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Review article
Pregledni članak

THE CLINICAL IMPACT OF WHO STANDARDIZATION OF PSA ASSAYS

KLINIČKI UTICAJ WHO STANDARDIZACIJE NA PSA TESTOVE

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Summary: The determination of serum level of the prostate specific antigen (PSA) is widely used for detection and management of prostate cancer. Analytical variability between the various PSA assays on the market has been reported. This discrepancy in the PSA results was shown to be related to non-equimolar detection of total PSA (tPSA) but also to a lack of assay standardization and could have serious clinical repercussions on the diagnostic performance of PSA testing. The recalibration of equimolar assays to common reference preparations (tPSA WHO 96/670 and fPSA 96/668) was thought to promote standardization of PSA assays and limit the clinical implication of assay variability. Comparison studies have demonstrated that PSA assay calibration to the WHO standard certainly improves the harmonisation of PSA testing, but differences between assays remain. Recent evaluations of the clinical impact of analytical variations induced by a calibration to the WHO standard reported that 15% to 30% of prostate cancer could be missed if the historical tPSA cut-off was used. In order to avoid unacceptable erosion of the clinical diagnostic performance of PSA determination for the detection of prostate cancer with WHO calibrated assays, it is critical to define new specific clinical decision points.

Keywords: PSA, total PSA, free PSA, prostate cancer, WHO calibration, assay standardization

Kratak sadržaj: Određivanje nivoa antigena specifičnog za prostatu (PSA) u serumu ima široku primenu u otkrivanju i praćenju toka bolesti kod raka prostate. Pokazalo se da između različitih testova za određivanje PSA koji postoje na tržištu postoji analitička varijabilnost. Utvrđeno je da je neslaganje u rezultatima PSA u vezi sa neekvimolarnim određivanjem ukupnog PSA (tPSA), ali i sa nedostatkom standardizacije testova za određivanje, i može imati ozbiljne kliničke posledice na dijagnostičke karakteristike testiranja PSA. Veruje se da rekalibracija ekvimolarnih testova do uobičajenih referentnih standarda (tPSA SZO 96/670) i tPSA 96/688) pomaže standardizaciju testova za određivanje PSA i ograničava kliničke implikacije varijabilnosti testova. Uporedne studije pokazale su da kalibracija testova za PSA prema standardima SZO značajno unapređuje harmonizaciju analiziranja PSA, ali razlike između testova i dalje postoje. Nedavna ispitivanja kliničkog uticaja analitičkih varijacija zbog kalibracije prema standardu SZO pokazala su da bi se moglo prevideti 15 do 30% slučajeva raka prostate ako bi se koristile zastarele »cut-off« vrednosti. Kako bi se izbegla neprihvatljiva erozija kliničke dijagnostičke performase određivanja PSA u cilju otkrivanja raka prostate pomoću testova kalibrisanih prema SZO, neophodno je odrediti nove specifične tačke za kliničko odlučivanje.

Ključne reči: PSA, ukupni PSA, slobodni PSA, karcinom prostate, WHO kalibracija, standardizacija testa

Introduction

Prostate specific antigen (PSA) is the most widely used serum biomarker for the screening and monitoring of prostate cancer (PCa). PSA was first described in 1971 as a marker for human semen in forensic medicine and was termed »γ-seminoprotein« (1). This biomarker was purified and subsequent studies reported its detection in human serum with potential utility in PCa detection (2, 3). In 1987, Stamey et al. (4) reported that serum level of PSA increased with advancing clinical stage of PCa and was proportional to the estimated volume of the tumor. However, the serum level of PSA was also increased in a variety of clinical conditions affecting the prostate gland including benign hyperplasia and prostatitis. This lack of specificity and low positive predictive value of the PSA marker raised doubts on the clinical utility of PSA in detecting PCa. But several studies demonstrated the

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use of serum PSA in association with DRE as a first-line test for PCa screening (5–7). In 1994 serum PSA determination was approved by the U.S. Food and Drug Administration (FDA) as an aid for early detection of PCa. To improve the clinical specificity of PSA, the determination of serum level of PSA molecular isoforms such as free PSA (fPSA) and its ratio to total PSA (%fPSA) were introduced (8). Variation of total PSA (tPSA) measurement including kinetic parameters (PSA velocity, PSA doubling time), and PSA density were also considered for the management of PCa patients (9). The subsequent widespread use of PSA testing has deep consequences on the diminution of age-adjusted incidence and mortality of PCa (9).

