

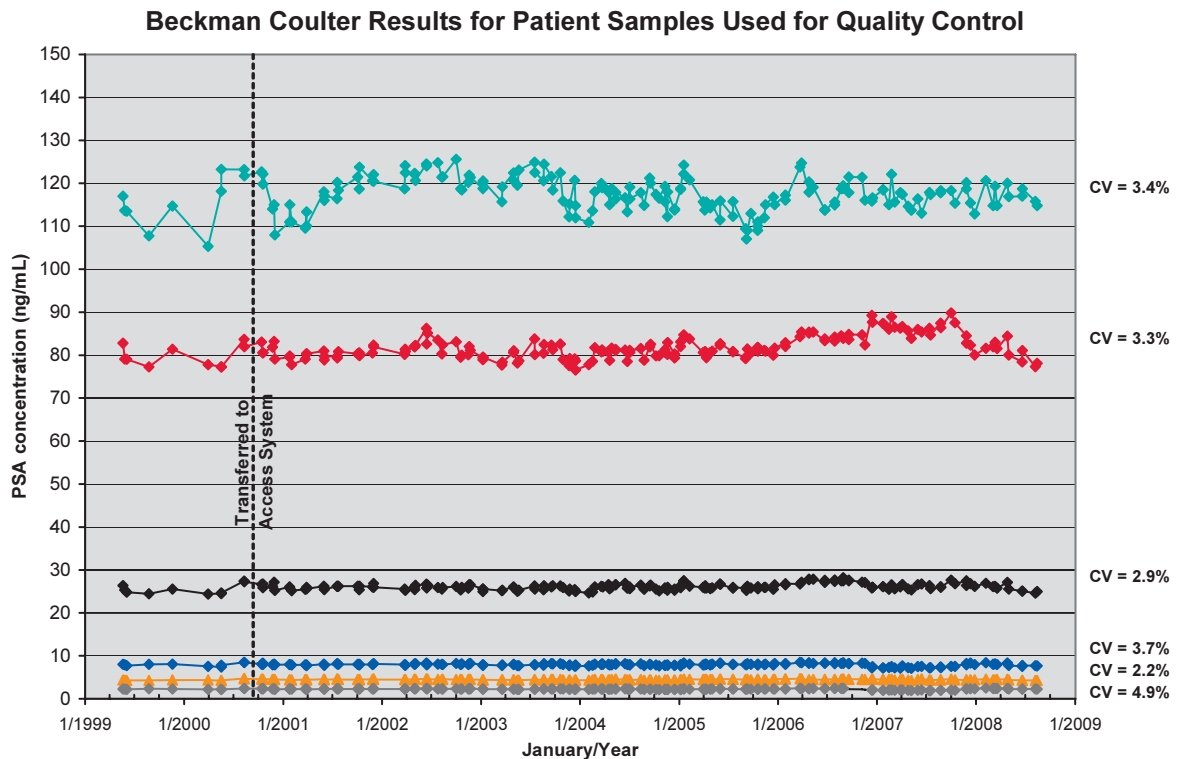
## Access<sup>®</sup> Hybritech<sup>®</sup> PSA and free PSA Hybritech and WHO Calibrations

To provide you with additional information about the difference in PSA assay calibrations, Beckman Coulter has gathered together the answers to some frequently asked questions. If you have additional questions, please send them to [PSA@beckman.com](mailto:PSA@beckman.com) for a personal reply.

### Standardization Background

#### Q. What is the origin of the Hybritech standard?

- A. Beckman Coulter's Hybritech PSA assay has a rich history. In 1986, the Hybritech Tandem<sup>®</sup>-R assay became the first PSA assay to be approved by the FDA. In 1994, 4.0 ng/mL was identified as the clinical decision point using the Hybritech PSA assay in a multicenter study on 6,630 men. In 2000, the Hybritech Tandem-R assay migrated to the Access Immunoassay System. The assay performance between Access and Tandem-R systems has remained stable since the assay migration, and is monitored by rigorous QC programs to assure equivalent clinical performance.



**Q. Why was the WHO standard introduced?**

**A.** WHO standardization was intended to harmonize PSA assays.

**Q. What is the origin of the WHO standard?**

**A.** Thomas Stamey, M.D., a clinical urologist and leading prostate cancer researcher at Stanford University, organized two conferences in 1992 and 1994, respectively, to harmonize PSA assays. A PSA standard containing a 90:10 mixture of complexed PSA and free PSA was proposed at the second Stanford conference and became the basis for the reference preparation, designated WHO IRP 96/670.

