

## SUMMARY OF SAFETY AND EFFECTIVENESS DATA

### I. GENERAL INFORMATION

**Device Generic Name:**

Kit, Test, Prostate specific antigen (PSA)

**Device Trade Name:**

Access® Hybritech® PSA reagents on the Access® Immunoassay Systems  
(calibrators)

**Applicant's Name and Address:**

Beckman Coulter, Inc.  
1000 Lake Hazeltine Drive  
Chaska, MN 55318

**Date(s) of Panel Recommendation:** None

**Premarket Approval Application (PMA) Number:** P850048/S021

**Date of Notice of Approval to Applicant:** May 9, 2008

### II. INDICATIONS FOR USE

The modifications described in this supplement do not affect the intended use and indications for use.

The Access® Hybritech® PSA assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of total prostate specific antigen (PSA) levels in human serum, using the Access® Immunoassay System.

The Access® Hybritech® PSA assay is indicated for the measurement of serum PSA in conjunction with digital rectal examination (DRE) as an aid in the detection of prostate cancer in men aged 50 or older. Prostate biopsy is required for the diagnosis of cancer. This device is further indicated for the serial measurement of PSA to aid in the prognosis and management of patients with prostate cancer. This device is further indicated for the serial measurement of PSA to aid in the prognosis and management of patients with prostate cancer.

### III. CONTRAINDICATIONS

Not applicable

### IV. WARNINGS AND PRECAUTIONS

The PSA assay results based on the original Hybritech calibration should not be converted directly with the three calibration factors to that of WHO traceable calibration with the three calibration factors without actual measurement on the ACCESS analyzer using

WHO derived calibration function.

V. DEVICE DESCRIPTION

A. Background and rationale for addition of WHO calibration traceability to WHO PSA Reference Preparation 96/670

The Hybritech Tandem-R PSA assay (P850048) was approved by FDA in February 1986 with calibration linked to an established internal reference preparation of human PSA purified from seminal plasma. The original clinical cut-off of 4.0 ng/mL for the Hybritech Tandem-R PSA assay was established using this calibration material. With the acquisition of Hybritech and the transfer of the Tandem-R PSA assay to the Access Immunoassay Systems, Beckman Coulter maintained the standardization of the assay as was originally approved by FDA, and received approval for the Access version of the Hybritech assay (P850048/S016) in February 2000.

The assignment of mass to the WHO 96/670 standard was carried out using a different quantitative method than that used to assign the mass for the original Hybritech standard. The concentration of the WHO 96/670 standard was assigned by quantitative amino acid analysis resulting in a molar extinction coefficient of 1.84, while the Hybritech standard was assigned by Lowry colorimetric total protein method with a corresponding molar extinction coefficient of 1.42. As a result of this difference in extinction coefficients, the apparent mass of PSA in the WHO 96/670 standard is higher than that of the original Hybritech standard. The result of this mass difference is that patients' PSA values based on the WHO 96/670 standard read approximately 20% lower compared to results based on the Hybritech standard.

B. Device

The Access® total PSA WHO calibrators are the same set of calibrators as the original Access® Hybritech® PSA calibrators derived from purified free PSA materials of human seminal fluid except the PSA values were traceable to the WHO 96/670 total PSA standards. The concentration of the WHO 96/670 standard was determined by quantitative amino acid analysis resulting in a molar extinction coefficient of  $0.99 \text{ L} \times \text{g}^{-1} \times \text{cm}^{-1}$  at 280 nm(1) . Three conversion factors were used to align the original Hybritech Access total PSA calibrator values with the WHO standard.

**Table 1. Hybritech to WHO Calibration Factors**

Hybritech Calibrator Dose Range (ng/mL)	Hybritech to WHO Calibration Factor
0-2.0	0.845
2.01-75.0	0.779
>75.0	0.809

VI. ALTERNATIVE PRACTICES OR PROCEDURES

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The original assay calibration used in the measurement of total PSA on Beckman Access analyzer employed the same six calibrators as the Access® PSA WHO calibrators except the Lowry colorimetric total protein method with a molar extinction coefficient of  $1.42 \text{ L} \times \text{g}^{-1} \times \text{cm}^{-1}$  at 280 nm was used to assign PSA values. These calibrators were approved for the Access analyzer in P850048/S016.

### VII. MARKETING HISTORY

The Access® Hybritech® PSA assay with both the Hybritech and WHO calibrations was first made commercially available outside the United States in September 2006. The required regulatory submissions, including those required by the European Union and Canada, have been submitted and approved.

### VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

As the result of different methods for assigning total PSA values for the Hybritech and WHO calibrators, the original clinical cut-off is changed from 4.0 ng/mL with the Hybritech calibration to 3.1 ng/mL with the WHO calibration. If the wrong cut-off is used due to confusion of the calibration method used with the assay, a potential risk of false positive result is subject a patient to unnecessary repeated biopsies and follow-up. Alternatively, a false negative test result may delay a patient from timely diagnosis and treatment.

### IX. SUMMARY OF PRECLINICAL STUDIES

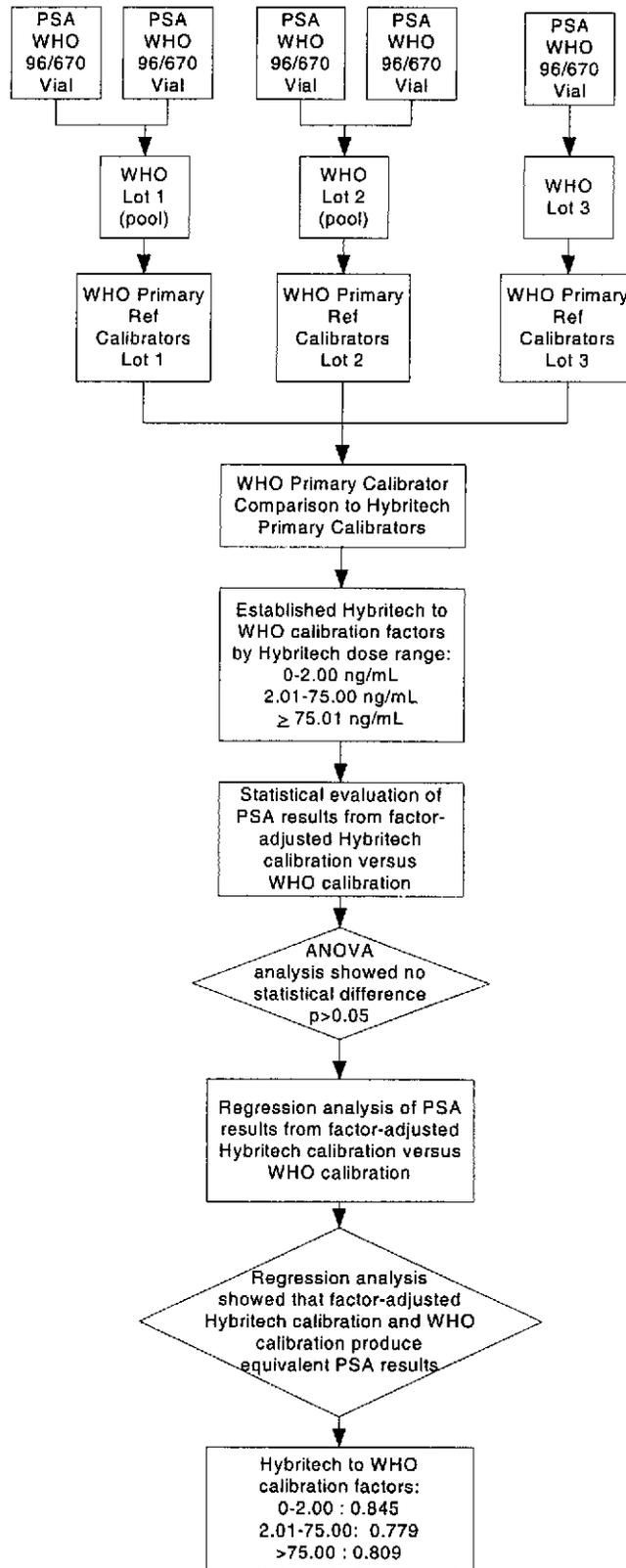
#### A. Establishment and verification of Access PSA WHO calibration

1. Summary of process
  - a. Establishment of WHO primary calibrators to the World Health Organization 1st International Reference Preparation 96/670.
  - b. Establishment of WHO calibration factors relative to the Access Hybritech PSA calibration to align with the Access free PSA assay WHO standardization. Statistical analysis of verification data included:
    - i. Analysis of variance (ANOVA) to confirm factor equivalence and poolability over the three WHO primary calibrator/Hybritech primary calibrator comparisons.
    - ii. Deming regression to confirm that PSA results from both Hybritech and WHO calibrations are equivalent.
  - c. Establishment of a clinical cut-off for use with the WHO calibration that is directly traceable and clinically equivalent to the original clinical data used to establish the efficacy of the Hybritech PSA assay.
  - d. Verification of the safety and efficacy of a WHO calibration 3.1 ng/mL cut-off for clinical interpretation of PSA results relative to the Hybritech calibration 4.0 ng/mL cut-off. Statistical analysis of clinical data included:
    - i. Concordance analysis to confirm equivalence of the WHO cut-off for clinical interpretation of PSA results.

