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# Prostate Specific Antigen Assay Standardization Bias Could Affect Clinical Decision Making

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**Purpose:** Although prostate specific antigen is widely used to detect and manage prostate cancer, many patients and physicians are unaware of which prostate specific antigen assay is being used. Most commercial prostate specific antigen assays are standardized to the WHO 90:10 standard or aligned with the original Hybritech® assay with potentially disparate results.

**Materials and Methods:** A total of 1,916 men participated in a prostate cancer screening study in 2007. On the day of collection prostate specific antigen was tested from the same serum sample using the Access® (Hybritech standard) and ADVIA Centaur® (WHO 90:10 prostate specific antigen standard) assays. We examined the differences between the 2 assays and the effect that this might have on clinical decisions.

**Results:** Median prostate specific antigen was 0.9 and 1.05 ng/ml for the Centaur and Access assays, respectively, representing a 17% difference. Mean prostate specific antigen was 3.45 and 4.79 ng/ml, respectively, representing a 38% difference. Using a prostate specific antigen threshold of 2.5 ng/ml 5% of men would have been recommended to undergo biopsy using the Access but not the Centaur assay. Furthermore, prostate specific antigen differed by greater than 0.4 ng/ml in 26%, greater than 0.75 ng/ml in 14.5% and greater than 2 ng/ml in 4.5% of men in the same sample simply by using the different assays.

**Conclusions:** In our prospective screening population median prostate specific antigen was 17% lower using WHO vs Hybritech based assay standardization. As such, if these assays were instead used on a serial basis in the same patient, this could lead to false acceleration or false deceleration in prostate specific antigen velocity. Thus, the assay may influence the likelihood of prostate biopsy and, thereby, prostate cancer detection.

*Key Words: prostate, prostate-specific antigen, prostatic neoplasms, biopsy, reference standards*

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It is estimated that in 2008 there would be 186,320 new prostate cancer cases diagnosed and 28,660 deaths from prostate cancer in the United States.<sup>1</sup> The National Comprehensive Cancer Network recommends offering a baseline PSA test and digital rectal examination at age 40 years, which is used to determine the subsequent screening protocol.<sup>2</sup> The American Cancer Society and American Urological Association recommend annual prostate cancer screening in all men beginning at age 50 years or in men in the fifth decade of life who are deemed to be at high risk for prostate cancer due to a strong family history of prostate cancer or black American heritage.<sup>3</sup> There are more than 54 million men in the United States older than 50 years. Thus, prostate cancer screening issues affect a large proportion of the American male population. According to industry estimates more than 35 million PSA tests are performed each

year, of which about 20 million are used for prostate cancer screening (Beckman Coulter, Inc., personal communication).

The original Tandem®-R PSA assay was developed at Hybritech and approved by the United States Food and Drug Administration in 1986. PSA testing was initially approved to aid in the treatment of patients who were already diagnosed with prostate cancer. In 1991 a large study demonstrated that prostate cancer screening using PSA (Hybritech assay) and digital rectal examination was superior to that of digital rectal examination alone.<sup>4</sup> Based on the results of this study and others the Food and Drug Administration approved the PSA test as an aid to the early detection of prostate cancer in 1994, using a threshold value of 4.0 ng/ml.

Since that time, there has been a proliferation of commercial PSA assays, frequently yielding appreciably different results in the same patient and engendering legitimate concern over the interchangeability of PSA measurements. Based on a report from the College of American Pathologists Ligand Assay Survey Committee major differences in PSA results were observed among different manufacturers.<sup>5</sup> In 1999 the WHO Expert Committee on Biological Standardization adopted a mixture of 90% complexed PSA and 10% free PSA as one of the new standards (first IS 96/670 stan-

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dard material). Currently most PSA tests in the United States are aligned to the original Hybritech standard and/or the WHO 90:10 PSA standard.