Taking advantage of the pioneer work on PSA, Hybritech Corporation developed the Tandem-R assay, that was the first FDA approved PSA assay, in 1986. Using this method, a multicenter prospective study validated a clinical decision point of 4.0 ng/mL of serum PSA for early detection of PCa (10). Referring to the Hybritech standard, numerous PSA assays were subsequently developed and brought to the market promoting the 4.0 ng/mL PSA cut-off.

Several studies have showed that very discordant results of tPSA and fPSA concentrations are obtained with different assays (11, 12). The clinical impact of these analytical discrepancies between the assays was also reported (13–15). Non-equimolar detection of tPSA and fPSA and non-uniform assay calibration were suggested to explain the assays variability (13, 16). In an attempt to harmonize the PSA detection across manufacturers, reference materials were developed for assay calibration and were later adopted by the World Health Organization (WHO) as reference material for tPSA assays (WHO 96/670) and a separate standard for fPSA was adopted (WHO 96/668) (17–19).

Despite assay calibration of PSA assays to common WHO reference material, a recent study reported that interchangeability of PSA measurements is not achieved and uniform clinical decision points cannot be used without serious clinical implication for the diagnosis of PCa (20). In this review, we are aiming to summarise some results presented at the 2008 annual meeting of the European Association for Urology held in Milan, demonstrating the clinical implications of recalibrating PSA assays to the WHO standard when the clinical decision point is not correctly adjusted to the assay calibration.

PSA assays equimolarity

The major form of PSA in serum is complexed to the α1-anti-chymotrypsin. This complexed PSA is present in a higher proportion in patients with PCa. Conversely, the ratio of unbound PSA (fPSA) to tPSA is lower in patients with PCa compared with patients with benign prostate disease. Therefore, accurate

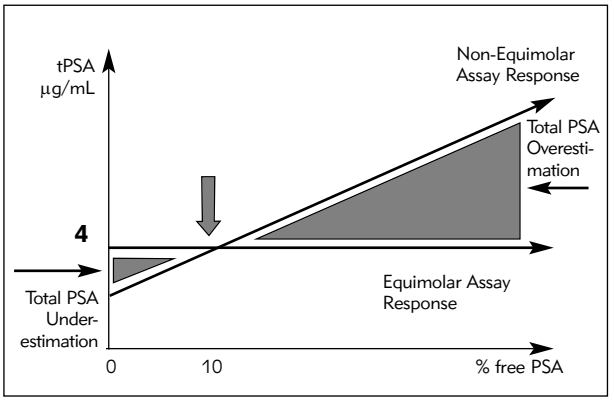

Figure 1 Equimolar detection of complexed and free PSA. A non-equimolar PSA assay tends to preferentially recognize free PSA, therefore the total PSA concentration is overestimated with a high proportion of free PSA and underestimated with a low proportion of free PSA leading to both false positive and false negative results.

determination of the serum level of tPSA and fPSA is critically important for the diagnosis of PCa. Some early assays for tPSA were inclined to preferentially detect fPSA and overestimate the tPSA concentration in sera of patients with benign prostate disease compared to those with PCa (*Figure 1*) (21). Thus it was recommended that tPSA assays should measure the detectable forms of PSA in an equimolar basis (15, 22). Equimolar recognition of free and complexed PSA is essential for accurate PSA testing, especially in a PSA concentration near to the 4.0 ng/mL cut-off. Non-equimolar determination of PSA could lead to false positive but also false negative results (*Figure 1*) (15, 22).

After long-lasting and intense discussions on the clinical relevance of equimolar and non-equimolar measurement of the immunologically detectable forms of PSA, a standard material was proposed with the goal of assay standardissation (23). This preparation contains both complexed and fPSA in a ratio of 90:10 respectively with a molar extinction coefficient used for mass assignment different from the original Hybritech standard (24, 25). This material was later adopted by the WHO as the reference preparation for PSA assay standardization. Despite the original intention to establish this reference material as an »equimolarity standard«, it became apparent that only inter-assay variability was improved and all the challanges of non-equimolar assays were not corrected (18, 19). Only modifications of the assays by manufacturers were able to improve equimolar testing of PSA (22). Nevertheless, seeking a legitimate goal toward greater uniformity of PSA results and PSA assays interchangeability, most immunodiagnostic companies offer PSA assays calibrated to the new international WHO standard. The WHO reference preparation became the new »mass standard« for the calibration of PSA assays.