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- ii. Deming regression to confirm the relationship (i.e. calibration factors) established between the Hybritech and WHO calibrations.
  - iii. Receiver Operating Characteristic (ROC) analysis to confirm equivalence of the WHO cut-off for clinical interpretation of PSA results, testing the null hypothesis:  $H_0: AUC_{Hyb} = AUC_{WHO}$ .
  - iv. Clinical sensitivity, clinical specificity, and accuracy analysis using McNemar's test of equality to confirm equivalence of the WHO cut-off for clinical interpretation of PSA results.
2. Establishment of WHO primary calibrator sets  
Three independently prepared WHO primary calibrator sets (i.e. preps or lots) from multiple vials of the WHO 96/670 standard were used for value assignment. Based on the mass assigned by WHO, the final PSA concentration of the reconstituted WHO 96/670 standard (stock) was 500 ng/mL. WHO calibrator levels S3, S4, and S5 were prepared directly from the reconstituted WHO 96/670 standard stock using single dilutions to target PSA concentrations similar to Relative Light Unit (RLU) levels as the Hybritech primary calibrator levels (8.0 ng/mL for S3, 58.0 ng/mL for S4, and 121 ng/mL for S5). Similarly, WHO calibrator levels S1 and S2 were prepared using single dilutions of a 1:20 dilution of the reconstituted WHO 96/670 standard stock (stock 1) to target concentrations of 0.40 ng/mL for S1 and 1.70 ng/mL for S2. Access Hybritech PSA Sample Diluent was used as the diluent in the preparation of the WHO calibrators.
3. Establishment of WHO calibration factors  
Figure 1 summarizes the process by which calibrator factors were established to align the Hybritech calibrators to the WHO standard.

**Figure 1. Calibration Factor Establishment Process Summary**



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The master calibration curve technique was used to establish the Access WHO calibration curve from the Access Hybritech primary calibration curve. This is a statistically-based method used to accurately and precisely measure the difference between corresponding Hybritech and WHO calibrator levels to establish the relationship between the WHO primary calibrator preps and the Hybritech primary calibrators.

Using the master curve technique, each of the three WHO primary calibrator preps was tested side-by-side in three independent studies with the Hybritech primary calibrator set, patient serum samples, and controls in the Access PSA assay to determine the relationship (i.e. calibration factors) between Hybritech and WHO calibrations.

The Hybritech master calibration curve and the WHO master calibration curve created were used to convert the replicate serum sample RLUs to PSA concentrations. The mean concentration of each serum sample replicate set was calculated.

The calibration factors, defined as the ratio WHO/Hybritech, were derived using 100 patient serum sample concentrations calculated from the Hybritech master calibration curve and the WHO master calibration curve. Three factors were computed in ranges defined by the location of the Access PSA calibrators in the Hybritech calibration curve; 0 – 2.0 ng/mL PSA (Hybritech), 2.01 – 75.0 ng/mL PSA (Hybritech) and >75.0 ng/mL PSA (Hybritech).

The mathematical formula used to compute the different calibration factors for the three calibration ranges is illustrated below.

### Calibration Factor Computation Mathematical Formula for Each Range

For each sample:

$$C = A \div B$$

For each range:

Calibration Factor = Average (C) of all samples in that range

Where:

A = Serum Sample Concentration (ng/mL PSA) calculated from the WHO master calibration curve

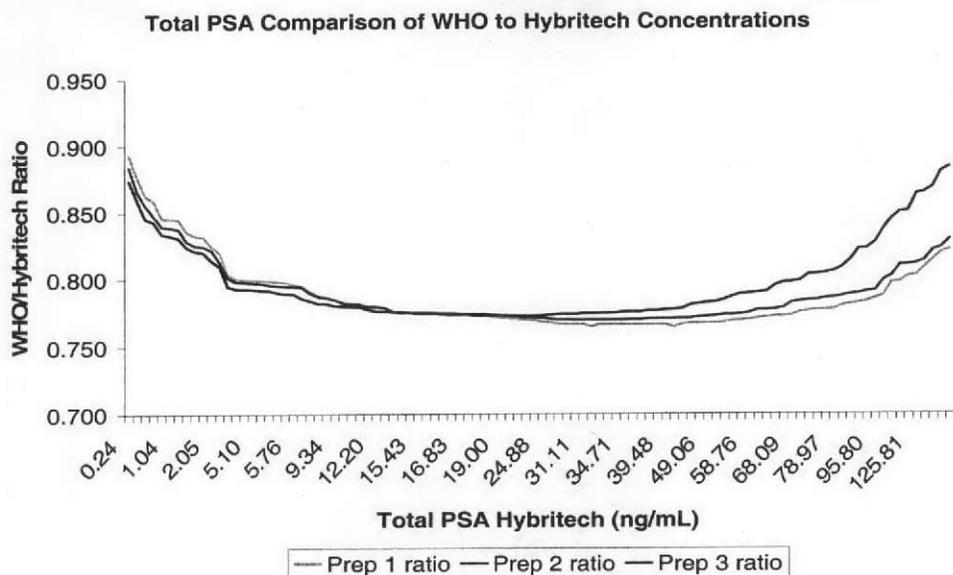
B = Serum Sample Concentration (ng/mL PSA) calculated from the Hybritech master calibration curve

C = Ratio WHO/Hybritech

Within each WHO primary calibrator prep, all means were sorted by Hybritech concentration and used to compute the WHO/Hybritech PSA concentration ratio. The relative differences between WHO and Hybritech were plotted over the range of the Access Hybritech PSA primary calibrator

range (0 – 160 ng/mL) for the three WHO primary calibrator preps. The results of this analysis are shown in Figure 2.

**Fig. 2. Total PSA Comparison of WHO to Hybritech Concentrations**



The plot shows that the WHO to Hybritech PSA concentration ratio is almost consistent across the range of 2.01 to 75.0 ng/mL for the set of 100 patient samples with three different WHO/Hybritech PSA concentration ratios.

The results for the WHO to Hybritech calibration curve comparisons were evaluated by calculating the WHO/Hybritech ratio for serum samples run with both WHO and Hybritech primary calibrator curves for three WHO primary calibrator sets. Serum samples spanned the assay range. The WHO/Hybritech ratios are summarized in Table 2.

**Table 2. WHO/Hybritech Calibration Factor Summary by WHO Primary Calibrator Sets and Hybritech PSA Assay Ranges**

WHO/Hybritech Lot 1	N	Mean	SD	SE	95% CI of mean	Median
0-2.0 ng/mL Hybritech	10	0.853	0.020	0.006	0.838-0.867	0.846
2.01-75 ng/mL Hybritech	72	0.777	0.013	0.002	0.774-0.780	0.772
75.01-160 ng/mL Hybritech	18	0.793	0.015	0.004	0.785-0.800	0.786
<b>WHO/Hybritech Lot 2</b>						
0-2.0 ng/mL Hybritech	10	0.839	0.017	0.005	0.826-0.851	0.833
2.01-75 ng/mL Hybritech	72	0.781	0.009	0.001	0.779-0.784	0.777
75.01-160 ng/mL Hybritech	18	0.836	0.028	0.007	0.823-0.850	0.832
<b>WHO/Hybritech Lot 3</b>						
0-2.0 ng/mL Hybritech	11	0.843	0.019	0.006	0.830-0.856	0.839
2.01-75 ng/mL Hybritech	69	0.778	0.010	0.001	0.776-0.781	0.774
75.01-160 ng/mL Hybritech	20	0.799	0.015	0.003	0.791-0.806	0.791

An Analysis of Variance (ANOVA) was performed to determine if the PSA

results from the factor-adjusted Hybritech calibration are statistically different from the WHO calibration PSA results (Table 3)

**Table 3. Summary of ANOVA P Value Results Across the Access PSA Calibration Curve Ranges**

	Hybritech PSA ng/mL Range			
	Overall	0-2.0	2.01-75.0	>75.0
<b>Factor vs. Calibrator</b>	0.8501	0.9635	0.7477	0.9105
<b>Lot-to-Lot</b>	0.5334	0.8427	0.4533	0.1262
<b>Interaction</b>	0.9809	0.9979	0.9971	0.8540

Based on the outcome of these analyses, a mean factor was established using three WHO primary calibrator sets for each of the three Hybritech calibration curve ranges (0-2.0, 2.01-75.0, and >75.0 ng/mL). The mean factors and statistics for each Hybritech range (0-2.0, 2.01-75.0 and >75.0 ng/mL) are summarized in Table 4.

**Table 4. Summary of Calibration Factor Means**

Mean/Median	n	Mean	SD	SE	95% CI of mean	Median
0-2.0 ng/mL Hybritech	3	<b>0.845</b>	0.0072	0.0042	0.827-0.863	0.839
2.01-75 ng/mL Hybritech	3	<b>0.779</b>	0.0021	0.0012	0.773-0.784	0.774
75.01-160 ng/mL Hybritech	3	<b>0.809</b>	0.0233	0.0134	0.751-0.867	0.791

For the calibration uncertainty analysis, the replicate mean, standard deviation, and standard error were calculated for each calibrator level. The uncertainty was calculated as the mean RLU of the replicates divided by the standard error. The uncertainty of the RLU estimate of each non-zero calibrator level was found to be  $\leq 1\%$  in all cases.

Table 5 compares the assigned values of the WHO calibrators after adjustment with the conversion factors to that of the Hybritech calibrators.

