Comparison of Hybritech and WHO standardization applied to access Hybritech total PSA assay on UniCel®

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Summary

Background. **It is widely accepted that the introduction of the First International Standard for PSA assay (WHO96/670) improved the agreement between total PSA immunoassay. Less attention was given to the fact that the results obtained with WHO calibration turned out to be lower by 16-20% than the original Hybritech assay with the inevitable consequences on positive/negative false results. The aim of the study was to verify, on UniCel®DxI800, the misalignment between Hybritech and WHO calibration for total PSA assay and to calculate the new cut-off value if WHO calibrated.**

Methods. **We measured total PSA values, in 500 samples, both with Hybritech and WHO calibration on UniCel®DxI800 (Beckman Coulter).**

Results. **Our data confirm the negative bias of WHO values with respect to Hybritech values and on the grounds of Passing-Bablok regression, the 4.0 µg/L cut-off, obtained according to Hybritech total PSA assay, corresponds to a WHO-calibrated cut-off of 3.2 µg/L.**

Conclusions. **When WHO calibration is used for a PSA assay, a new assay-dependant cut-off should be calculated or, if the laboratory decides to maintain the same cut-off value of PSA-Hyb with a WHO calibration, a redefinition of the sensibility/specificity of 4.0 µg/L cut-off is needed.**

Key Words: **WHO96/670, prostate carcinoma, cut-off, Hybritech**

Riassunto

Dosaggio Access Hybritech per il PSA totale: doppia standardizzazione, Hybritech e WHO, su UniCel®

Premesse. E' ampiamente dimostrato che l'introduzione del primo standard internazionale per il dosaggio dell'antigene prostatico specifico (PSA-WHO 96/670) ha contribuito ad armonizzare i risultati ottenuti con metodi diversi. Meno attenzione è stata data al fatto che con la calibrazione WHO i risultati sono inferiori del 16-20% rispetto al dosaggio originale Hybritech portando ad inevitabili falsi positivi/negativi. Lo scopo del lavoro è stato di verificare, su UniCel®DxI800, la concordanza tra i campioni dosati con calibrazione Hybritech e quelli con calibrazione WHO e di calcolare il cut-off nel caso in cui il dosaggio venga effettuato contro la calibrazione WHO.

Metodi. Il PSA è stato dosato, su 500 campioni, con doppia calibrazione (Hybritech e WHO) su UniCel®DxI800 (Beckman Coulter).

Risultati. I nostri dati confermano un bias negativo dei valori di PSA ottenuti con la calibrazione WHO rispetto a quella Hybritech e, sulla base della retta di regressione di Passing e Bablok, il cut-off di 4.0 µg/L secondo il dosaggio Hybritech PSA totale, corrisponde ad un cut-off con una calibrazione WHO pari a 3.2 µg/L.

Conclusioni. Quando viene effettuato un dosaggio per il PSA totale con la calibrazione WHO un nuovo cut-off dosaggio-specifico deve essere calcolato o, nel caso si mantenga il medesimo cut-off del dosaggio Hybritech anche usando la calibrazione WHO è necessario ridefinire sensibilità e specificità del cut-off in esame.

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Table I. The intra-assay and inter-assay variation for total PSA assay according to WHO and Hybritech calibration.

	INTRA - ASSAY			INTER-ASSAY		
	LEVEL 1	**LEVEL 2**	**LEVEL 3**	**LEVEL 1**	**LEVEL 2**	**LEVEL 3**
PSA-Hyb (μg/L)	0.35	3.48	27.98	0.37	3.50	28.01
%CV	5.04	5.40	5.78	7.57	5.30	5.70
PSA-WHO (μg/L)	0.30	2.94	21.53	0.32	2.97	22.96
%CV	4.55	5.81	6.62	6.25	5.78	6.58

PSA-Hyb: PSA assay calibrated with the original Hybritech Tandem-R method; PSA-WHO: PSA assay calibrated according to the First International Standard adopted by the World Health Organization; CV%: coefficient of variation in percent = (standard deviation/mean)*100.

