Additional information on the proposed method is available on the Clinical Chemistry Online web site (http://www.clinchem.org/content/vol48/issue6).

References

Clinical Evaluation of the Elecsys Total Prostate-specific Antigen Assay on the Elecsys 1010 and 1010 Systems, Alexander Haese,1 Robert T. Dworschack,3 Steven P. Piccoli,2 Lori J. Sokoll,2 Alan W. Partin,2* and Daniel W. Chan2 (1 The James Buchanan Brady Urological Institute and 2 The Department of Pathology, The Johns Hopkins University School of Medicine, Baltimore, MD 21287;3 Roche Diagnostics, Indianapolis, IN 46256;4 Diagnostic Oncology, Seymour, CT 06483; * address correspondence to this author at: The Johns Hopkins University School of Medicine, James Buchanan Brady Urological Institute, 600 N. Wolfe St., Baltimore, MD 21287; fax 410-614-8096, e-mail apartin@jhmi.edu)

Prostate cancer (PCa) is a leading cause of cancer-related deaths. The American Cancer Society expects >198,000 newly diagnosed cases and 31,500 deaths related to PCa in the US in 2001 (1).

The indolent clinical course of PCa makes its early detection a challenge. Symptoms typically do not occur until PCa is locally advanced or metastasized, at which time cure is not usually possible. Early detection is therefore mandatory to reduce the mortality of PCa. Prostate-specific antigen (PSA) is a serine protease synthesized in prostatic epithelium. Introduced into clinical use in the late 1980s (2), PSA today is recognized as the most useful tumor marker in urologic oncology and as indispensable for early detection, staging, and monitoring of PCa (3).

Digital rectal examination (DRE) alone has a poor sensitivity of 2.2–25% (4, 5) and specificity of 39% (6) in detecting PCa. Measurement of serum PSA concentrations in combination with DRE markedly improves the detection of PCa and increases the lead time for diagnosis (4, 5).

This clinical study was designed to demonstrate the clinical utility of the newly developed Roche Diagnostics Elecsys® total PSA Assay on the Roche/Hitachi Elecsys® 2010 and 1010 systems. This assay is a quantitative in vitro diagnostic test for detection of total PSA (tPSA) and is indicated for measurement of tPSA in combination with DRE to aid in the diagnosis of PCa.

A total of 1121 serum samples for PSA analysis were prospectively taken from men referred for urologic evaluation (mean age, 66 years; range, 50–91 years). DRE was performed by urologists. Symptoms leading to referral were, e.g., voiding dysfunction, lower back pain, or perineal discomfort. No previous PSA values were available, nor had any patient undergone a previous biopsy. No DRE was performed <15 days and no medical treatment for benign prostatic hyperplasia was administered <90 days before serum collection.

All patients underwent DRE and transrectal ultrasound-guided biopsy of the prostate. A total of 1097 (97.7%) men received six or more biopsies. DRE and biopsy results were classified as either benign or cancerous.

The Elecsys PSA assay is a dual monoclonal antibody sandwich assay that recognizes free PSA and PSA complexed with α1-antichymotrypsin on an equimolar basis. The method is based on an electrochemiluminescent immunoassay technique in which a biotinylated anti-PSA monoclonal antibody and second anti-PSA monoclonal antibody labeled with a ruthenium-based reporter molecule react with PSA-α1-antichymotrypsin and free PSA to form a sandwich. This complex is bound to streptavidin-coated microparticles via the biotin-streptavidin interaction. The microparticles are magnetically captured on the electrode induces a chemiluminescent emission, which age to the electrode induces a chemiluminescent emission, which age to the electrode induces a chemiluminescent emission, which age to the electrode induces a chemiluminescent emission, which age to the electrode induces a chemiluminescent emission, which age to the electrode induces a chemiluminescent emission, which age to the electrode induces a chemiluminescent emission, which age to the electrode induces a chemiluminescent emission, which age to the electrode induces a chemiluminescent emission, which age to the electrode induces a chemiluminescent emission, which age to the electrode induces a chemiluminescent emission, which age to the electrode induces a chemiluminescent emission, which age to the electrode induces a chemiluminescent emission, which age to the electrode induces a chemiluminescent emission, which age to the electrode induces a chemiluminescent emission, which age to the elec...
curve recorded on the reagent barcode. The measurable ranges are 0.002–100 μg/L for the Elecsys 2010 and 0.006–100 μg/L for the Elecsys 1010. Total assay imprecision for the Elecsys PSA assay is 2.4–3.8% over the range 0.3–91.1 μg/L.