**Table 5. Comparison of Assigned Value of WHO and Hybritech Calibration**

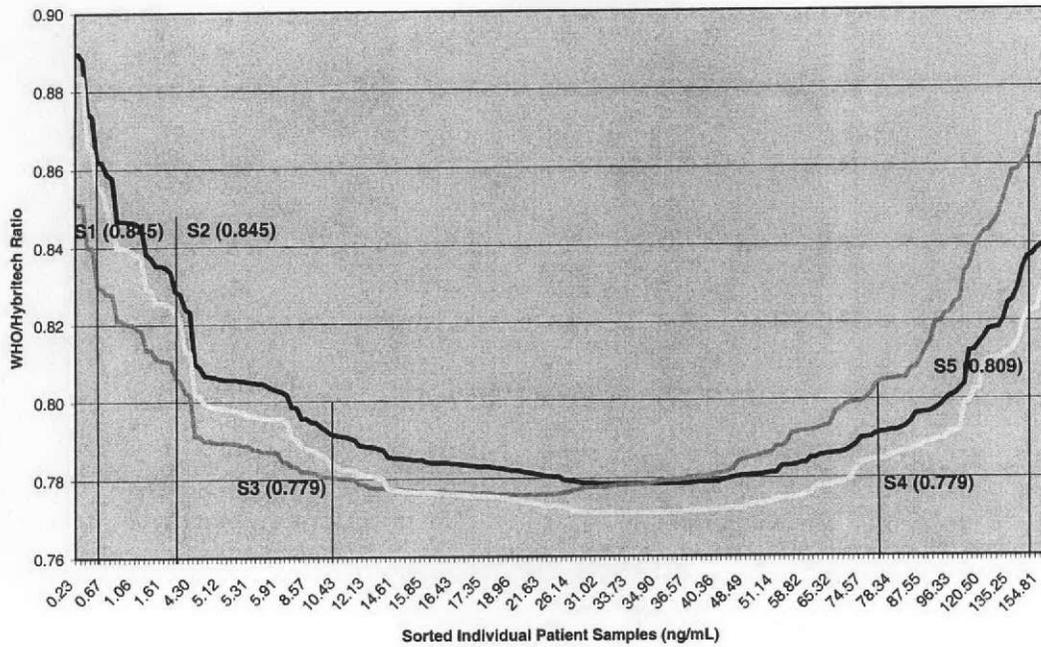
Calibrator Level	WHO assigned Values (ng/mL)	Hybritech Assigned Values (ng/mL)
S0	0.00	0.00
S1	0.42	0.47
S2	1.60	1.95
S3	7.8	9.9
S4	60.0	74.7
S5	125	151

Figure 3 shows a plot of percent difference between Hybritech and WHO calibration values for the 100 patient samples used to establish the WHO calibration for three WHO primary calibrator lots. The plot shows a non-constant difference over the assay range, indicating a need for range-dependent

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adjustment factors.

### Comparison of WHO to Hybritech Results (3 factor)



#### 4. Laboratory Validation Studies

Laboratory validation studies were performed to compare the three factors adjusted Hybritech calibration curve to the original Hybritech calibration curve.

##### a. Calibration curve comparison

Figure 4 shows the six-point curve fits of both the Hybritech and WHO calibrations. The WHO calibrators contain less PSA mass for its assigned dose than the Hybritech calibrators, therefore, the WHO curve provides lower doses for a given RLU than does the Hybritech curve.

**Figure 4. Hybritech and WHO Calibration Curves**

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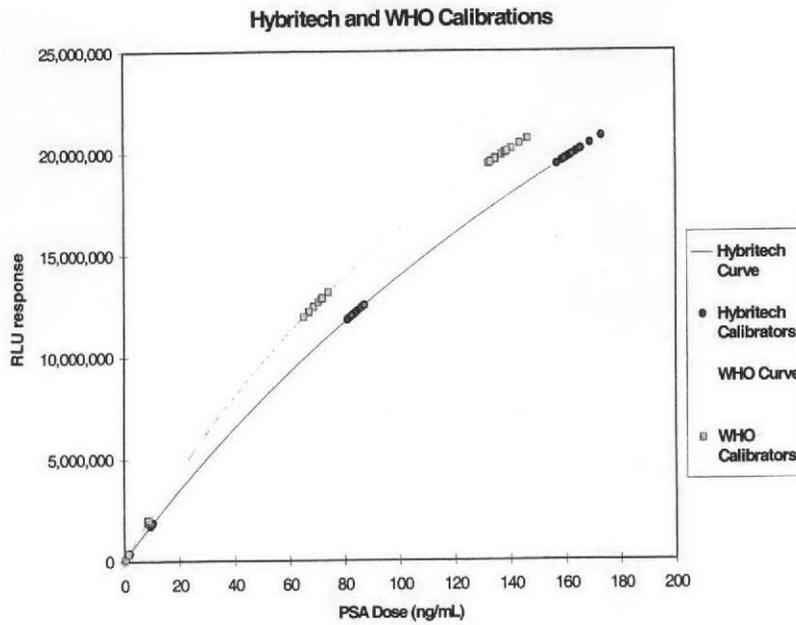
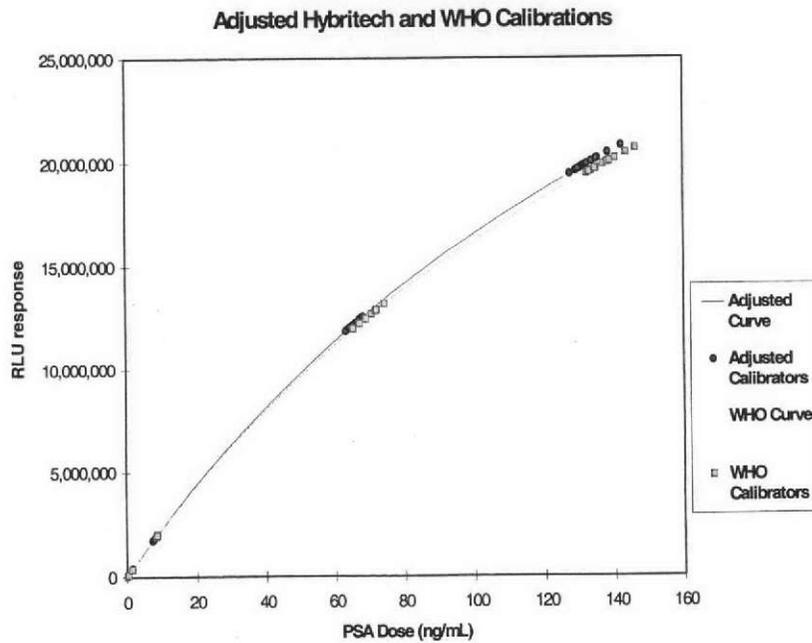


Figure 5 shows the six-point curve fits of both the WHO calibration and the 3-factor adjusted Hybritech calibrations. It was demonstrated that the two curves match each other well, indicating the adjustment can take the place of using actual WHO calibrator material.

**Figure 5. Comparison of Adjusted Hybritech and WHO Calibrators**



b. Calibration verification

i. Calibration recovery

The relative light units (RLUs) generated for each calibrator level

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replicate was read as an unknown off of its respective calibration curve (e.g. Hybritech calibrator level S1 read off the Hybritech calibration curve, and WHO calibrator level S1 read off the WHO calibration curve) to provide a dose value for each replicate. The two replicate doses were averaged for each calibrator level of each calibrator set (Hybritech and WHO) to provide a mean reported calibrator value. The percent recovery for each calibrator level of each calibrator set (Hybritech and WHO) was determined by dividing the mean reported calibrator value by the assigned calibrator value.

Results showed acceptable recovery of individual calibrator levels from their respective calibration curve with the grand average recovery  $100.0 \pm 2.0\%$  (See Tables 6 and 7).

**Table 6. Access Hybritech Calibrator Recovery**

Calibrator Level	Hybritech Assigned Concentration (ng/mL)	RLU	Hybritech Reported Concentration (ng/mL)	Percent Recovery (%)	Mean Percent Recovery (%)
S0	0.00	10,224	0.00	N/A	N/A
		10,104			
S1	0.47	104,331	0.46	98.7	99.5
		105,875	0.47	100.4	
S2	1.95	391,846	1.99	102.2	103.2
		398,981	2.03	104.2	
S3	9.9	1,791,170	10.1	102.4	100.8
		1,738,000	9.8	99.2	
S4	74.7	10,843,400	77.9	104.3	104.9
		10,946,700	78.8	105.5	
S5	151	17,811,100	150	99.5	101.1
		18,229,500	155	102.8	
GRAND AVERAGE					101.9

**Table 7. Access WHO Calibrator Recovery**

Calibrator Level	WHO Assigned Concentration (ng/mL)	RLU	WHO Reported Concentration (ng/mL)	Percent Recovery (%)	Mean Percent Recovery (%)
S0	0.00	11,410	0.00	N/A	N/A
		11,667			
S1	0.42	104,472	0.44	104.3	103.2
		102,554	0.43	102.2	
S2	1.60	352,457	1.59	99.6	99.9
		354,608	1.60	100.2	
S3	7.8	1,647,810	7.7	99.4	101.9
		1,729,560	8.1	104.5	
S4	60.0	10,055,000	57.3	95.5	95.6
		10,075,000	57.5	95.8	
S5	125	17,878,200	128	102.6	104.2
		18,224,800	132	105.8	

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GRAND AVERAGE	101.0
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ii. Calibration validation between mathematically assigned and observed values

A commercial set of Hybritech calibrators with WHO outputs (mathematically assigned WHO dose values) were run as unknowns in duplicate. The relative light units (RLUs) generated by each calibrator level replicate was read off the WHO calibration curve generated from the WHO calibrator set for a reported WHO dose value. The reported WHO dose values were averaged for each Hybritech calibrator level to provide a mean reported WHO dose value. The percent difference for each calibrator level was determined by subtracting the mathematically assigned WHO dose value from the mean reported WHO dose value, then dividing by the mathematically assigned WHO dose value as described in the following equation.

$$\frac{(\text{Mean Reported WHO Dose Value} - \text{Mathematically Assigned WHO Dose Value})}{\text{Mathematically Assigned WHO Dose Value}} \times 100$$

The data as summarized in Table 8 demonstrate that the percent difference between the mean reported WHO dose values and the mathematically assigned WHO dose values are acceptable based on the fact that all the individual percent differences centered around the grand average percent difference of  $6.87 \pm 1.5\%$ . The difference across individual calibrator levels may be reflective of the expected lot-to-lot differences inherent in the WHO calibrator set preparation process.