Introduction

The prostate specific antigen (PSA) is currently the most used serum marker for monitoring patients affected by prostatic diseases. In 1986 the Hybritech (Hyb) Tandem-R PSA assay was the first widely used FDA-approved commercial assay for total PSA determination and in 1994 an upper cut-off of 4.0 μg/L, based on a prospective analysis carried out on 6600 males[1], was identified as the clinical decision point for this method. In 2000, such method was automatized on the Access Immunoassay System Beckman Coulter analyzer and the results obtained with the Access method were calibrated to give equivalent results with respect to the original Hyb Tandem-R method, so that the 4.0 μg/L cut-off was maintained. However, the low interchangeability of measurements of total PSA among commercial PSA assays, based above all on the lack of equimolar response of some PSA assays and the use of non uniform calibrations, led to the possible misinterpretation of individual PSA values, especially if referred to 4.0 μg/L cut-off[2].

Consequently, the development of equimolar-response assays and the calibration with a new standard for PSA were improved and finally in 1990 the World Health Organization (WHO) adopted the First International Standard for PSA assay (WHO96/670), a standard which contains a mixture of 90% complexed PSA (bound to alfa-1-antichimotripsin) and 10% free PSA[3] and that has a mass assigned using a recalculated molar extinction coefficient[4], considerably higher than the original one. The result of the introduction of this standard was an harmonization among the different commercial PSA assays[5-8] even if the interchangeability of PSA values remained inadequate[5-9].

Less attention, instead, was given to the concern that the results obtained with the WHO calibration turned out to be lower by 16-20% if compared to the original Hybritech assay. In order to maintain the same sensitivity and specificity of the original PSA assay Hybritech Tandem-R method, a new lower cut-off was necessary.

At present, Beckman Coulter gives the option to calibrate the Access Hybritech PSA method also according to the WHO standard.

On the basis of the differences highlighted using both standards with the inevitable effects on positive false and negative false results, especially if referred to 4.0 μg/L cut-off[10], we deemed necessary to verify the information of the manufacturer about the demonstrated misalignment between the original Access® Hybritech® Tandem R and the Access Hybritech PSA-WHO calibration on UniCel®DXI800 Beckman Coulter, our in-house instrument.

Materials and methods

Daily, we selected the samples among those of the routine with PSA-Hyb values between 2.5 and 20.0 μg/L and, immediately after, we also performed the PSA Access Hybritech assay according to the WHO calibration. We collected a total amount of 500 samples that were frozen after the assays were done.

The assays were performed, according to the instructions of the manufacturer, on our two in-house UniCel®DXI800 (Beckman Coulter, Fullerton, CA) and three reagent lots and one calibrator lot were used. Moreover, daily quality controls on three levels, low, medium and high, (BIO-RAD Liquichek Immunoassay Plus Trilevel) were performed with both the calibrations before every analytical session.

The data analysis (MedCalc Softer, Mariakerke, Belgium) was performed with Bland-Altman test for assessing agreement between the tested methods, and Passing Bablok regression, for testing the equality of measurements considering the original Hybritech assay as the reference method.

Results

The intra-assay and inter-assay variations of each assay are summarized in Table I.

The Bland-Altman plot of the differences (Fig. 1), expressed as percentage with respect to the mean concentration, shows that the values are distributed about a mean value of 20.6%, with a 95% confidence interval (C.I.) ranging from 3.7% to 37.5%.

The Passing-Bablok regression (Fig. 2) analysis evidences that the WHO values are significantly lower than

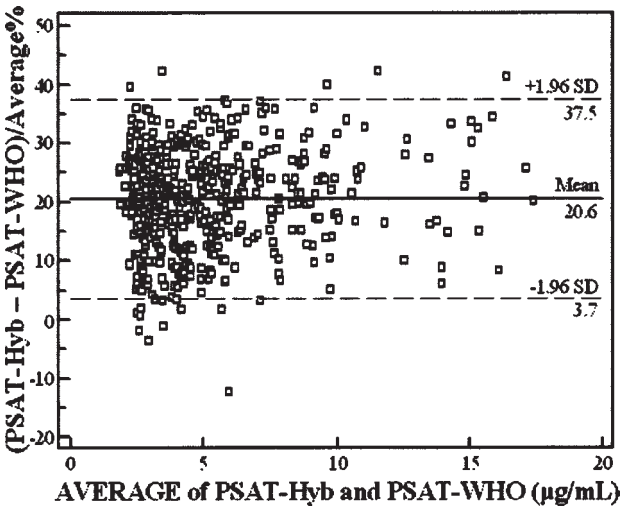

Figure 1. Bland-Altman plot of the differences, expressed as percentage, with respect to the mean concentration.