Despite reports suggesting as much as a 20% difference between PSA measurements based on the WHO 90:10 and Hybritech PSA standards the clinical community remains largely unaware of this issue. Indeed, many clinicians use PSA measurements to make patient treatment decisions without considering which assay was used. Therefore, we further examined the differences in PSA measurements between a WHO based and a Hybritech based assay in the same serum sample in a large prospective screening population. Furthermore, we evaluated the effect that these differences could have on clinical cutoff points and determinations of PSA kinetics.

## MATERIALS AND METHODS

In April 2007 community dwelling men in the Chicago metropolitan area were invited to participate in a free prostate cancer screening in collaboration with the National Prostate Cancer Coalition. All participants provided informed consent and the study protocol was approved by the institutional review board.

A total of 1,916 men underwent a PSA test and digital rectal examination as part of the initial screening round in this program. On the day of collection PSA was tested from the same serum sample using the Hybritech Access assay with Hybritech standardization and the ADVIA Centaur assay with WHO 90:10 PSA standardization.

These data were used to examine differences between the 2 PSA measurements and the effect that this might have on patient treatment decisions. Specifically we created a Bland-Altman plot of the agreement between assays. In addition, descriptive statistics were performed to evaluate the number of men who would have met different clinical cutoff points using each PSA assay. Subgroup analysis was performed to evaluate the distribution of PSA in the 1,746 men between ages 40 and 75 years who might represent a typical screening population.

To examine the potential confounding of PSA velocity by assay standardization bias we used dynamic measurements simulation. Specifically we calculated the absolute difference between Access and Centaur total PSA measurements in the same serum sample to simulate a situation in which the 2 assays would be used on a serial basis. We also created a Bland-Altman plot of agreement. SAS® 8.2 was used for all statistical analyses.

## RESULTS

The [table](#) lists study population demographics. Median age was 57 years and the mean body mass index was 28.72 kg/m<sup>2</sup>. Of the men 55% were white.

The [figure](#) shows a Bland-Altman plot of the agreement between PSA assays. Median PSA was 0.9 and 1.05 ng/ml for the Centaur and Access assays, respectively, representing a 17% difference. Mean PSA was 3.45 and 4.79 ng/ml, respectively, representing a 38% difference. In the subset of men 40 to 75 years old median PSA was 0.8 ng/ml for the Centaur assay and 1.03 ng/ml for the Access assay. Mean PSA in this subgroup was 3.12 and 4.43 ng/ml, respectively.

<i>Study population characteristics</i>	
Median age (range)	57 (30–98)
Mean ± SD body mass index (kg/m <sup>2</sup> )	28.72 ± 5.08
% Race:	
White	55.6
Black	21.6
Other	22.8
PSA (ng/ml):	
Mean Access (range)	4.79 (0–109)
Mean Centaur (range)	3.45 (0–75)
Median Access	1.05
Median Centaur	0.9

Using a PSA threshold of 2.5 ng/ml 94 men (5%) would have been recommended to undergo prostate biopsy using 1 assay but not the other. Specifically 82 men (4.3%) would have been recommended to undergo biopsy using only the Access assay, whereas 12 (0.6%) would have been recommended to undergo biopsy using the Centaur but not the Hybritech assay.

Likewise, using a PSA threshold of 4 ng/ml 87 men (4.5%) would have been recommended to undergo prostate biopsy using 1 assay but not the other. Specifically 78 (4%) and 9 men (0.5%) would have been recommended to undergo biopsy using only the Access and only the Centaur assay, respectively.

Assay standardization also had the potential to confound clinically useful PSA kinetic measurements. A total of 499 men (26%) had a PSA difference of greater than 0.4 ng/ml. This apparent PSA increase would have been found in 470 men (24.5%) if the Centaur assay had been used before the Access assay, and in 29 (1.5%) if the Access assay had been used before the Centaur assay.