Passing–Bablok regression analysis was performed for PSA values obtained from the Elecsys 2010 vs 1010 assay. Because of the wide range, we separately analyzed the ranges <50 μg/L and >50 μg/L. The mean and median tPSA, the SD, and the mean difference for PSA were calculated for patients with DREs suspicious and nonsuspicious for PCa and for patients with and without cancer on biopsy. Each group was subcategorized into established PSA ranges (0.2–2.5, >2.5–4, >4–10, and >10 μg/L). The Wilcoxon signed-rank test was applied to detect statistically significant differences in PSA concentrations by both assays between the respective DRE or biopsy result groups.

Patients were divided into age groups (50–60, 60–70, and >70 years) for analysis of the influence of age on serum PSA for patients with and without evidence of cancer. The Kruskal–Wallis test was performed to evaluate the significance of age differences.

The incidence of PCa was evaluated for the entire patient cohort and in PSA ranges <2.5, ≥2.5–4, >4–10, >10–20, and >20 μg/L.

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) to detect PCa were calculated for DRE, PSA >2.5 μg/L, and PSA >4 μg/L on both the Elecsys 1010 and 2010 assays. Subsequently, the combination (DRE-positive or PSA >2.5 μg/L and DRE-positive or PSA >4 μg/L) for both assays was evaluated to check for improved diagnostic accuracy.

ROC curve analysis was performed for both assays, and areas under curve (AUCs) were calculated in the entire cohort of 1121 patients.

We then analyzed the subgroup of patients with unremarkable DRE to evaluate the sensitivity, specificity, PPV, and NPV for PSA at cutoffs of 2.5 and 4 μg/L in detecting those cancers that would have been missed if DRE was applied as a single diagnostic test. Mean and median PSA values were calculated for patients positive or negative for PCa, and the statistical significance of differences between groups was tested using Mann–Whitney U-analysis. Box-and-whisker plots were used to depict results. ROC analysis was applied to evaluate the utility of PSA in identifying patients with PCa in patients with a benign DRE.

DRE was nonsuspicious and suspicious for cancer in 917 (81.8%) and 204 (18.2%) patients, respectively. Prostate biopsy revealed no evidence of PCa in 730 (65.2%) and evidence of PCa in 391 (34.8%) men. These distributions are consistent with previously reported studies (4,7,8).

PSA values obtained on the Elecsys 1010, when compared with those obtained on the Elecsys 2010, yielded regression equations of \( y = 1.035x - 0.0226 \) \( (R^2 = 0.994) \) for 1106 samples with PSA <50 μg/L and \( y = 1.033x + 0.782 \) \( (R^2 = 1.0) \) for 15 samples with PSA >50 μg/L, suggesting measured concentration differences in PSA between the two assays of ~3% (Fig. 1).

PSA values obtained on the Elecsys 1010 were 0.03–2.20 μg/L (1.9–4.8%) higher than results obtained on the Elecsys 2010. This difference was statistically significant \( (P = 0.0158 \text{ to } <0.0001) \), but clinically, this difference is likely to be meaningless [see Table 2 in the data supplement, available with the electronic version of this Technical Brief on the Clinical Chemistry Online web site (http://www.clinchem.org/content/vol48/issue6)].

PSA values in patients with and without PCa displayed age dependency throughout the entire cohort of patients \( (P<0.0001; \text{ Table 3 in the data supplement}) \).

The incidence of PCa was 34.8% for all patients. In PSA ranges ≤2.5 μg/L to >20 μg/L, the incidence increased from 16.9% to 71.6% on the Elecsys 1010 and from 17.8% to 74.0% on the Elecsys 2010 (see Table 4 in the data supplement).

The sensitivity of DRE to detect PCa was poor. Only 103 of 391 cancers were detected by DRE (sensitivity, 26.3%), whereas 629 of 730 patients with a benign DRE did not have PCa on biopsy (specificity, 86.2%). At a cutoff of 4 μg/L, the sensitivity for PCa detection was 85.9% on the Elecsys 2010 and 87.5% on the Elecsys 1010. Sensitivities were >90–95% when the cutoff for PSA was lowered to 2.5 μg/L or when a combination of DRE and PSA cutoff of either 4 or 2.5 μg/L was applied. Complete data are shown in Table 1.

Calculation of ROC curves showed virtually identical curves for both assays. The AUCs [95% confidence interval (CI)] were 0.651 (0.617–0.685) and 0.650 (0.616–0.684) for the Elecsys 1010 and 2010, respectively, which was not statistically different.

