Standardization of Prostate-Specific Antigen (PSA) Assays: Can Interchangeability of PSA Measurements Be Improved?

The landscape of prostate cancer has changed since the appearance of the first prostate-specific antigen (PSA) assay. PSA testing has gained rapid recognition since M. Kuriyama et al. *(1)* of Roswell Park first reported clinical studies using an enzyme immunoassay with anti-PSA rabbit antibody. Mikolajczyk and coworkers *(2, 3)* reported the presence of several free PSA isoforms and the potential application of free PSA isoforms as serum markers. Today, more than 30 types of total PSA assay reagent sets and ~10 types of free PSA and PSA–α_1-antichymotrypsin (ACT) assays, based on various principles, are available.

Key elements in PSA measurement are interchangeability of assays and stability of serum samples before tests *(4, 5)*. Efforts to standardize PSA assays were initiated in 1992 at the First Stanford Conference, organized by T. Stamey. At the Second Stanford Conference on International Standardization of Prostate-Specific Antigen (1994), Stamey et al. *(6)* proposed a primary calibrator consisting of 90% purified PSA-ACT and 10% free PSA (90:10) on a molar basis. Subsequently, the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) issued a document *(7)* recommending a set of 3 distinct materials containing 100% free PSA, 100% PSA-ACT, and 90% PSA-ACT:10% free PSA. This document led to further activity in the harmonization of PSA assays.

In 1999, Robert M. Nakamura, a member of the International Consultation Committee on Prostate Cancer, made recommendations, with his colleagues *(8)*, on standardization and quality assessment of PSA immunoassays. The recommendations included the following: (*a*) free PSA should be the primary standard for free PSA and total PSA immunoassays; (*b*) assays should exhibit an equimolar response to free PSA and PSA-ACT complexes; (*c*) assay antibodies to free PSA should not cross-react with human kallikrein 2 (hK2); (*d*) reference values for free PSA/total PSA need to be established for each assay combination; and (*e*) clinical laboratories should specify the name of the assay on their reports.

Kuriyama et al. *(9)* studied 9 commercially available assays for total PSA and described equations to calculate the expected Tandem-R PSA (Beckman Coulter) results from the PSA concentrations obtained with the other methods studied. That report has been widely used in clinical studies. At the time of the report by Kuriyama et al., however, the concept of equimolarity of assays was not well established, and the risk of converting results among assays, without knowing the reactivities of the antibodies in the different reagent sets, was not assessed. The heterogeneity of PSA forms in serum was later described in detail *(10)*, and the effect of that heterogeneity on results of immunoassays became fully appreciated.

In 1997, the Committee of Investigation on PSA Assay (sponsored by the Japanese Urological Association and the Japanese Society of Laboratory Medicine) carried out a survey of the variability among assays. Patient serum, purified 100% free PSA, and purified 100% PSA-ACT were prepared for survey samples. We studied 28 total PSA assays from 22 manufacturers and analyzed the reactivity ratios of free PSA to PSA-ACT in those assays by dilution tests using purified 100% free PSA and purified 100% PSA-ACT. The study revealed that the differences among measurement schemes were related to differences in the reactivities of antibodies for free PSA and PSA-ACT caused by the variety of PSA molecular forms.

In 2000, we performed another survey focusing on the clinically important total PSA range up to 20 μg/L *(11)*. In that survey, 26 assays from 18 companies were investigated. During the 3 years between our surveys, the reactivities of most assays for total PSA had been improved. In the initial survey, only 34% of the assays showed an equimolar response to free PSA and PSA-ACT. The minimum and maximum measured values were 4.1 and 32.3 μg/L for the serum sample containing >40% free PSA *(11)*.

Large disparities persist among assays, however. We attribute this variability, in part, to matrix differences between serum samples and the WHO buffer-based reference material used for assignment of values to the assay calibrators. Anti-PSA antibodies show different reactivities and affinities for the various forms of free PSA and PSA-ACT in buffer- or serum-based samples.

The goal of PSA standardization efforts is to improve harmonization of results for patient serum samples, but the WHO primary standard materials use buffer with added bovine serum albumin, and assay calibrators use various matrices. Ideally, the total PSA assay should have an equimolar response for free PSA and PSA-ACT.

We examined the matrix effect among the buffer- and serum-based samples by measuring purified free PSA and PSA-ACT. The differences in the measured concentrations for buffer-based vs serum-based samples reported by the AxSYM (Abbott Diagnostics) and ADVIA Centaur (Bayer) were <4%, in contrast to those reported by the Elecsys (Roche Diagnostics), Access (Beckman Coulter), and Immulite (Diagnostic Products Corp.), which were 9%, 7%, and 18%, respectively. In all cases, PSA-ACT showed greater reactivity than did free PSA.

The standardization of PSA assays is limited because only primary reference materials (WHO reference materials) have been prepared and because PSA does not have a total reference measurement system including reference materials and a reference measurement procedure. To further promote standardization, a reference measurement system should be established to transmit the accuracy of the buffer-based primary standard material to patient serum, which is the final goal.

In a comprehensive study in this issue of *Clinical Chemistry*, Stephan et al. *(12)* show that interchangeability

among methods is a problem for clinical samples. In that study, they took great care when storing samples and performing the assay, and the reliability of the measured values was high. The percentage free PSA (%fPSA) is related to both total PSA and free PSA and needs to be evaluated carefully. As Stephan et al. indicate, for patients in whom the %fPSA determines whether a prostate biopsy is performed, the result might depend on the assay used. Thus it seems important that total and free PSA be measured by compatible methods.

Some important requirements for studies that compare several assays include that the comparison method chosen gives equimolar responses for free PSA and PSA-ACT and that it has minimal susceptibility to matrix effects. We have confirmed that the Access (Hybritech) total PSA assay, which was used as the comparison method in the study by Stephan et al. *(12)*, does not have an adequate equimolar response to free PSA and PSA-ACT in spite of using the same antibodies as the Tandem-R PSA assay.

One difficulty in the study of Stephan et al. *(12)* may be the calculation of free PSA from the PSA-ACT and total PSA values obtained by the ADVIA Centaur assay. Although most molecules of complexed PSA are combined with ACT, some complexed PSA that is measurable by immunoassay is combined with other protease inhibitors, such as α_1-proteinase inhibitor, inter-α-trypsin inhibitor, and others *(13)*. Thus, the concentrations of total PSA, free PSA, and PSA-ACT measured by assays of Eiken Chemical Company do not necessarily satisfy the equation fPSA + cPSA = tPSA in our study (where fPSA is free PSA, cPSA is complexed PSA, and tPSA is total PSA).

In conclusion, at present, samples should be preserved until measurement is carefully performed, and the assay name and reference interval should be listed on reports.

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DOI: 10.1373/clinchem.2005.061325