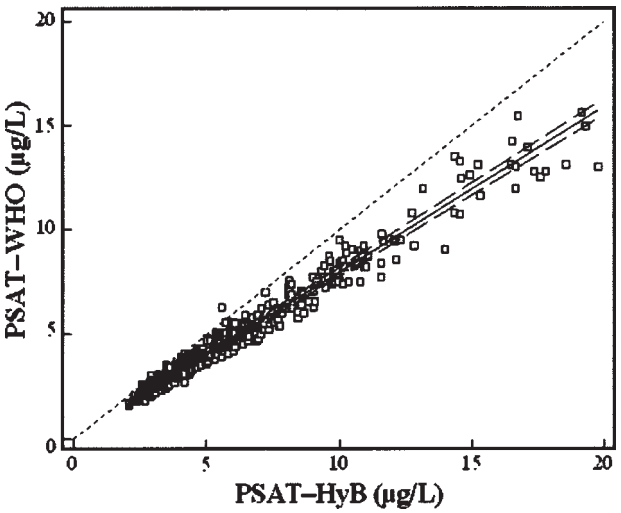

Figure 2. Passing-Bablok regression plot of PSA-WHO values with respect to PSA-Hyb values.

those of Hybrytech. In particular, the intercept of the regression line is significantly different from zero and the slope is significantly different from 1: PSA-WHO = 0.078 + 0.790 PSA-Hyb (C.I. 95% intercept: 0.011 – 0.145; C.I. 95% slope: 0.775 – 0.806).

On the grounds of Passing-Bablok regression, the 4.0 µg/L cut-off, obtained according to Hybritech total PSA assay, corresponds to a WHO-calibrated cut-off of 3.2 µg/L.

The scatter-plot of PSAT-WHO against PSAT-HyB in the range from 2.5 µg/L and 5 µg/L (Fig. 3) shows that 1.6% of PSA-WHO values are positive false and 2.0% are negative false with respect to the original PSA-HyB method, considering the 4.0 µg/L and 3.2 µg/L cut-offs for PSAT-HyB and PSA-WHO respectively. Moreover it shows that 0.0 % of PSA-WHO values are positive false and 16.3% are negative false with respect to PSA-HyB, considering the 4.0 µg/L cut-off both for PSAT-HyB and PSA-WHO. Finally, setting the PSA-WHO cut-off to 3.1 µg/L lowers the number of negative falses to one subject.

Discussion

The Bland-Altman plot evidences the presence of a negative bias of WHO values with respect to Hyb values consistent with literature[11]. The marked variability about the mean value is likely due to the fact that this study was not originally intended as part of a controlled study, namely different reagent lots were used, data were collected daily for about 6 months, during the laboratory routine work and two DxI800 were used. Moreover, the variability about the mean value characterises the whole studied interval (PSAT-Hyb: 2.5-20 µg/L) suggesting that it isn't dependent on the PSAT concentrations.