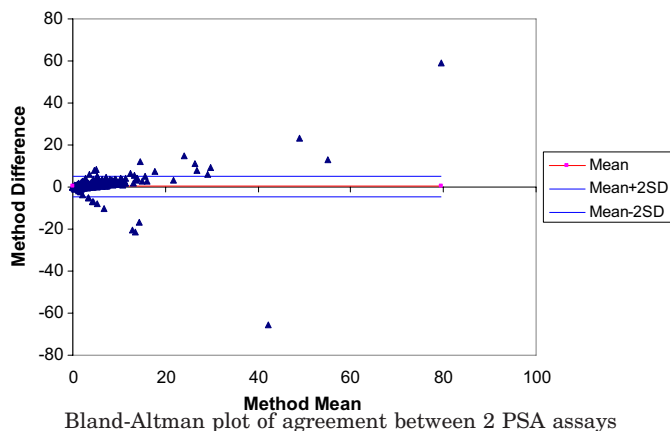
A difference of greater than 0.75 ng/ml was present in 277 men (14.5%) men. This kinetics pattern would have been found in 253 men (13%) using the Centaur assay followed by the Access assay and in 24 (1.3%) using the Access assay followed by the Centaur assay.

Finally, 86 (4.5%) men had a PSA difference of more than 2 ng/ml in the same sample simply by using the 2 assays. This pattern would have been found in 74 men (3.8%) using the Centaur assay followed by the Access assay and in 12 (0.6%) using the Access assay followed by the Centaur assay.

## DISCUSSION

PSA is used widely for important clinical decisions. Currently available commercial PSA tests are primarily aligned to the WHO 90:10 PSA standard or to the original Hybritech standard. Although many patients and physicians are unaware of which PSA test is being used when interpreting a given result, our study shows that PSA may be considerably different when using WHO based and Hybritech based standardization.

Our results are in agreement with prior studies suggesting that total PSA results based on the WHO 90:10 PSA standard are typically about 20% lower than those based on the Hybritech standard. Overall the effect of differences in PSA standardization on clinical outcomes has been the subject of several recent studies. For example, Link et al observed that PSA measurements made with Access, an automated version of the original Hybritech Tandem-R, were 1.23 times higher than those of the Centaur assay ( $p < 0.0001$ ).<sup>6</sup> In their study differences would have potentially altered the biopsy



recommendation in 19% of men in their prostate cancer screening program, which was even higher than the rate in our study.

Similarly in the European Randomized Study of Screening for Prostate Cancer biopsy was recommended for a PSA of greater than 3.0 ng/ml using the Hybritech Tandem-E assay.<sup>7</sup> Blijenberg et al noted on statistical modeling that using a hypothetical assay X with results that differed from those of the Tandem-E by 5%, 10% or 20% would have changed the recommendation for biopsy in 155, 504 and 1,133 men, respectively.<sup>7</sup>

Finally, Stephan et al compared free and total PSA results among the AxSYM®, Access, Immulite® 2000, Elecsys® 2010 and ADVIA Centaur assays.<sup>8</sup> After assigning the Access assay a value of 100% the other assays yielded total PSA results of 87% to 115%. Similar variability was observed for free PSA, leading Stephan et al to likewise conclude that commercially available assays are not interchangeable and can result in different clinical decisions.

In addition to total PSA measurements, PSA based adjunctive parameters to predict prostate cancer and its aggressiveness have become increasingly popular in recent years and the effect of the PSA standardization dilemma on these parameters is underappreciated. For example, PSA velocity is a measurement of the change in PSA per year. PSA velocity greater than 0.75 ng/ml per year has traditionally been used as a means to distinguish prostate cancer from benign prostatic conditions.<sup>9</sup> More recently lower PSA velocity thresholds in the range of 0.4 ng/ml per year have been recommended to help predict prostate cancer, particularly in men with a total PSA of less than 4 ng/ml.<sup>10</sup> However, our results demonstrate that 14.5% and 26% of men, respectively, would have exceeded these thresholds merely based on differences in PSA assay standardization.