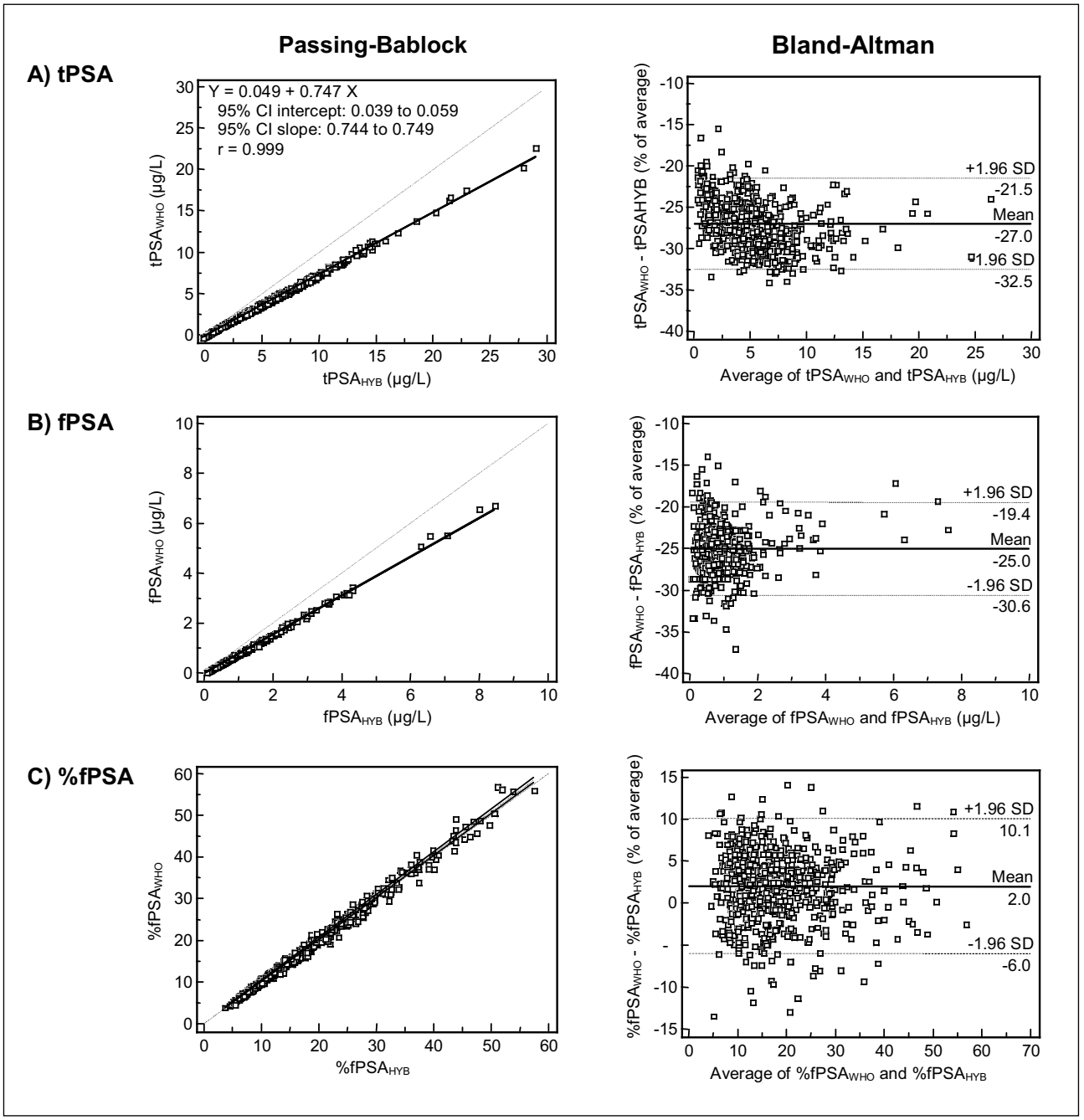

Figure 2 Passing and Bablock and Bland-Altman comparison plots of A) tPSA, B) fPSA and C) %fPSA measured either with the WHO or Hybritech calibrated Access assays. The comparison analysis was performed on sera of 641 patients with tPSA concentration between 0.26 and 29.5 ng/mL. Courtesy of Dr. Stephan.