**Table 8. Percent Differences between the Mean Reported WHO Dose Values and the Mathematically Assigned WHO Dose Values**

Calibrator Level	Assigned WHO dose Value (ng/mL)	Reported WHO Dose Value (ng/mL)	Mean Reported WHO Dose Value (ng/mL)	Percent Difference (%)
S0	0.00	0.00	N/A	N/A
		0.00		
S1	0.40	0.43	0.43	7.50
		0.43		
S2	1.65	1.76	1.7	5.45
		1.72		
S3	7.7	8.1	8.3	7.34
		8.4		
S4	58.2	60.9	61.6	5.87
		60.4		
S5	122	133	132.0	8.20
		131		
GRAND AVERAGE				6.87

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iii. Calibration stability

Calibration curve stability was not tested. Calibration curve stability was validated during development of the Access PSA assay and approved in P850048/S016. Since the matrix, antigen, and mass of antigen in each calibrator are unchanged and WHO calibration is derived by applying the three calibration factors, the calibration stability is not affected..

B. Analytical validations

1. Equimolarity

The purpose of this study was to demonstrate that equimolarity of the Access PSA assay established as part of the FDA approved Access PSA PMA supplement (P850048/S016) is not affected as result of conversion to the WHO 96/670 total PSA standard which is 90:10 of complexed PSA:Free PSA whereas the original Hybritech Total PSA standard is 100% free PSA.

The data presented in Tables 9 and 10 demonstrate that variable proportions of PSA-ACT and free PSA in a given sample do not affect the recovery of total PSA in the Access Hybritech PSA assay using either a Hybritech or WHO calibration. The mean Hybritech molar response ratio for the 10, 4, and 2 ng/mL target total PSA concentrations was 1.02 as compared to the mean WHO calibration molar response ratio of 1.00. A paired samples t-test shows that the difference between the Hybritech calibration mean molar response ratio and the WHO calibration mean molar response ratio is not statistically significant (p=0.1339).

**Table 9. Access Hybritech PSA Molar Response Ratios Hybritech Calibration**

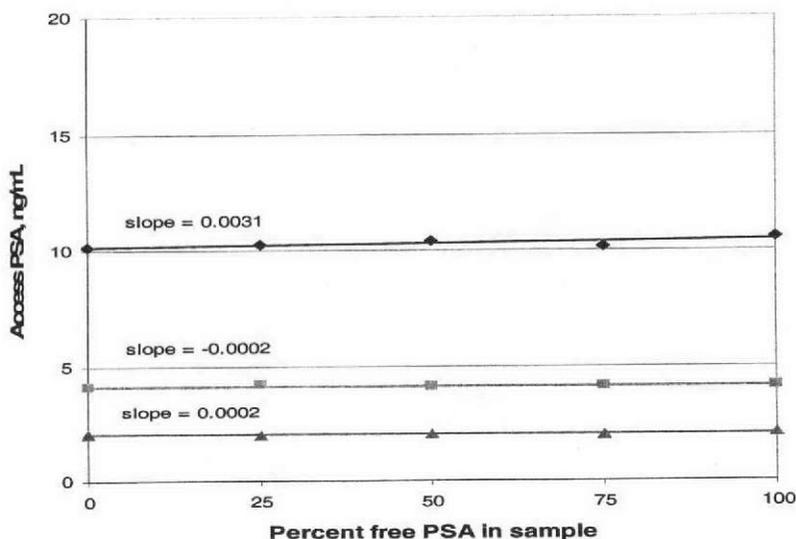
Test Sample Containing:		10 ng/mL Target Total PSA Test Concentration	4 ng/mL Target Total PSA Test Concentration	2 ng/mL Target Total PSA Test Concentration
%PSA-ACT	%fPSA	Total PSA Mean Recovery (ng/mL)		
100	0	10.14	4.11	2.05
75	25	10.23	4.21	2.00
50	50	10.38	4.11	2.06
25	75	10.16	4.12	1.99
0	100	10.57	4.13	2.08
<b>Molar Response Ratio</b>		1.04	1.01	1.02
		<b>Mean Molar Response Ratio</b>		
		1.02		

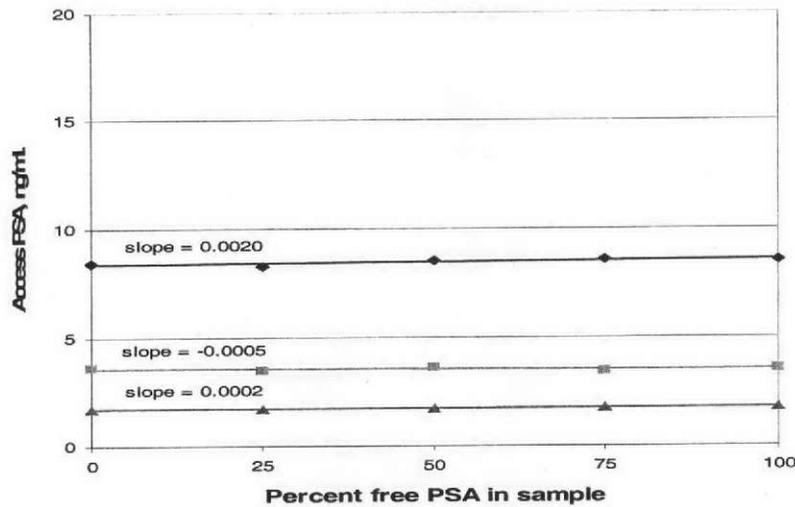
**Table 10. Access Hybritech PSA Molar Response Ratios WHO Calibration**

Test Sample Containing:		10 ng/mL Target Total PSA Test Concentration	4 ng/mL Target Total PSA Test Concentration	2 ng/mL Target Total PSA Test Concentration
% PSA-ACT	% fPSA	Total PSA Mean Recovery (ng/mL)		
100	0	8.42	3.60	1.74
75	25	8.29	3.45	1.71
50	50	8.53	3.62	1.73
25	75	8.58	3.43	1.75
0	100	8.53	3.55	1.74
<b>Molar Response Ratio</b>		1.01	0.98	1.00
		<b>Mean Molar Response Ratio</b>		1.00

The results are also presented graphically in Figures 6 and 7. A linear regression test shows that there are no statistically significant trends over the range from 0% free PSA to 100% free PSA for either the Hybritech (p=0.3692) or WHO (p=0.6745) calibrations.

**Figure 6. Access Hybritech PSA Molar Response Ratios Hybritech Calibration**



**Figure 7. Access Hybritech PSA Molar Response Ratios WHO Calibration**

In summary, the mean molar response ratio for both the Hybritech and WHO calibrations is within the specified range of 0.85 – 1.15 established in P850048/S016. A paired samples t-test shows that the difference between the Hybritech calibration mean molar response ratio and the WHO calibration mean molar response ratio is not statistically significant. A linear regression test shows that there are no statistically significant trends over the range from 0% free PSA to 100% free PSA for either the Hybritech or WHO calibrations.

## 2. Imprecision

Imprecision of the Access Hybritech PSA assay was evaluated with a Hybritech commercial calibrator set (Hybritech calibration and 3-factor adjusted Hybritech calibration) and a WHO calibrator set (WHO calibration). The study design was based on CLSI Document EP5-A2 (Evaluation of Precision Performance of Quantitative Measurement Methods: Approved Guideline – Second Edition, 2004).

Imprecision of the Access Hybritech PSA assay with the Hybritech, 3-factor adjusted Hybritech, and WHO calibrations was assessed by testing three patient samples at WHO concentrations approximating 8, 3.1, and 1.6 ng/mL. Each patient sample was run in replicates of 3 with each of the Hybritech, 3-factor adjusted Hybritech, and WHO calibrations for a total of 9 replicates per sample. Testing occurred over 20 days (2 runs per day) for a total of 40 runs and 360 replicates on one instrument. Each replicate run over 40 runs was used to calculate standard deviation (SD) and coefficient of variation (CV) of within-run (repeatability) and total imprecision.

Results showed the total %CV ranged from 4.74% to 4.13% for the

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Hybritech calibration, 4.75% to 3.44% for the 3-factor Adjusted Hybritech calibration, and 5.53% to 4.33% for the WHO calibration (Tables 11 - 13).

**Table 11. Hybritech Calibration Imprecision**

Sample	Mean (ng/mL)	Within Run SD (ng/mL)	Within Run CV (%)	Total SD (ng/mL)	Total CV (%)
1	1.91	0.057	3.01	0.090	4.74
2	3.98	0.113	2.85	0.184	4.64
3	10.12	0.288	2.84	0.418	4.13

**Table 12 Three-factor Adjusted Hybritech Calibration Imprecision**

Sample	Mean (ng/mL)	Within Run SD (ng/mL)	Within Run CV (%)	Total SD (ng/mL)	Total CV (%)
1	1.58	0.044	2.80	0.075	4.75
2	3.26	0.094	2.87	0.148	4.52
3	7.98	0.167	2.09	0.275	3.44

**Table 13 WHO Calibration Imprecision**

Sample	Mean (ng/mL)	Within Run SD (ng/mL)	Within Run CV (%)	Total SD (ng/mL)	Total CV (%)
1	1.70	0.050	2.94	0.094	5.53
2	3.59	0.089	2.48	0.156	4.33
3	8.84	0.236	2.67	0.412	4.66

In conclusion, results of this analysis confirm that imprecision of the Access Hybritech PSA assay is not significantly different when using the Hybritech, 3-factor adjusted Hybritech, or WHO calibration.