The PSAT-WHO cut-off value calculated according to the Passing Bablok regression, (3.2 µg/L) agrees with the cut-off value proposed by the manufacturer (3.1 µg/L). The scatter plot of PSAT-WHO against PSAT-HyB, evidences that the PSAT-WHO cut-off value introduces a small amount of negative and positive false values with respect to the original Hybritech method; however, negative false values are reduced to a single observation using the PSAT-WHO cut-off suggested by the manufacture. Moreover, if we refer to 4.0µg/L cut-off for both the calibrations, Figure 3 evidences that 16.3% of patients results, considered

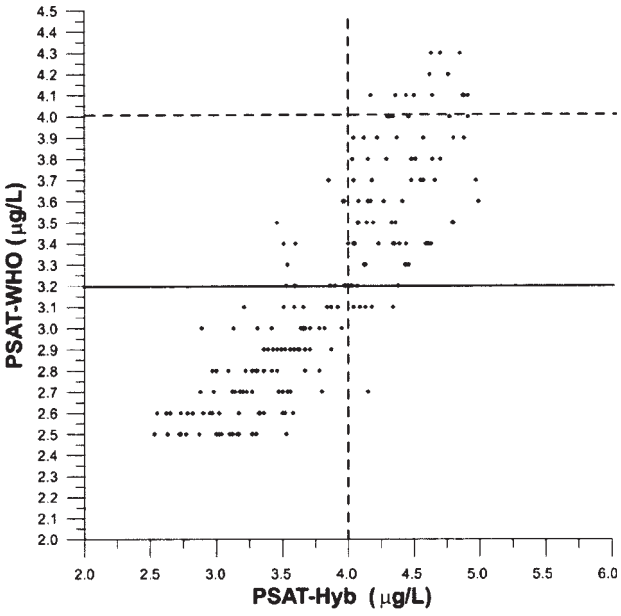

Figure 3. Scatter-plot of PSAT-WHO values against PSAT-HyB values in the range from 2.5 µg/L and 5 µg/L. The horizontal and vertical dashed lines represent the original Hybritech cut-off value (4.0 µg/L); the horizontal solid line represents the WHO cut-off (3.2 µg/L) calculated on the grounds of Passing-Bablok regression.

abnormal by PSAT-Hyb, are considered normal by PSAT-WHO, underlining that when WHO calibration is used for a PSA assay, a new assay-dependant cut-off should be adopted to maintain the same sensibility and the specificity of the original Hybritech method; on the other hand, if the laboratory decide to maintain the same cut-off value of PSA-Hyb with a WHO calibration, a redefinition of the sensibility and the specificity of 4.0 μg/L cut-off is needed.

Lastly, if the laboratory position is to maintain the PSA assay based on Hybritech calibration with the cut-off of 4.0 μg/L, it should be clear that in the external quality program (VEQ) PSA results obtained with Access Tandem-R calibration are higher than both the results obtained with WHO calibration and than most of the other methods not yet standardized. Moreover, if the WHO calibration is used, the communication to clinicians about the new calibration and, consequently the new cut-off adopted could minimize misinterpretations of individual PSA values as well as, throughout a period of time, the indication on the hospital reports of both results and cut-offs (Hybritech and WHO).

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References

1. Catalona WJ, Hudson MA, Scardino PT, Richie JP, Ahmann FR, Flanigan RC, et al. Selection of optimal prostate specific antigen cut-offs for early detection of prostate cancer: receiver operating characteristic curves. J Urol 1994;152:2037-42.
2. Semjonow A, De Angelis G, Oberpenning F, Schmid P, Brandt B, Hertle L. The clinical impact of different assays for prostate specific antigen. BJU Int 2000; 86:590-7.
3. Stamey TA. Second Stanford Conference on International Standardization of Prostate-Specific Antigen Immunoassays: September 1 and 2, 1994. Urology 1995; 45:173-84.
4. 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.
5. 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.
6. Stephan C, Klaas M, Müller 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.
7. 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.
8. Dorizzi RM, Meneghelli S, Rizzotti P, Giavarina D. Differences of prostate-specific antigen assays: a small light at the end of the tunnel? Ann Clin Biochem 2006; 43:420-1.
9. 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.
10. Semjonow A, Oberpenning F, Brandt B, Zechel C, Brandau W, Hertle L. Impact of free prostatie-specific antigen on discordant measurement results of assays for total prostate-specific antigen. Urology 1996; 48S:10-5.
11. Vignati G, Giovanelli L. Standardization of PSA measures: a reappraisal and an experience with WHO calibration of Beckman Coulter Access Hybritech total and free PSA. Int J Biol Markers 2007; 22:295-301.