Recalibration to WHO standards

Since the introduction of tPSA testing in the mid 1980's, differences in tPSA concentrations determined with assays from various manufacturers were rapidly identified (26). Diagnostic companies have improved the analytical performance of the PSA assays by using monoclonal antibodies, but also aimed to enhance the comparability of the assays. Nevertheless, differences among the assays still persist due to the use of different antibodies, assay format or calibration. The WHO calibration of PSA assays was supposed to reduce this inter-assay variability to the minimum to limit the possible clinical implications of such variations. There were lots of expectations that WHO calibration of PSA assays would solve the problem of assay discrepancies and would lead to a greater consistency between assays. Aiming for a better standardization of PSA testing, several countries require that laboratories report the tPSA and fPSA concentrations from assays

calibrated to the WHO 96/670 and the WHO 96/668 respectively.

Recalibration of an immunoassay to a new standard is expected to modify the concentration values obtained following the calibration. In fact, the calibration of a PSA assay to the WHO international standard could lead to under-recovery of PSA values (27, 28). Several studies have shown that calibration to the WHO standard could lead to a proportional negative bias in mass units of approximately 20% compared with the non-WHO calibrated assay (5, 29). As an example, when the Hybritech tPSA assay is calibrated to the WHO reference material, lower tPSA concentrations are detected, compared with the values obtained with an assay calibrated to the historical Hybritech calibrator (*Figure 2*). In a recent study, 641 sera of patients were tested for tPSA and fPSA using the Access tPSA and fPSA assay calibrated to the traditional Hybritech calibrator or the WHO preparation. A Passing and Bablock analysis clearly shows a slope of the regression line of 0.75 and 0.78 for tPSA and fPSA, respectively (*Figure 2*). The Bland-Altman difference plots confirm the proportional negative bias of 27% and 25% for tPSA and fPSA, respectively (*Figure 2*). The under-estimation of tPSA and fPSA by the WHO calibrated assays were quite similar. The direct consequence of this concordance in the negative bias for tPSA and fPSA is that the %fPSA ([fPSA/tPSA] x100) values are not different between the two assays (*Figure 2C*).

After several WHO calibrated assays became available, analytical comparison studies were performed to test the assays comparability. Improvement in the tPSA WHO calibrated assays variability was observed while the results of the fPSA assays comparison were reported to be still divergent (11). Not surprisingly, analytical variations between WHO calibrated assays and non-WHO calibrated assays were also reported (11, 12, 28, 29). But most importantly, it was shown that even WHO calibrated assays do not deliver similar tPSA values (11, 28, 29). Stephan and colleagues reported that the tPSA median values obtained from the analysis of 596 serum samples differed by up to 19% between two WHO calibrated methods (28, 29).

The differences between assays were larger when the WHO reference preparations were analysed than when the assays were compared against clinical samples (11). It was shown that the bias between artificial samples and clinical samples could account for 25% of difference across the methods compared (28). This observation could have important implications for method comparison studies and has led to controversies where differences between assays might have been overestimated due to the use of artificial samples (12, 28).

The calibration to a common WHO reference preparation improved but did not eliminate inter-assay variability. Moreover, this analytical adjustment of a PSA assay to the WHO introduces a negative bias when compared to classical calibration leading to an under-recovery of tPSA and fPSA concentrations. Therefore this shift in mass unit of tPSA and fPSA could inevitably have consequences on the clinical performance of the PSA assays in detecting PCa if the clinical decision point based on tPSA concentration is not adjusted.

Clinical impact of WHO recalibration

The initial clinical studies demonstrating the utility of PSA determination for the detection of PCa defined an optimal clinical decision point based on the tPSA concentration of 4.0 ng/mL using a Hybritech assay (10). In conjunction with digital rectal examination (DRE), this tPSA concentration cut-off was recommended as a biopsy indication, which subsequently determines the proportion of men referred for biopsy by which amongst them PCa will be detected. As previously discussed, the WHO calibration of an assay leads to lower tPSA concentrations detected (by approximately 20%), therefore if the initial 4.0 ng/mL cut-off is considered a biopsy indication, a fewer proportion of men will be referred, and as a consequence, fewer PCa will be detected (*Figure 3*). From the graph shown in *Figure 3*, it is clear that for maintaining the same proportion of PCa detected in the population screened using a WHO calibrated assay, the clinical decision point has to be adjusted to a lower concentration than the initial cut-off defined with a non-WHO calibrated assay.