Recent studies have also shown that men with a pretreatment PSA velocity of more than 2 ng/ml per year are at 9.8 and 12-fold increased risk for prostate cancer death after radical prostatectomy and radiation therapy, respectively.<sup>11,12</sup> It is important to note that these studies were calculated based on the Hybritech PSA standard. The same patient using a WHO based standardized test would have seen an increase of only about 1.6 ng/ml per year and, therefore, would have had a false perception of lower risk.

Furthermore, using different assays on a serial basis could also confound the application of PSA kinetics to predict prognosis. If a PSA test based on the Hybritech stan-

dard is used 1 year, followed by a WHO based test the next year, it may appear that patient PSA has remained constant, when in fact there has been a significant increase in PSA. Conversely using a WHO based assay, followed by a Hybritech based assay would falsely exaggerate the apparent PSA velocity. Thus, if the 2 patients were subsequently diagnosed with prostate cancer, the latter would be considered to be at greater risk for aggressive disease. Indeed, 4.5% of the men in our study would have falsely appeared to have the fatal PSA velocity of more than 2 ng/ml per year simply due to using different assays on a serial basis.

A contributing factor to the differences in assay results observed in this study is likely to be PSA standardization and differences in mass assignment between the 2 materials.<sup>13</sup> The WHO 90:10 PSA standard was based on the PSA molecular weight of 28,430 Da and an extinction coefficient at 280 nm of 1.84 l/gm/cm instead of the Hybritech standard of a molecular weight of 32,000 Da and 1.45 l/gm/cm, respectively.<sup>14</sup> In addition to the standard materials used, the combination of assay reagent components, assay design and instrumentation contribute to the final PSA results. Some early PSA assays were not equimolar, ie they measured free and complexed PSA differently.<sup>15</sup> Since not all patients have an equal percent of free and complexed PSA, nonequimolar assays might produce different total PSA results.

This study suggests that differences in assay standardization as well as assay configuration may compromise the accuracy and interpretation in a man of PSA and PSA derivatives frequently used in clinical practice. Patient PSA is approximately 20% lower when testing is performed using WHO standardized commercial tests compared to Hybritech standardization based assays. As such, the relatively recent adoption of WHO based assays by numerous manufacturers without a corresponding change in the recommended clinical cutoff can confound the informed use of PSA based parameters to make patient treatment decisions. For example, cross-application of a specific threshold value such as 4.0 ng/ml to WHO based measurements may not be appropriate. To account for standardization related differences manufacturers should provide validated assay specific threshold values or offer dual standardization.

A limitation of our study is that the data were derived from the first round of a contemporary screening study. Additional followup would allow us to further explore the potential effects of assay standardization bias on cancer detection and long-term outcomes. In addition, PSA measurement was higher with the Centaur assay in about 0.5% of cases, likely representing laboratory error.

Overall to treat men according to generally accepted standards of care physicians should be aware of these significant differences between PSA results. An initial PSA measurement using a Hybritech standard based assay and a repeat measurement based on the WHO standard could lead to confounding values with the second test lower than the first test or to delayed treatment, while the reverse testing order could lead to delayed diagnosis or more aggressive treatment than necessary. Thus, greater awareness of the differences in PSA assay standardization is crucial to enable the correct interpretation of PSA results. To make this possible manufacturers and clinical laboratories should provide information about which standardization material is used in their test. The laboratory report should state clearly the name of the assay manufacturer. More importantly the rec-

ommended threshold values relevant to that standardization method should be provided.

## CONCLUSIONS

In our 2007 prospective screening population mean PSA results obtained with a WHO standardized assay were 38% lower (median 17%) than with an assay based on Hybritech standardization. Thus, clinical PSA cutoff points derived from studies using 1 standard may not be applicable to the other standard. Approximately 1/20 men (5% of screening participants) would have been recommended to undergo prostate biopsy with the Hybritech standardized test but not with the WHO standardized test.