3. Limit of Blank/Detection/Quantitation

Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) of the Access Hybritech PSA assay was evaluated with a Hybritech commercial calibrator set (Hybritech calibration and 3-factor adjusted Hybritech calibration) and a WHO calibrator set (WHO calibration). The study design was based on CLSI document EP17-A "Protocols for Determination of Limits of Detection and Limits of Quantitation: Approved Guideline", 2004.

Limit of Blank (LoB)

Twenty-two female patient serum samples were obtained to evaluate LoB. These 22 female samples were run once on the Access Immunoassay System with the Access Hybritech PSA assay in replicates of 3 with each the Hybritech, 3-factor adjusted Hybritech, and WHO calibrations for a total of 198 female serum sample replicates. In addition to the female samples, Access Hybritech PSA Sample Diluent was run 22 times on the Access Immunoassay System with the Access Hybritech PSA assay in replicates of 3 with each of the Hybritech, 3-factor adjusted Hybritech, and WHO calibrations for a total of 198 replicates. Three controls were run 11 times in replicates of 3 with each of the Hybritech, 3-factor

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adjusted Hybritech, and WHO calibrations for a total of 99 replicates per control level.

### Limit of Detection (LoD)

Eight samples bracketing the Access Hybritech PSA assay analytical and functional sensitivity values ( $\approx 0.008$  to  $0.030$  ng/mL) were prepared to evaluate LoD. These samples were gravimetrically prepared by spiking Hybritech PSA Calibrator S2 (approximate concentration of  $2$  ng/mL) into Access Hybritech PSA Sample Diluent. These 8 samples were run 10 times on the Access Immunoassay System with the Access Hybritech PSA assay in replicates of 6 with each of the Hybritech, 3-factor adjusted Hybritech, and WHO calibrations for a total of 180 replicates per sample.

For the 8 LoD samples, the mean and standard deviation were computed for the replicates for each sample (minimum of 54 per sample) for the Hybritech, 3-factor adjusted Hybritech, and WHO calibrations. The relationship between mean and standard deviation was established for the Hybritech, 3-factor adjusted Hybritech, and WHO calibrations using a linear regression.

The LoD was determined using this relationship by finding the dose at which the 5<sup>th</sup> percentile of the distribution of a low dose sample would match the LoB. This was determined for the Hybritech, 3-factor adjusted Hybritech, and WHO calibrations using the LoB determined from the Access Hybritech PSA Sample Diluent and female sample study results.

### Limit of Quantitation (LoQ)

The LoQ was determined using the same relationship to find the dose at which 20% CV was achieved.

### Data Analysis

For the 66 Access Hybritech PSA Sample Diluent replicates for each calibration, the mean and standard deviation of their RLUs were determined. The RLU at the 95<sup>th</sup> and 97.5<sup>th</sup> percentiles were determined and reduced to dose using the Hybritech, 3-factor adjusted Hybritech, and WHO calibration curves established on the instrument. These two doses are respectively the LoB for the Access Hybritech PSA Sample Diluent study for the Hybritech and WHO calibrations.

For the 22 female samples (9 replicates each), the RLUs were inspected. For one sample, the RLUs were twice the magnitude of the other 21 samples. This sample was excluded from further analysis. In addition, 1 replicate outlier for one of the remaining 21 samples was removed. The mean and standard deviation were determined for the remaining 188 replicates. The RLU at the 95<sup>th</sup> and 97.5<sup>th</sup> percentiles were determined and reduced to dose using the Hybritech, 3-factor adjusted Hybritech, and WHO calibrations established on the instrument. These two doses are

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respectively the LoB for the female sample study for the Hybritech, 3-factor adjusted Hybritech, and WHO calibrations.

Results

a. In the sample diluent study:

The three calibrations provided no significant difference for analytical sensitivity, LoB, and LoD and all results were below the published Access Hybritech PSA assay analytical sensitivity value of <0.008 ng/mL (which used Access Hybritech PSA Sample Diluent to establish this claim).

b. In female sample study:

The three calibrations provided no significant difference for LoB and LoD. Results were above the published limit of detection but acceptable because results of such a study are expected to vary depending on the population of female samples used in the study

c. The LoQ showed no significant difference between the three calibrations.

Table 14 summarizes the sample diluent and female sample study LoB, LoD, and LoQ results for the Hybritech, 3-factor adjusted Hybritech, and WHO calibrations.

**Table 14 Limit of Detection/Limit of Blank/Limit of Quantitation**

Parameter	Hybritech Calibration Result (ng/mL)	3-factor Adjusted Hybritech Calibration Result (ng/mL)	WHO Calibration Result (ng/mL)
Published Analytical Sensitivity (using Access Hybritech PSA Sample Diluent)	<0.0080	<0.0080	<0.0080
Limit of Blank (using Access Hybritech PSA Sample Diluent)	0.0016	0.0016	0.0018
Limit of Blank (using female samples)	0.0092	0.0097	0.0109
Limit of Detection (using Access Hybritech PSA Sample Diluent)	0.0030	0.0030	0.0033
Limit of Detection (using female samples)	0.0107	0.0112	0.0126
Limit of Quantitation	0.0043	0.0043	0.0049

4. Dilution Recovery

Dilution recovery of the Access Hybritech PSA assay with both the Hybritech and WHO calibrations was assessed using a testing protocol based on the CLSI document EP6-A "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline", 2003. Five serum samples with elevated levels of PSA were diluted with the Access Hybritech PSA Zero Calibrator (S0) to various levels throughout the assay range and tested in replicates of four.

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The average dose for each sample was determined and compared to the expected recovery for each level. Percent recovery was calculated comparing the actual dose versus the expected dose. The grand percent recovery mean must be 100% ± 10% for both the Hybritech and WHO calibrations. Each dilution study was also analyzed by Deming regression. The analysis was performed for both the Hybritech and WHO calibrations.

The neat ranges of the samples ranged from 92 ng/mL to 135 ng/mL PSA with the Hybritech calibration. The mean percent recoveries for the 5 samples ranged from 103.7% to 112.8% for the Hybritech calibration and 105.4% to 113.3% for the WHO calibration. The grand percent recovery mean was within the specification 100% ± 10% for both the Hybritech and WHO calibrations. Correlation coefficients for the WHO calibration were similar to the Hybritech calibration correlation coefficients with overlapping 95% confidence intervals. Results are presented in Tables 15 and 16.

**Table 15. Dilution Recovery**

Sample	Hybritech Average Recovery	Hybritech Slope	Hybritech 95% CI	WHO Average Recovery	WHO Slope	WHO 95% CI
1	108.02	1.008	0.982-1.034	107.94	0.991	0.964-1.017
2	103.73	0.962	0.912-1.012	105.42	0.962	0.910-1.013
3	107.54	1.006	0.986-1.025	109.07	1.005	0.984-1.025
4	112.78	1.009	0.956-1.062	113.31	1.001	0.947-1.055
5	108.11	1.012	0.982-1.041	108.43	0.998	0.968-1.028
Grand	108.04	1.007	0.993-1.021	108.83	0.996	0.982-1.009

**Table 16. Dilution Recovery Deming Regression Results**

Sample	Hybritech Correlation	Hybritech 95% CI	WHO Correlation	WHO 95% CI
1	0.9998	0.9984-1.0000	0.9998	0.9982-1.0000
2	0.9993	0.9933-0.9999	0.9993	0.9929-0.9999
3	0.9999	0.9990-1.0000	0.9999	0.9990-1.0000
4	0.9993	0.9932-0.9999	0.9992	0.9928-0.9999
5	0.9998	0.9979-1.0000	0.9998	0.9977-1.0000

The results are consistent with current performance claims and confirm that dilution recovery is equivalent when using the Hybritech or WHO calibration.

5. Linearity

Linearity of the Access Hybritech PSA assay was evaluated with a Hybritech commercial calibrator set (Hybritech calibration and 3-factor adjusted Hybritech calibration) and a WHO calibrator set (WHO

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calibration). The study design was based on CLSI document EP6-A “Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline”, 2003.

Five serum samples with elevated levels of PSA were diluted with the Access Hybritech PSA Zero Calibrator (S0) to various levels throughout the assay range. Each neat sample and associated sample dilutions were tested on the Access Immunoassay System with the Access Hybritech PSA assay in replicates of 4 with each of the Hybritech, 3-factor adjusted Hybritech, and WHO calibrations for a total of 12 replicates per sample. Controls were run 3 times in replicates of 2.

For each calibration, the expected result and the observed result for each replicate were log transformed to assure equal spacing between each diluted sample level. CLSI document EP6-A procedure was used to create a linear fit and a quadratic or cubic fit to the data. The fit to each line was computed for each of the diluted levels and transformed back to dose (ng/mL). The percent difference was computed and presented as percent non-linearity. Where a higher order fit was insignificant, this percent difference is presented as 0.00%.

The neat ranges of the samples ranged from approximately 4 ng/mL to 117 ng/mL PSA with the Hybritech calibration. The resultant lowest dilution level was less than 0.2 ng/mL. The maximum non-linearity seen over all three calibrations was 5.40%. Results are presented in Tables 17-19.