The theoretical clinical implications of assay discrepancies due to calibration were confirmed by comparison studies using retrospective analysis of patient sera with various methods. A recent study aimed to

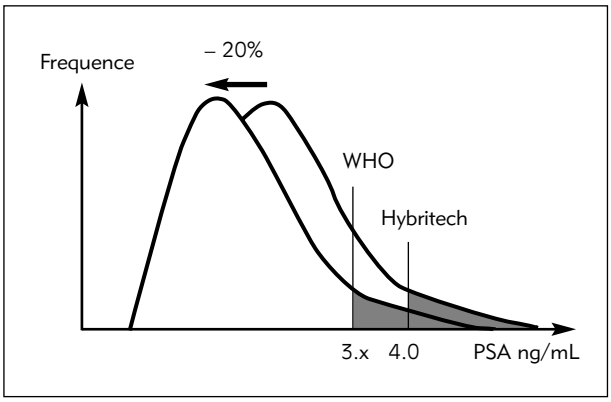

Figure 3 Impact of different PSA standardization on the clinical interpretation of results and on the cut-off definition. The hatched zone corresponds to the proportion of men referred for biopsy. If the 4.0 ng/mL cut-off is used as a clinical decision point with a WHO calibrated assay, the proportion of men referred for biopsy will be smaller than with the Hybritech calibrated assay.

determined the implication of several tPSA and fPSA inter-assays variations of three WHO and two non-WHO calibrated assays for differentiating benign and malignant prostate disease (5, 20). The results confirmed the discrepancies in tPSA and fPSA values especially between WHO and non-WHO calibrated assays but also between WHO calibrated assays from different manufacturers. The WHO calibrated assays tended to detect lower tPSA concentrations. Consequently, when using a fixed tPSA thresholds of 4.0 ng/mL, the number of patients with or without prostate cancer who were classified as true-positive (cancer) or true-negative (no cancer) significantly differed among the five assays. These differences in the number of patients classified as true-positive or true-negative modify the assay performance in terms of specificity and sensitivity. When considering a fixed 4.0 ng/mL tPSA clinical decision point, due to the assays discrepancies, the sensitivity of the test varied from 70% to 85%, while the specificity ranged from 23% to 57% (20). Similar trends were observed for the %fPSA. At a fixed threshold of %fPSA<20%, the sensitivity of the assays was from 79% to 98% and the specificity observed was ranging from 28% to 64%. A larger difference in sensitivity and specificity was observed between WHO and non-WHO assays, but such modifications of the clinical performance of PSA assays can also be demonstrated between WHO calibrated assays. These results are extremely important from a clinical perspective as these decreases in sensitivity and specificity of the PSA determination indicate that either a significant number of PCa would be missed or a substantial number of unnecessary biopsies would not be spared if a fixed tPSA or %fPSA cut-off is used with various assays.

The modification of clinical performance of the PSA assays due to inter-assay variability was previously demonstrated in the past (13–15). Unfortunately, the standardization of the assays to the WHO standard probably reduces assay discrepancies, but certainly did not eliminate the inter-assay variability and its clinical implications.

As previously described in this review, the recalibration of a given assay to the WHO standard could also be a source of variation in tPSA and fPSA concentrations detected (*Figure 2*). Therefore, independently from inter-assay variability, the standardization of a PSA assay to the WHO standard could have serious clinical implications if the clinical decision point is not adapted to the analytical characteristics of the newly calibrated assay.

Recently three studies were presented at the 2008 European Association for Urology (EAU) congress in Milan describing the clinical impact of the standardization of the »Beckman Coulter Access tPSA and fPSA Hybritech« assays to the WHO reference materials with unadjusted clinical decision point. All three studies confirmed the analytical negative bias of approximately 20% when comparing the tPSA and fPSA values of the Hybritech calibrated assays versus the WHO calibrated assays.

In the first study performed at UCL in Brussels, the clinical impact of analytical variations observed between the WHO and Hybritech calibrated Access assays was evaluated by a retrospective analysis of a database of 4,548 patients (30). The tPSA and fPSA values from these patients were determined in serum using the Hybritech calibrated Access assay. Based on the regression analysis of results from a comparison study on the serum of 155 patients, the theoretical tPSA and fPSA values for the WHO calibration were calculated. Then, using ERSPC data to evaluate risk of PCa, the clinical consequences of a determination of the PSA concentrations with a WHO or a non-WHO calibrated assay were analysed (31). This simulation showed that using the WHO calibrated assay, in 210 patients biopsy, will be postponed for 3 to 5 years, and 129 patients will not be offered a biopsy among whom 30 will develop PCa. Depending on the age of the patients, and for fixed tPSA levels > 3 ng/mL, 61 PCa – 15% to 18% of PCa would have been be missed (30).