Furthermore, if serial PSA measurements do not come from tests aligned to the same standard, there could be the false impression of increasing or decreasing PSA when there is actually no change. Indeed, 26%, 14.5% and 4.5% of men had a potentially misleading PSA difference between the 2 tests (greater than 0.4, 0.75 and 2.0 ng/ml, respectively), confounding the use of PSA kinetics measurements. A greater recognition of PSA standardization issues is warranted among patients, physicians and testing laboratories to enable the accurate and judicious use of PSA based parameters for clinical decision making.

### Abbreviations and Acronyms

PSA = prostate specific antigen

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## EDITORIAL COMMENTS

These authors report that despite the existence of an international standard for PSA there remain significant differences among commercial assay systems. The analysis clearly demonstrates the impact that such differences can have on the patients being tested. For example, when using a threshold of 4 ng/ml, the Access assay recommends almost 9 times as many men for biopsy compared with the Centaur assay.

It is important to remember that assays suffer not only from the lack of calibration to a single standard, but also from a nonequimolar response to PSA isoforms, making the interpretation of results even more challenging for physicians and patients alike. The authors claim that clinicians should have greater awareness of the differences that currently exist among assay methods and they suggest using method specific cutoffs to ensure optimum patient care. While such additional information can alleviate problems in the short term, it will only serve to maintain a confusing and complex system for interpreting results in the longer term. A more appropriate solution is for all manufacturers to ensure that their assays are calibrated against the WHO standard and equimolar in response to PSA isoforms, avoiding the need for method specific cutoffs and simultaneously enhancing patient care.

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This study underlines a topic that has been widely discussed in the last years and summarized in our recent publication, mainly from an analytical point of view.<sup>1</sup> Despite the introduction of the WHO standards for total and free PSA, to which many manufacturers declare that they are aligned,

several groups have reported discordant results and patient misclassification between methods.<sup>2</sup>

When the new WHO calibration is adopted, there is as a consequence the problem of maintaining the 4.0 ng/ml traditional cutoff, which is widely accepted but referred to as the original Hybritech calibration. Considering that a documented negative bias exists between WHO and Hybritech calibration material, it is necessary to redefine a population cutoff for WHO calibration (reference 6 in article). We can agree with the conclusion of these authors regarding the strong message that laboratories should declare the method and calibration standard that were used and clinicians should pay attention to this information and refer to appropriate cutoffs to avoid misleading patient classifications.

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**REPLY BY AUTHORS**

Consistent PSA measurements are essential and must be used in an informed way to make valid biopsy and treatment recommendations. PSA levels frequently are 20% to 25% lower using WHO aligned assays compared to Hybritech aligned assays but the difference may vary considerably

from patient to patient. The adoption of different PSA standards has created new issues in applying established clinical parameters determined largely with the traditional Hybritech standard. Therefore to correctly interpret PSA results, physicians and patients must be aware of which standard is being used. Repeat PSA testing in a patient using a different assay can underestimate or overestimate total PSA, free PSA, PSA density, PSA velocity and PSA doubling time, which could potentially delay diagnosis, affect treatment choice, and confound monitoring and assessment of treatment outcomes.

We agree that not all differences between PSA assay results are due solely to standardization bias and that there are other possible sources of discordance. Although standardization bias usually can be corrected for within the same manufacturer assay platform, not all differences can be eliminated by a correction factor across different manufacturer assay platforms. Therefore, we do not believe that using only the WHO standard could provide valid information concerning prostate cancer risk and aggressiveness in all patients.

We recommend that when using a WHO aligned assay, physicians should consider using a total PSA cutoff of 3 instead of 4 ng/ml or 2 instead of 2.5 ng/ml. If physicians wish to rely on parameters established using a Hybritech standardized assay, they should consider retesting patients with PSA between the age specific median and either 2.5 or 4.0 ng/ml with a Hybritech standardized assay, depending on which cutoff they use to recommend biopsy. Similarly physicians should consider assay standardization bias when evaluating PSA kinetics and percent free PSA. Patients with values near critical thresholds should be retested with a Hybritech aligned assay if clinical decisions are to be based on parameters established with that assay.