**Q. What is the difference between Hybritech and WHO IRP 96/670 standard materials?**

**A.** The basis for the difference in the two standard materials can be simply explained:

- The quantitation method is different. PSA in the original Hybritech calibration material was quantified using the widely accepted standard Lowry total protein method. The PSA concentration in WHO was measured using amino acid analysis and ion-spray mass spectrometry.
- The molecular weight of PSA quantified by the two methods is different. The PSA molecular weight in the classic Hybritech calibration material is approximately 34,000 daltons. Dr. Stamey and his collaborators proposing the WHO standard derived a different molecular weight of PSA (28,430 daltons), which resulted in a molar absorption coefficient of 1.84, approximately 20% higher than the previously reported coefficient of 1.42 calculated by Hybritech scientists.

**Q. What calibration was used to establish the NCCN\* prostate cancer guidelines?**

**A.** The Hybritech PSA assay with the classic Hybritech calibration served as the basis to establish the National Comprehensive Cancer Network\* (NCCN) guidelines.

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**Cutoffs and Clinical  
Decision Points**

**Q. Why is Beckman Coulter, Inc. recommending a change in the cutoff and clinical decision points for the WHO calibration?**

**A.** There is a 22% difference between the Hybritech and the WHO cutoffs.

Beckman Coulter evaluated the impact of WHO calibration through analysis of a 6,630 subject prospective clinical study. That study provided the basis for the approval of Hybritech PSA in conjunction with DRE as an aid in the detection of prostate cancer, and identified 4.0 ng/mL as a value at which to consider a biopsy.

\* NCCN and National Comprehensive Cancer Network are trademarks of National Comprehensive Cancer Network, Inc.

It was determined that a different clinical cutoff for the WHO calibration is necessary to provide the **same clinical sensitivity and specificity** as the classic Hybritech calibration as shown in the table below.

**WHO Calibration 3.1 Total PSA Cutoff is Clinically Equivalent to Hybritech Calibration**

Calibration (ng/mL)	Hybritech $\leq 4.0$	Hybritech $> 4.0$	Total Samples
WHO $\leq 3.1$	5616	0	5616
WHO $> 3.1$	0	1014	1014
Total Samples	5616	1014	6630

Relative Agreement                      100%                      100%

- Q. If my lab wants to stay with the Hybritech calibration, do I need to change how I process the samples today?**
- A.** No. If you continue using the Hybritech calibration, there is no change to how your system is calibrated, no change to the QC values, and no change to the cutoff.
- Q. My lab already uses a lower cutoff; do I still need to change the values if I adopt the WHO calibration?**
- A.** Yes, your lab needs to adjust the clinical decision points accordingly. Please see the table below.

**Hybritech Calibration and WHO Calibration PSA Values**

Hybritech Calibration PSA Value (ng/mL)	WHO Calibration PSA Value (ng/mL)	Description
0.00	0.00	Not Applicable
0.35	0.30	PSA velocity to trigger biopsy if PSA $< 4.0$ ng/mL <sup>1</sup>
0.75	0.64	PSA velocity suspicious for prostate cancer if PSA 4.0–10.0 ng/mL <sup>1</sup>
2.0	1.6	PSA velocity for aggressive prostate cancer <sup>2</sup>
2.5	2.0	Total PSA value to trigger biopsy <sup>1</sup>
4.0	3.1	Total PSA value to trigger biopsy <sup>1</sup>
10.0	7.8	Upper end of threshold for biopsy <sup>1</sup>
20.0	15.6	Prostate cancer risk stratification <sup>2</sup>

1 NCCN Clinical Practice Guidelines in Oncology \*: Prostate Cancer Early Detection v.2.2007

2 NCCN Clinical Practice Guidelines in Oncology: Prostate Cancer v.1.2008

\* Clinical Practice Guidelines in Oncology is a trademark of National Comprehensive Cancer Network, Inc.

**Q. If my lab is not using Hybritech PSA, what should I do?**

**A.** Please refer to your assay manufacturer's instructions for use. If you have questions, contact your assay manufacturer.

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**Equimolarity**

**Q. What is the definition of an equimolar PSA assay?**

**A.** Equimolar-response assays detect the free and complexed forms of PSA equally. Non-equimolar or skewed-response assays have been shown to produce two to three times more signal per free PSA molecule than with PSA-ACT. The Access Hybritech PSA assay is an equimolar assay in which sample recovery is unaffected by the ratio of PSA forms in serum. Therefore, the reported result is not changed by the relative concentrations of free PSA and PSA-ACT in the sample.