The second study from the Erasmus Medical Center in Rotterdam followed a similar approach, using a large cohort of unscreened and asymptomatic men (n=2.283) selected from the database of the local center of the ERSPC (32). The serum tPSA concentration was measured with the Hybritech calibrated Access assay and the theoretical tPSA values for the WHO calibrated assay were calculated using the results of a comparison study. Differences in prostate biopsy rates, PCa detection and characteristics of the missed cancers were calculated while maintaining a hypothetical biopsy threshold of 4.0 ng/mL for both the original Access Hybritech tPSA and Access tPSA calibrated to the WHO 96/670 standard. A 32% decrease in the number of prostate biopsies and a 31% decrease in detected cancers were observed with the WHO calibration compared with the original Hybritech calibration. Differences in tumor characteristics between the missed and detected cancers were not significant.

In the third comparison study performed at the University Hospital Charité in Berlin, sera from 641 patients were tested for tPSA and fPSA using either the Hybritech or the WHO calibrated Access assays. The patients were classified into two groups for the presence or absence of PCa, all histologically confirmed. The results from this study demonstrated that the equimolarity of the Access Hybritech tPSA assay was not influenced by the calibration. The negative bias for tPSA and fPSA between the Hybritech and the WHO calibrated assays was confirmed. Interestingly, the receiver operating characteristic (ROC) analysis, for tPSA and %fPSA and both assays calibrations showed totally overlapping ROC curves. This ROC analysis, which is not dependent on cut-off definition, demonstrated that the diagnostic performance of the assays is not altered by the calibration. Nevertheless,

for clinical decision making, the definition of a cut-off point is necessary. This study also showed that for a cut-off point of 4.0 ng/mL, the sensitivity of the Hybritech calibrated assay is 76%, while for the same cut-off point the sensitivity of the WHO calibrated assay is only 55%. The definition of a new clinical decision point at 3.0 ng/mL could restore 76% sensitivity for the WHO calibrated assay. Therefore, in clinical practice, the definition of a new clinical decision point is critical when a WHO calibrated assay is used to maintain equivalent clinical performance.

The appropriate clinical decision point

To avoid erosion of the clinical performance of PSA determination in the detection of PCa, it is absolutely critical that the appropriate clinical decision point is used according to the calibration of the assay. In a retrospective analysis of the clinical data used for the validation of the 4.0 ng/mL tPSA cut-off with the Access Hybritech assay (10), it was possible to demonstrate that 38 (15%) PCa may have been missed if this clinical decision point would have been applied to tPSA values obtained with the Access WHO calibrated assay (*Table IA*). From this analysis an appropriate clinical decision point of 3.1 ng/mL was identified to preserve the clinical performance of the assay calibrated to the WHO standard (*Table IB* and *Table II*). Using appropriate tPSA cut-offs, a similar number of PCa are detected with the two methods and the relative agreement between Hybritech and WHO calibrated Access assays reaches 100% (*Table II*). These results were independently confirmed by the comparison study performed by Stephan and colleagues on a different cohort of patients (33, 34).

At this point, it is important to realise that this 3.1 ng/mL cut-off cannot be applied to any WHO calibrated tPSA assay, since analytical variations between WHO calibrated assays from various manufacturers were described (11, 20). Therefore, an appropriate clinical decision point should be defined for each tPSA assay.

Table I Distribution of patients according to the tPSA cut-off value. A) Unadjusted WHO clinical cut-off. B) Adjusted WHO clinical cut-off.

A)

	Hybritech Calibration		
WHO Calibration	4.0 ng/mL	> 4.0 ng/mL	Total
≤ 4.0 ng/mL	47	**38 (15%)**	85
> 4.0 ng/mL	0	170	170
Total	47	208	255

B)

	Hybritech Calibration		
	≤4.0 ng/mL	> 4.0 ng/mL	Total
≤ 3.1 ng/mL	47	**0**	85
> 3.1 ng/mL	0	208	170
Total	47	208	255

Table II Clinical performance of the WHO and Hybritech calibrated Access tPSA assays using an appropriate cut-off.

tPSA Calibration (ng/mL)	Hybritech ≤ 4.0	Hybritech > 4.0	Total samples
WHO ≤ 3.1	5616	0	85
WHO > 3.1	0	1 014	1 014
Total samples	5 616	1 014	6 630
Relative agreement	100%	100%	255

Conclusion

The highly desirable goal of assay-independent, interchangeable fPSA and tPSA results has not been achieved today. Despite the standardization of the various PSA assays to common WHO reference materials, discrepancies between tPSA and fPSA results obtained from various assays persist. Even the recalibration of a given assay to the WHO standard could lead to analytical bias with possible clinical implications for the interpretation of the PSA results. The PSA assays interchangeability might be a difficult objective to achieve due to PSA molecular heterogeneity and structural diversity observed in malignant or benign prostate diseases. Analytical constraints related to the use of numerous antibodies with different epitope specificities and affinities (35), and the different technical principles underlying the various analyzers might also prevent the complete standardization of PSA assays. The introduction of the WHO calibration aims to improve the harmonization of PSA assays, but a uniform clinical decision point cannot be recommended and the requirement for assay specific and clinically validated cut-offs remains (13, 20).