In the subgroup of 917 men with benign DRE, PCa was found in 288 (31.4%) men. The incidence of PCa in this group thus was slightly lower than in the overall cohort (34.9%). Analysis of the 288 patients with PCa and unremarkable DRE showed that median PSA values were significantly higher in patients with cancer compared with no cancer (7.36 and 7.28 μg/L vs 5.72 and 5.49 μg/L; \( P <0.0001 \)) on both the Elecsys 2010 and 2010 assay (see Fig. 2 in the data supplement). With the Elecsys 2010 assay, 252 of 288 (87.5%) patients had a PSA >4 μg/L and 276 of 288 (95.8%) had a PSA >2.5 μg/L. The Elecsys 2010 measured a PSA >4 μg/L in 256 of 288 (88.8%) and a PSA >2.5 μg/L in 277 of 288 (96.1%) patients.

Calculation of sensitivity, specificity, PPV, and NPV revealed sensitivities between 87.5% (Elecsys 2010 with a cutoff of 4 μg/L) and 96.2% (Elecsys 1010 with a cutoff of 2.5 μg/L). Specificity was 9.9–25.1% (see Table 5 in the data supplement). The AUCs of the ROC curves for both assays were not significantly different [AUCs (95% CI) of 0.632 (0.593–0.670) and 0.633 (0.595–0.672), respectively].

In summary, PSA measurements on the Elecsys 2010 and 2010 systems provide comparable results. The incidence of PCa in a referral population in a PSA range <2.5 μg/L was 16.9%, whereas in the range 2.5–4 μg/L, it was 23%. DRE identified only 26.3% of all cases of PCa. A total of 629 of 730 (86.2%) men with a benign DRE were
negative for PCa on biopsy. The combination of PSA and DRE has a sensitivity of 91.8–97.2%, which increases the sensitivity of PCa detection by 60–70% when cutoffs of 4 and 2.5 μg/L are used, respectively. PSA has a high sensitivity (87.5–96.2%) in detecting those cancers that DRE fails to identify, at the same time reducing the number of unnecessary biopsies by ~25%. PSA measurements using the Elecsys 1010 and 2010 tPSA assays

![Regression plot for Elecsys 2010 vs Elecsys 1010.](image)

**Fig. 1.** Regression plot for Elecsys 2010 vs Elecsys 1010.

Passing–Bablok analysis (n = 1106) yielded a slope of 1.035 (95% CI, 1.030–1.040) and a y-intercept of −0.0226 (95% CI, −0.0433 to 0.0009). (Inset) analysis for PSA range >50 μg/L (n = 15). Slope, 1.033 (95% CI, 1.017–1.067); y-intercept, 0.782 (95% CI, −3.952 to 2.008).

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**Table 1.** Diagnostic performance of DRE and PSA cutoffs of 2.5 and 4 μg/L, alone or in combination, to detect PCa (all patients).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
<th>PPV, %</th>
<th>NPV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRE</td>
<td>26.3 (22.0–31.0)</td>
<td>86.2 (83.4–88.6)</td>
<td>50.5</td>
<td>68.6</td>
</tr>
<tr>
<td>E2010a &gt;4 μg/L</td>
<td>85.9 (82.1–89.2)</td>
<td>28.1 (24.8–31.5)</td>
<td>39.0</td>
<td>78.8</td>
</tr>
<tr>
<td>E1010 &gt;4 μg/L</td>
<td>87.5 (83.8–90.6)</td>
<td>25.9 (22.7–29.2)</td>
<td>38.7</td>
<td>79.4</td>
</tr>
<tr>
<td>E2010 ≤2.5 μg/L</td>
<td>95.1 (92.5–97.0)</td>
<td>12.1 (9.8–14.6)</td>
<td>36.7</td>
<td>82.2</td>
</tr>
<tr>
<td>E1010 ≤2.5 μg/L</td>
<td>95.4 (92.8–97.2)</td>
<td>12.1 (9.8–14.6)</td>
<td>36.7</td>
<td>83.0</td>
</tr>
<tr>
<td>DRE(+) or E2010 &gt;4 μg/L</td>
<td>91.8 (88.6–94.3)</td>
<td>19.7 (16.9–22.8)</td>
<td>38.0</td>
<td>81.8</td>
</tr>
<tr>
<td>DRE(+) or E1010 &gt;4 μg/L</td>
<td>90.8 (87.5–93.5)</td>
<td>21.6 (18.7–24.8)</td>
<td>38.3</td>
<td>81.4</td>
</tr>
<tr>
<td>DRE(+) or E2010 ≤2.5 μg/L</td>
<td>96.9 (94.7–98.4)</td>
<td>8.49 (6.6–10.8)</td>
<td>36.2</td>
<td>83.8</td>
</tr>
<tr>
<td>DRE(+) or E1010 ≤2.5 μg/L</td>
<td>97.2 (95.0–98.6)</td>
<td>8.50 (6.6–10.8)</td>
<td>36.3</td>
<td>84.9</td>
</tr>
</tbody>
</table>

*a E2010, Elecsys 2010; E1010, Elecsys 1010.
provided high sensitivity for PCA detection in this referral group of men.