**Q. Is equimolarity maintained with the Access PSA WHO calibration?**

**A.** Yes, the Access PSA WHO calibration maintains the equimolarity of the Access Hybritech assay.

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**Percent Free PSA**

**Q. Can I use different calibrations to calculate %fPSA?**

**A.** No. Percent free PSA (%fPSA) can only be calculated if the total PSA and free PSA results were derived from the same type of calibration (Hybritech or WHO). Therefore, never mix Hybritech and WHO calibrations when calculating %fPSA.

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**Proficiency Surveys**

**Q. Which category should I use for proficiency reporting?**

**A.** Proficiency surveys such as CAP (College of American Pathologists) are or will be providing a new category for reporting results for the Beckman Coulter Hybritech PSA assay with WHO calibration. If you change to the WHO calibration, you will need to check the master list to identify the correct codes for your proficiency reporting agency.

CAP reporting codes for PSA and free PSA on Access and UniCel® DxI® immunoassay instruments are provided in the table below.

Hybritech Calibration		WHO Calibration	
Access	DxI	Access	DxI
BECKMAN ACCESS/2	BECKMAN UNICEL DxI	BECKMAN ACCESS (WHO)	BECKMAN UNICEL DxI (WHO)

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**For More Information****Q. Where can I find more information on PSA standardization?**

**A.** You can visit [www.beckmancoulter.com/PSAvalue](http://www.beckmancoulter.com/PSAvalue) to learn more about this topic.

You can also send your questions to [PSA@beckman.com](mailto:PSA@beckman.com)

**Q. Are there scientific publications that address the effect of standardization?**

**A.** There are many scientific publications in peer reviewed journals. Some of these are named in the following list.

- 1 Slev PR, La'ulu SL, Roberts WL. Intermethod differences in results for total PSA, free PSA, and percentage of free PSA. *Am J Clin Pathol* 2008 Jun; 129(6):952–958.
- 2 Cook B. PSA Testing: the disconnect between standardization and interpretive criteria. *Clin Lab News* 2008 Jun; 34(6):10–12.
- 3 Stephan C, Kahrs A, Klotzek S, et al. Toward metrological traceability in the determination of prostate-specific antigen (PSA): calibrating Beckman Coulter Hybritech Access PSA assays to WHO standards compared with the traditional Hybritech standards. *Clin Chem Lab Med* 2008 May; 46:623–629.
- 4 Loeb S, Chan DW, Sokoll LJ, et al. Differences in PSA measurements due to assay standardization bias. *J Urol* 2008 Apr; 179(4S):721.
- 5 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 Oct-Dec; 22 (4):295–301.
- 6 Sotelo RJ, Mora KE, Pérez LH, et al. Assay Standardization Bias: Different Prostate Cancer Detection Rates and Clinical Outcomes Resulting from Different Assays for Free and Total Prostate-Specific Antigen. *Urology* 2007 Jun; 69(6):1143–1146.
- 7 Thompson IM, Ankerst DP, Chi C, et al. Assessing Prostate Cancer Risk: Results from the Prostate Cancer Prevention Trial. *J Natl Cancer Inst* 2006 Apr; 98:529–534.
- 8 Link RE, Shariat SF, Nguyen CV, 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 Jun; 171:2234–2238.
- 9 Babaian R, et al. National Comprehensive Cancer Network. *Clinical Practice Guidelines in Oncology* 2004 Mar.
- 10 Klee GG, Schryver PG, Kisabeth RM. Analytic bias specifications based on the analysis of effects on performance of medical guidelines. *Scand J Clin Lab Invest* 1999; 59:509–512.
- 11 Catalona WJ, Hudson MA, Scardino PT, et al. Selection of optimal prostate specific antigen cutoffs for early detection of prostate cancer: receiver operating characteristic curves. *J Urol* 1994; 152:2037–2042.

- 12 Catalona WJ, Smith DS, Ratliff TL, et al. Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. *N Engl J Med* 1991; 324:1156–1161.
  - 13 Graves HCB, Kamarei M, Stamey TA. Identity of prostate specific antigen and the semen protein p30 purified by a rapid chromatography technique. *J Urol* 1990; 144:1510–1515.
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