It is the responsibility of diagnostic companies to clearly communicate on the possible clinical consequences of recalibration of PSA assays to the WHO standard when an inappropriate clinical decision point is used. Adaptation of the clinical interpretation of the PSA results to the assay's calibration is critically important to avoid unacceptable erosion of the PSA testing clinical performance. It is essential to raise awareness amongst laboratory managers and more importantly amongst clinicians of the potential clinical impact of PSA assay recalibration to the WHO standard.

References

1. Hara M, Koyanagi Y, Inoue T, Fukuyama T. Some physico-chemical characteristics of »g-seminoprotein«, an antigenic component specific for human seminal plasma. Forensic immunological study of body fluids and secretion. VII. Nihon Hoigaku Zasshi 1971; 25: 322–4.
2. Papsidero LD, Wang MC, Valenzuela LA, Murphy GP, Chu TM. A prostate antigen in sera of prostatic cancer patients. Cancer Res 1980; 40: 2428–32.
3. Wang MC, Valenzuela LA, Murphy GP, Chu TM. Purification of a human prostate specific antigen. Invest Urol 1979; 17: 159–63.
4. Stamey TA, Yang N, Hay AR, McNeal JE, Freiha FS, Redwine E. Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. N Engl J Med 1987; 317: 909–16.
5. Catalona WJ, Smith DS, Ratliff TL, Dodds KM, Coplen DE, Yuan JJ, et al. Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. N Engl J Med 1991; 324: 1156–61.
6. Catalona WJ, Richie JP, Ahmann FR, Hudson MA, Scardino PT, Flanigan RC, et al. Comparison of digital rectal examination and serum prostate specific antigen in the early detection of prostate cancer: results of a multicenter clinical trial of 6,630 men. J Urol 1994; 151: 1283–90.
7. Carter HB, Pearson JD, Metter EJ, Brant LJ, Chan DW, Andres R, et al. Longitudinal evaluation of prostate-specific antigen levels in men with and without prostate disease. JAMA 1992; 267: 2215–20.
8. Catalona WJ, Partin AW, Slawin KM, Brawer MK, Flanigan RC, Patel A, et al. Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease: a prospective multicenter clinical trial. JAMA 1998; 279: 1542–7.
9. Loeb S, Catalona WJ. Prostate-specific antigen in clinical practice. Cancer Lett 2007.
10. Catalona WJ, Hudson MA, Scardino PT, Richie JP, Ahmann FR, Flanigan RC, et al. Selection of optimal prostate specific antigen cutoffs for early detection of prostate cancer: receiver operating characteristic curves. J Urol 1994; 152: 2037–42.
11. Kort SA, Martens F, Vanpoucke H, Van Duijnhoven HL, Blankenstein MA. Comparison of 6 automated assays for total and free prostate-specific antigen with special reference to their reactivity toward the WHO 96/670 reference preparation. Clin Chem 2006; 52: 1568–74.
12. Roddam AW, Rimmer J, Nickerson C, Ward AM. Prostate-specific antigen: bias and molarity of commercial assays for PSA in use in England. Ann Clin Biochem 2006; 43: 35–48.
13. Semjonow A, Oberpenning F, Brandt B, Zechel C, Brandau W, Hertle L. Impact of free prostate-specific antigen on discordant measurement results of assays for total prostate-specific antigen. Urology 1996; 48: 10–5.
14. Semjonow A, De Angelis G, Oberpenning F, Schmid HP, Brandt B, Hertle L. The clinical impact of different assays for prostate specific antigen. BJU Int 2000; 86: 590–7.
15. Roddam AW, Price CP, Allen NE, Ward AM. Assessing the clinical impact of prostate-specific antigen assay variability and nonequimolarity: a simulation study based on the population of the United Kingdom. Clin Chem 2004; 50: 1012–6.
16. Blijenberg BG, Yurdakul G, Van Zelst BD, Bangma CH, Wildhagen MF, Schroder FH. Discordant performance of assays for free and total prostate-specific antigen in relation to the early detection of prostate cancer. BJU Int 2001; 88: 545–50.
17. Stamey TA, Chen Z, Prestigiacomo AF. Reference material for PSA: the IFCC standardization study. International Federation of Clinical Chemistry. Clin Biochem 1998; 31: 475–81.
18. Rafferty B, Rigsby P, Rose M, Stamey T, Gaines Das R. Reference reagents for prostate-specific antigen (PSA): establishment of the first international standards for free PSA and PSA (90:10). Clin Chem 2000; 46: 1310–7.
19. Chan DW, Sokoll LJ. WHO first international standards for prostate-specific antigen: the beginning of the end for assay discrepancies? Clin Chem 2000; 46: 1291–2.
20. Stephan C, Kramer J, Meyer HA, Kristiansen G, Ziemer S, Deger S, et al. Different prostate-specific antigen assays give different results on the same blood sample: an obstacle to recommending uniform limits for prostate biopsies. BJU Int 2007; 99: 1427–31.
21. Graves HC. Issues on standardization of immunoassays for prostate-specific antigen: a review. Clin Invest Med 1993; 16: 415–24.
22. Semjonow A, Oberpenning F, Weining C, Schon M, Brandt B, De Angelis G, et al. Do modifications of non-equimolar assays for total prostate-specific antigen improve detection of prostate cancer? Clin Chem 2001; 47: 1472–5.
23. Stamey TA. Second Stanford Conference on International Standardization of Prostate-Specific Antigen Immunoassays: September 1 and 2, 1994. Urology 1995; 45: 173–84.
24. Stamey TA, Teplow DB, Graves HC. Identity of PSA purified from seminal fluid by different methods: comparison by amino acid analysis and assigned extinction coefficients. Prostate 1995;27:198–203.
25. Belanger A, Van Halbeek H, Graves HC, Grandbois K, Stamey TA, Huang L, et al. Molecular mass and carbohydrate structure of prostate specific antigen: studies for establishment of an international PSA standard. Prostate 1995; 27: 187–97.
26. Chan DW, Bruzek DJ, Oesterling JE, Rock RC, Walsh PC. Prostate-specific antigen as a marker for prostatic cancer: a monoclonal and a polyclonal immunoassay compared. Clin Chem 1987; 33: 1916–20.
27. Link RE, Shariat SF, Nguyen CV, Farr A, Weinberg AD, Morton RA, et al. Variation in prostate specific antigen results from 2 different assay platforms: clinical impact