This work was supported by the Deutsche Forschungsgemeinschaft (GZ Ha3168 1/1). The study design was based on a proposal by Diagnostic Oncology for Roche Diagnostics. Specimen recruitment, data generation, and database management were performed by Diagnostic Oncology under the sponsorship of Roche Diagnostics. This collaboration led to the submission (P990056) and the premarket approval of the Elecsys total PSA Assay to the Food and Drug Administration. Roche Diagnostics provided the database to Dr. Partin and associates for analysis. Roche Diagnostics facilitated the writing of the manuscript by supporting inquiries regarding the database and participating in review of the conclusions of the manuscript.

References

High-Throughput Analysis of Hemoglobin from Neonates Using Matrix-assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry, Urban A. Kiernan,² Jeff A. Black,³ Peter Williams,² and Randall W. Nelson¹

The risks associated with sickle cell and other untreated hemoglobinopathies have led to mandatory neonatal screenings in 41 of the 50 states in the US (1). Preliminary screenings are often performed by electrophoresis at alkaline pH on cellulose acetate, with follow-up analysis of abnormal samples by acid electrophoresis on citrate agar (2). Alternatively, isoelectric focusing (IEF) or HPLC can be used in the screenings (3), but other techniques offer advantages.

In the past decade, new mass spectrometry (MS) approaches, including matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS, have been applied to the study of proteins (4). MALDI-TOF MS offers a potent means of analyzing constituent chains of hemoglobin A (HbA) directly from samples as small as a single red blood cell (5). Mass accuracy and resolution are sufficient to determine the presence of point mutations within chains differing in mass by as little as ~20 Da. This ability allows detection of the βGlu→Val variant (~30 Da lower in molecular mass) during direct analysis of the intact chains. Moreover, the site of mutation can be determined by combining MALDI-TOF MS with specific enzymatic digestion, i.e., “mass mapping.”

Thus, MALDI-TOF MS has the potential to be used in place of conventional approaches (6) for HbAS/HbS screening in the general population. To date, however, the mass spectrometric approaches have not been taken to the high-throughput mode necessary for efficient population screening, nor have they been applied to neonatal blood samples, which contain roughly equal portions of HbA and HbF. We report here progress in the development of a high-throughput mass spectrometric screening approach capable of screening the hemoglobin of neonates at rates of ~100 samples/h. In a blind analysis, 96 neonatal blood samples were prepared for both intact protein molecular mass determination and mass mapping using parallel robotics (96-well format) and analyzed by MALDI-TOF MS for point mutations. Results from these analyses were later confirmed by IEF.

We studied 96 neonatal heel-stick/blotter-paper samples isolated in individual wells of a 96-well titer plate. The samples were extracted and processed in parallel by soaking each disk in 200 µL of 1 mL/L trifluoroacetic acid (TFA) for 3 min with gentle mixing in a robotic workstation (Multimek 96; Beckman). The TFA liberated protein from the paper and disrupted the interactions between hemoglobin subunits. The acidified hemoglobin solutions were diluted 500-fold in a mixture of 1 mL/L TFA and 1 mmol/L N-octylglucoside and dispensed onto 96-well-formatted MALDI-TOF MS targets for either intact protein analysis or tryptic mass mapping.

For intact protein analysis, samples were prepared in parallel on the robotic workstation by depositing 2 µL of diluted hemoglobin solution and 2 µL of MALDI matrix solution [saturated sinapic acid in a 1:2 (by volume) mixture of acetonitrile–2 mL/L aqueous TFA] on a 96-well-format hydrophobic/hydrophilic contrasting MALDI-TOF MS target (7); samples were then allowed to air dry. The MS analysis was performed on a Bruker Biflex III MALDI-TOF mass spectrometer operating in linear delayed-extraction mode with 19.00 kV full accelerating potential. Draw-out pulses of 1.700 kV (300-ns delay) were used for parent chain analysis. Mass spectra were acquired automatically by summing 5–10 laser shot spectra while gauging spectral quality with fuzzy logic routines (8).