**Table 17 Hybritech Calibration Linearity**

Sample #3		Sample #6		Sample #8		Sample #14		Sample #12/7	
Dose (ng/mL)	Non-linearity								
4.354	-3.19%	12.403	-2.23%	19.913	-2.63%	57.066	-0.93%	117.112	0.00%
2.866	1.48%	8.128	1.07%	13.241	0.16%	38.007	-0.91%	78.134	0.00%
2.214	3.02%	6.162	2.17%	10.012	1.62%	28.544	-0.31%	58.992	0.00%
1.475	3.70%	4.090	2.56%	6.642	3.06%	18.986	1.00%	39.529	0.00%
0.754	1.78%	2.009	1.02%	3.357	3.51%	9.493	3.16%	20.198	0.00%
0.385	-1.18%	0.988	-1.13%	1.631	1.33%	4.897	3.10%	10.403	0.00%
0.193	-2.15%	0.423	-1.10%	0.726	-4.12%	2.009	-3.98%	3.929	0.00%

**Table 18. Three-factor Adjusted Hybritech Calibration Linearity**

Sample #3		Sample #6		Sample #8		Sample #14		Sample #12/7	
Dose (ng/mL)	Non-linearity								
3.557	-2.26%	9.730	0.00%	15.468	-2.61%	44.006	1.09%	91.878	0.00%
2.379	1.30%	6.461	0.00%	10.423	0.16%	29.523	-1.95%	61.406	0.00%
1.855	2.34%	4.941	0.00%	7.953	1.61%	22.286	-2.36%	46.418	0.00%
1.254	2.56%	3.322	0.00%	5.348	3.03%	14.933	-1.29%	31.158	0.00%
0.657	0.69%	1.669	0.00%	2.765	3.46%	7.561	2.50%	15.968	0.00%
0.343	-1.42%	0.841	0.00%	1.377	1.31%	3.948	4.48%	8.249	0.00%
0.175	-0.82%	0.371	0.00%	0.632	-4.07%	1.647	-3.32%	3.130	0.00%

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**Table 19 WHO Calibration Linearity**

Sample #3		Sample #6		Sample #8		Sample #14		Sample #12/7	
Dose (ng/mL)	Non-linearity								
3.812	-2.86%	10.588	0.00%	16.998	-2.92%	49.262	1.09%	103.231	0.00%
2.541	1.29%	7.005	0.00%	11.378	0.18%	32.837	-2.27%	68.568	0.00%
1.977	2.67%	5.344	0.00%	8.642	1.80%	24.676	-2.65%	51.611	0.00%
1.331	3.30%	3.579	0.00%	5.772	3.40%	16.427	-1.30%	34.431	0.00%
0.691	1.63%	1.787	0.00%	2.949	3.90%	8.226	3.19%	17.464	0.00%
0.356	-1.04%	0.893	0.00%	1.450	1.48%	4.249	5.40%	8.929	0.00%
0.177	-1.91%	0.390	0.00%	0.654	-4.56%	1.746	-4.18%	3.335	0.00%

In summary, results of this analysis confirm that linearity of the Access Hybritech PSA assay is not significantly different when using the Hybritech, 3-factor adjusted Hybritech, or WHO calibrations. The validated upper linearity range is 117 ng/mL.

6. Spike Recovery

A study was performed to assess recovery of exogenous PSA spiked into normal male serum as measured on the Access Hybritech PSA assay to confirm that the spike recovery of the Access Hybritech PSA assay when using either the Hybritech or WHO calibration is equivalent to original data approved in P850048/S016.

Concentrations of PSA spanning the range of the assay were spiked into each of 5 normal male serums to obtain 4 spiked levels for each serum. The PSA concentrations were measured in quadruplicate. The WHO calibration results were modeled from the original study data. The original Hybritech calibrator concentrations were assigned WHO calibrator concentrations based on the calibration factors. Original relative light units (RLUs) from raw data were calculated off new WHO calibration curves resulting in dose values based on the WHO calibration.

Recovery was calculated using the following formula:

$$\text{Percent Recovery} = (\text{Observed}/\text{Expected}) \times 100 \%$$

Acceptable recovery is defined as  $\pm 10\%$  of the sample mean, with no apparent trending.

The mean recoveries of the five samples ranged from 96.9% to 101.7% with a grand percent recovery mean of 98.5% with the Hybritech calibration, and 96.6% to 101.6% with a grand percent recovery mean of 98.2% for the WHO calibration. Results are consistent with current performance claims and confirm that spike recovery is equivalent when using the Hybritech or WHO calibration.

7. Hook Effect

To confirm that the hook effect of the Access Hybritech PSA assay is

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equivalent when using either the Hybritech or WHO calibration to the original data approved in P850048/S016, multiple concentrations of PSA (ranging from 19 to over 625,000 ng/mL) were prepared by serial dilution of a seminal fluid sample into zero calibrator (S0), and then assayed in duplicate with the Access Hybritech PSA assay along with a six-point calibration curve and assay controls. This experiment was performed on 3 lots of reagent packs.

The seminal fluid dilutions were assigned equivalent WHO concentrations based on the calibration factors shown. The WHO PSA concentrations ranged from 15 to over 500,000 ng/mL. The original mean RLUs and the WHO concentration were calculated for each sample. Results for the neat sample and the dilutions were compared to the value of the S5 calibrator. The acceptance criterion is no hook effect up to 50,000 ng/mL.

Results showed that the Access Hybritech PSA assay does not demonstrate a hook effect up to approximately 150,000 ng/mL for the Hybritech calibration and 126,000 ng/mL for the WHO calibration and are consistent with the approximately 20% off-set between the Hybritech and WHO calibrations.

### 8. Method comparison

#### Deming regression analysis

In order to show that the calibration curve and factors produced equivalent WHO PSA results, Deming regression analysis was performed between Hybritech and WHO PSA calibrations across the three WHO primary calibrator sets.

The Deming intercepts and slopes show an average slope of 0.9950 with 95% confidence intervals including 1.0 (range 0.9368 to 1.0533) for one lot of Hybritech versus three lots of WHO primary calibrator sets, thus verifying the suitability of the WHO 96/670 standard for use as a primary calibrator.

Table 20 shows a summary of Deming regression analysis Hybritech vs. WHO for PSA sample recoveries from the WHO calibration ("Cal Curve" in Table 20) and factor-adjusted Hybritech calibration ("Cal Factor" in Table 20) across combinations of the three WHO primary calibrator lots. Samples ranged from 0 to 130 ng/mL (Hybritech). Pearson correlation coefficients are also reported and show very high correlation with "r" ranging from 0.9814 to 0.9999.

**Table 20. Summary of Deming Regression for the 0 – 150 ng/mL Hybritech PSA Assay Range**

Cal Curve	Cal Factor	Intercept	SE	95% CI	Slope	SE	95% CI	r
Lot 1	Lot 1	0.1784	0.0241	0.1269-0.2227	0.9750	0.0006	0.9693-0.9718	0.9999
Lot 1	Lot 2	-0.3034	0.2635	-0.8266-0.2197	1.0748	0.0067	1.0614-1.0882	0.9953
Lot 1	Lot 3	-0.1950	0.2560	-0.7032-0.3133	1.0985	0.0065	1.0855-1.1115	0.9965
Lot 2	Lot 1	-0.3252	0.3838	-1.0870-0.4367	0.9307	0.0093	0.9121-0.9492	0.9814
Lot 2	Lot 2	-0.8690	0.4785	-1.8190-0.0811	1.0310	0.0116	1.0079-1.0541	0.9904
Lot 2	Lot 3	-0.7786	0.5424	-1.8554-0.2982	1.0540	0.0132	1.0278-1.0801	0.9954
Lot 3	Lot 1	0.3185	0.3248	-0.3262-0.9632	0.8630	0.0074	0.8483-0.0877	0.9983
Lot 3	Lot 2	-0.1492	0.4155	-0.9742-0.6757	0.9559	0.0095	0.9370-0.9747	0.9927
Lot 3	Lot 3	-0.0143	0.3056	-0.6481-0.5655	0.9771	0.0070	0.9632-0.9909	0.9976

Table 21 summarizes the overall mean and 95% confidence interval for Deming regression for the 0-150 ng/mL Hybritech PSA assay range. The 95% confidence interval for the intercept includes 0 and for the slope includes 1.0. These results are consistent and show that the WHO calibration and factor-adjusted Hybritech calibration are producing equivalent WHO PSA results.