on 2304 patients undergoing prostate cancer screening. J Urol 2004; 171: 2234–8.

28. Stephan C, Semjonow A. Accuracy assessment of PSA methods must be based on clinical instead of artificial samples – a response to AW Roddam et al., Ann Clin Biochem 2006; 43: 35–48. Ann Clin Biochem 2006; 43: 421–2; author reply 422–3.
29. Stephan C, Klaas M, Muller C, Schnorr D, Loening SA, Jung K. Interchangeability of measurements of total and free prostate-specific antigen in serum with 5 frequently used assay combinations: an update. Clin Chem 2006; 52: 59–64.
30. Fille C, Tombal B, Philippe M. Impact of total PSA WHO calibration on decision points in screening for prostate cancer [abstract]. EAU Congress, Milan. 2008: 218.
31. Postma R, Schroder FH. Screening for prostate cancer. Eur J Cancer 2005; 41: 825–33.
32. Jansen FH, Roobol M, Bangma CH, Van Schaik RHN. The clinical impact of the PSA assay WHO calibration on prostate biopsy rates and prostate cancer detection [abstract]. EAU Congress, Milan. 2008.
33. Stephan C, Kahrs A, Klotzek S, Reiche J, Müller C, Lein M, et al. Toward the metrological traceability in determination of prostate-specific antigen: the new Access Hybritech assays calibrated against WHO standards compared with the traditional Access Hybritech assays. CCLM 2008; in press.
34. Stephan C, Kahrs A, Klotzek S, Reiche J, Müller C, Lein M, et al. Comparison of the »gold« standard Access PSA (Hybritech) assay with the new Beckman Coulter WHO-standardized calibrated Access PSA assays [abstract]. EAU Congress, Milan. 2008.
35. Stenman UH, Paus E, Allard WJ, Andersson I, Andres C, Barnett TR, et al. Summary report of the TD-3 workshop: characterization of 83 antibodies against prostate-specific antigen. Tumour Biol 1999; 20 Suppl 1: 1–12.

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