not been proven by the studies presented here, we are engaged in several prospective investigations of this with promising preliminary results.

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