**Table 21. Mean and 95% Confidence Intervals for Deming Intercepts and Slopes for the 0-150 ng/mL Hybritech PSA Assay Range**

Intercept	Mean	95% CI		Slope	Mean	95% CI	
	-0.2409	-0.5424	0.0605		0.9950	0.9368	1.0533

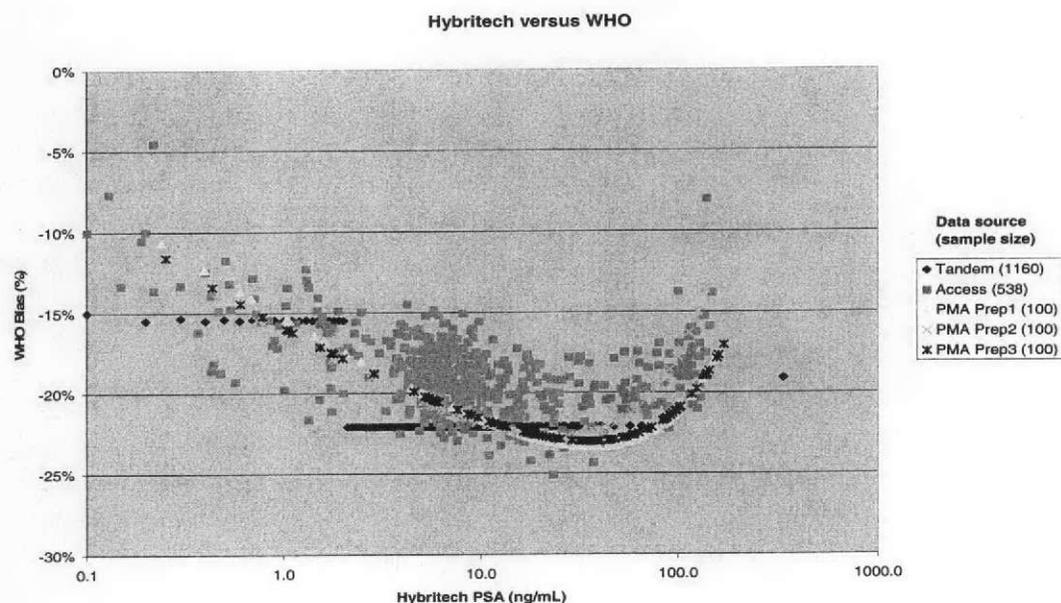
#### Percent Difference Plot

The data from the 1160 patients was reviewed in approved P850048/S009 (Hybritech Tandem-R cancer detection). The Deming regression in P850048/S021 was performed using the original 1160 Hybritech Tandem-R PSA results and WHO results. The WHO results were calculated by applying the applicable calibration factor (i.e. 0.845 for Hybritech Tandem-R values 0-2.0, 0.779 for Hybritech Tandem-R values 2.01-75.0, and 0.809 for Hybritech Tandem-R values 75.01 and greater) to the original 1160 Hybritech Tandem-R PSA results. Since the 1160 patients were not tested directly with the Access PSA assay, no Access PSA assay calibration curves (Hybritech or WHO) were available to use a calibration fit to convert the Hybritech Tandem-R PSA results to Access PSA WHO results. Therefore, in the percent difference plot, it appears that a stepwise function is associated with these results at the points in the assay range where the calibration factors change.

To demonstrate the PSA results are generated from the WHO calibration in a consistent fashion, the Deming regression results from the three original WHO calibrator sets (preps) used to establish the WHO calibration [identified as “PMA Prep1 (100)”, “PMA Prep2 (100)”, and

“PMA Prep3 (100)” in Figure 9], and the 538 sample clinical laboratory study used to validate the WHO calibration [identified as “Access (538)” in Figure 9] are included in the percent difference plot in Figure 8. The results illustrate PSA results are generated from the WHO calibration in a consistent fashion.

**Figure 8. Percent Difference Plot Hybritech versus WHO**



## 9. Cut-off

### Establishing cut-off by WHO Calibration

Application of the 2.01-75.0 ng/mL range WHO factor (0.779) to the established 4.0 ng/mL cut-off for clinical interpretation of PSA results yields a WHO calibration cut-off of 3.116 ng/mL. This value was rounded to 3.1 ng/mL. The safety and efficacy of using the WHO calibration 3.1 ng/mL cut-off for clinical interpretation of PSA results was confirmed by concordance evaluation of clinical data generated to validate the Hybritech 4.0 ng/mL cut-off approved in P850048. These data were gathered in a multi-center, prospective clinical trial conducted to test the effectiveness of PSA along with digital rectal examination (DRE) as an aid in the detection of prostate cancer. A total of 6,374 men 50 years of age and older participated in the study. Reference the Access Hybritech PSA directional insert, located in Section XI, for additional study detail.

The original Hybritech FDA PMA submission (P850048) was filed with 6,374 subjects, but enrollment continued in order to meet the compliance rate recommended by the Advisory Panel for the biopsy subject sub-population. The study enrollment grew to 6,630, which are used in the analyses within this supplement (P850048/S021).

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Table 22 shows the comparison of the distribution of the original 6,630 samples from the clinical study conducted to test the effectiveness of PSA along with digital rectal examination (DRE) as an aid in the detection of prostate cancer. There were no discrepant pairs between the Hybritech calibration 4.0 ng/mL cut-off and the WHO calibration 3.1 ng/mL cut-off with relative agreements of 100% for both positive and negative samples.

**Table 22. Comparison of the Distribution of the 6,630 Clinical Study Samples with the Hybritech 4.0 ng/mL Cut-off versus the WHO 3.1 ng/mL Cut-off**

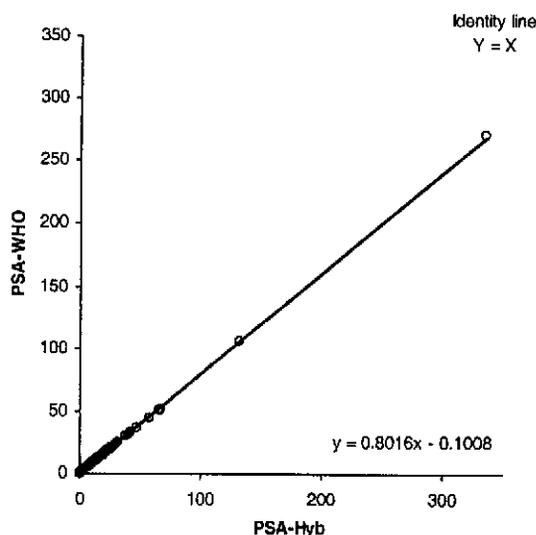
	Hybritech ≤ 4.0	Hybritech > 4.0	Total
WHO ≤ 3.1	5,616	0	5,616
WHO > 3.1	0	1,014	1,014
Total	5,616	1,014	6,630

Deming Regression Analysis

Deming regression analysis was conducted on the subgroup of subjects (1,160 out of the 6,630) in the P850048 dataset that had biopsies performed as a follow-up to DRE and PSA testing. This analysis was performed to assess the impact of the WHO calibration and associated clinical cut-off using Deming regression and Receiver Operating Characteristic (ROC) analysis comparing to the results originally derived using the Hybritech calibration.

The Deming plot in Figure 9 and accompanying data in Table 23 show a slope of 0.8017 between Access Hybritech and WHO calibrations. The results of this analysis demonstrated a 20% proportional bias and 10% constant bias between the two calibrations methods and confirmed the relationship (i.e. calibration factors) established between the Hybritech and WHO calibrations.

**Figure 9. Deming Plot of Hybritech to WHO Calibration**



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**Table 23. Deming Regression of Hybritech to WHO Calibration PSA Data**

	<b>Coefficient</b>	<b>SE</b>	<b>95% CI</b>
<b>Intercept</b>	-0.1008	0.0056	-0.1118 to -0.0897
<b>Slope</b>	0.8016	0.0004	0.8008 to 0.8025
<b>Pearson Coefficient <i>r</i></b>	0.9998	-	0.9998 to 0.9999

ROC Analysis

Refer to Clinical Study Section below.

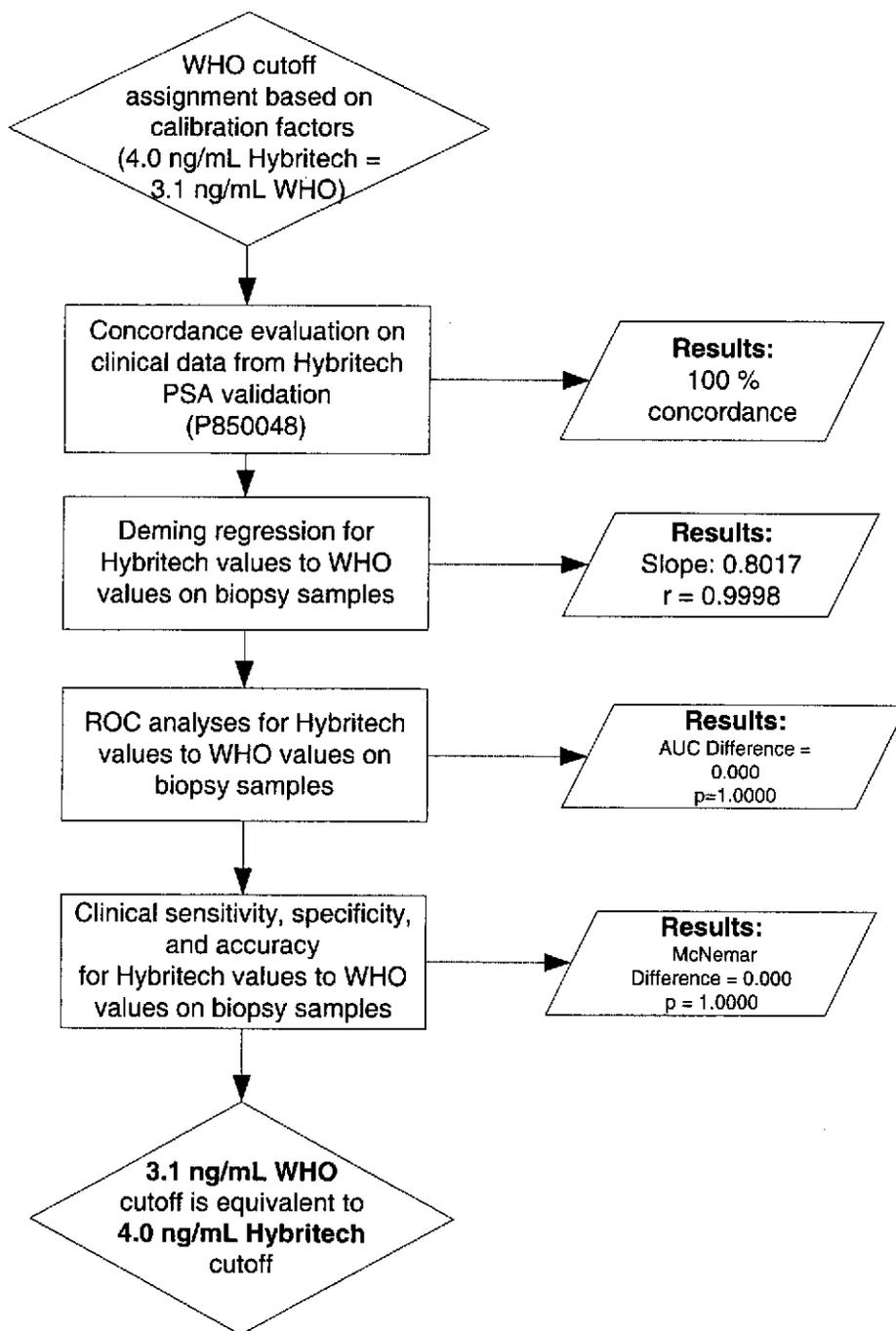
X. SUMMARY OF CLINICAL STUDIES

A. **Study Design**

The process of verifying the WHO cut-off of 3.1 ng/mL is shown in Figure 10.

**Figure 10. WHO Cut-off (3.1 ng/mL) Verification Process Summary**

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**B. Gender Bias**  
Not applicable.

**C. Patient Assessments**  
The reference standard for comparison was the Hybritech Access® Total PSA Calibration method. Histology obtained by PSA biopsy results provided clinical truth.

**D. Demographic Data**

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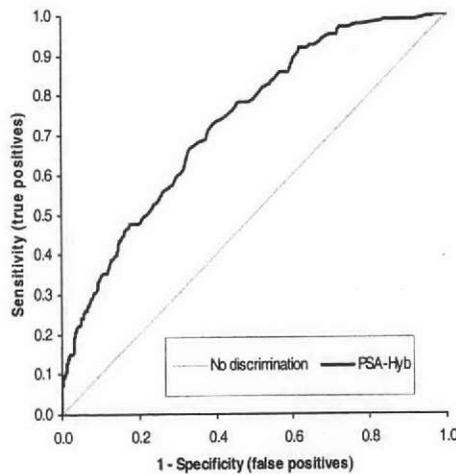
Demographic data for the banked specimens used in the clinical comparison study are provided in P850048/S016.

**E. Data Analysis and Results**

ROC Analysis

Receiver Operating Characteristic (ROC) analysis of both the Hybritech and WHO calibration data was performed on the 1,160 subject dataset. Hybritech calibration results are shown in Table 24 and Figure 11. WHO calibration results are shown in Table 25 and Figure 12.

**Figure 11. ROC Plot of Hybritech Calibration (n = 1160)**

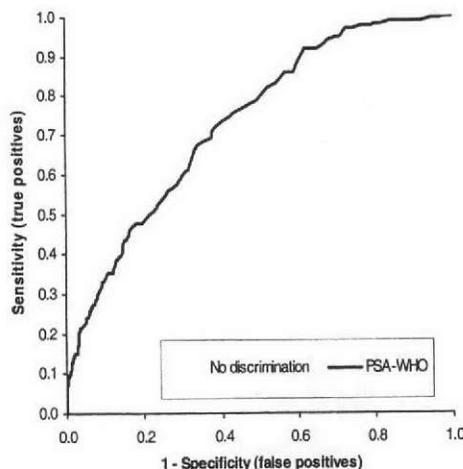


**Table 24. ROC Analysis Results of Hybritech Calibration (n = 1160)**

Curve	AUC	SE	95% CI
PSA-WHO	0.732	0.017	0.699-0.766

Non-PCA 905; PCA 255; Total 1160

**Figure 12. ROC Plot of WHO Calibration (n = 1160)**



**Table 25. ROC Analysis Results of WHO Calibration**

Curve	AUC	SE	95% CI
PSA-WHO	0.732	0.017	0.699-0.766

Non-PCA 905; PCA 255; Total 1160

The ROC analysis resulted in areas under the curve (AUC) of 0.732 and 0.732 for the Hybritech and WHO calibrations, respectively. The results of a statistical hypothesis test,  $H_0: AUC_{Hyb} = AUC_{WHO}$ , show no statistically significant differences ( $p=1.0000$ ) and are shown in Table 26. The results of this analysis also confirm the safety and efficacy of the 3.1 ng/mL cut-off for use with the WHO calibration.

**Table 26. Summary of Statistical Evaluation of AUC for Hybritech and WHO Calibrations**

Curve	AUC*	SE	95% CI
PSA-WHO	0.732	0.017	0.699-0.766
PSA-Hyb	0.732	0.017	0.699-0.766

\*  $p = 1.0000$

#### Clinical Sensitivity and Specificity Analysis

The computation of clinical sensitivity, specificity, and accuracy for the Hybritech 4.0 ng/mL and WHO 3.1 ng/mL cut-offs are presented in Table 27.

The clinical performance for Hybritech 4.0 ng/mL was approved in P850048 and is also confirmed using the WHO 3.1 ng/mL cut-off.

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**Table 27. Clinical Sensitivity, Specificity and Accuracy for Hybritech and WHO Calibration Cut-offs**

Hybritech_4.0	Non-PCA	PCA	Total
≤ 4.0	434	47	481
> 4.0	471	208	679
<b>Total</b>	<b>905</b>	<b>255</b>	<b>1160</b>

		95% LCL	95% UCL
Clinical Sensitivity	0.816	0.763	0.861
Clinical Specificity	0.480	0.447	0.513
Clinical Accuracy	0.553	0.524	0.582

WHO_3.1	Non-PCA	PCA	Total
≤ 3.1	434	47	481
> 3.1	471	208	679
<b>Total</b>	<b>905</b>	<b>255</b>	<b>1160</b>

		95% LCL	95% UCL
Clinical Sensitivity	0.816	0.763	0.861
Clinical Specificity	0.480	0.447	0.513
Clinical Accuracy	0.553	0.524	0.582

A McNemar test analysis shows no statistically significant difference between the Hybritech 4.0 ng/mL cut-off and the WHO 3.1 ng/mL cut-off. No discordant pairs were observed between the Hybritech and WHO calibrations. The results of this analysis are shown in Table 28.

**Table 28 McNemar Statistical Analysis Summary of Results**

Hybritech_4.0	WHO_3.1		Total
	≤3.1	>3.1	
≤ 4.0	481	0	481
> 4.0	0	679	679
<b>Total</b>	<b>481</b>	<b>679</b>	<b>1160</b>

Difference between proportions	0.000	
0% CI	0.000 to 0	(exact)
2-tailed p	1.0000	(exact)

These analyses support a 3.1 ng/mL WHO calibration cut-off to correspond to the Hybritech calibration 4.0 ng/mL cut-off. Further, the results of a rigorous validation of the relationship between the original Hybritech PSA calibration and WHO calibration clearly support a conclusion that the use of the WHO calibration does not introduce any new concerns of safety or effectiveness for the Access Hybritech PSA assay.

XI. CONCLUSIONS DRAWN FROM THE STUDIES

Risk and Benefit Analysis

Risks of introduction of a dual calibration scheme for the Beckman Total PSA assay include:

1. Adding the WHO calibration may adversely affect clinical and non-clinical assay performance characteristics (e.g. cut-off, imprecision, analytical sensitivity, linearity, hook effect, etc.).
2. May lead to confusion in laboratory personnel resulting from the available option of the Hybritech or WHO calibrations.
3. Confusion between laboratory personnel and physician regarding whether results originated from the Hybritech (4.0 ng/mL cut-off) or WHO (3.1 ng/mL cut-off) calibration

Potential benefits are the PSA assay being traceable to WHO 96/670 standard renders the PSA assay results comparable to that of different assay platforms of other manufacturers and the results could be exchangeable and easier being interpreted.

Hazard Analysis

Hazard analysis was performed on the two scenarios below:

1. The PSA results using the cut-off threshold at 3.1 ng/mL instead of 4.0 ng/mL would likely result in higher false positive results if the PSA values with Hybritech calibration were confused with PSA values with WHO calibration. For example, consider a subject with PSA value of 3.5 ng/mL with Hybritech calibration ( $3.5 < 4.0$ ) but if one thinks that this value is obtained with WHO calibration then this value, 3.5 ng/mL, will be incorrectly compared with the threshold 3.1 ng/mL for WHO calibration ( $3.5 > 3.1$ ). What would be the tolerable false positive rate for clinician and patient in referring non-suspicious DRE for biopsy?
2. The PSA results using the cut-off threshold at 3.1 ng/mL instead of 4.0 ng/mL would likely result in higher false negative results if the PSA values with WHO calibration were confused with PSA values with Hybritech calibration. For example, consider a subject with PSA value 3.5 ng/mL with WHO calibration ( $3.5 > 3.1$ ) but if one thinks that this value is obtained with Hybritech calibration then this value, 3.5 ng/mL, will be incorrectly compared with the threshold of 4.0 ng/mL for Hybritech calibration. What would be the tolerable false negative rate for clinician and patient in non-referring for biopsy?

Controls mitigating risk

1. Appropriate warnings and limitations have been updated in and added to the directional insert:
  - a. PSA concentrations are dependent on the standard used to calibrate the assay. PSA concentrations based on calibration to the WHO 96/670 Reference Preparation will differ significantly from PSA concentrations based on

## SUMMARY OF SAFETY AND EFFECTIVENESS DATA

- calibration to the original Hybritech Tandem™-R assay. The concentrations are not interchangeable. If the calibration is changed, accepted laboratory practice is to establish a new baseline for patient monitoring.
- b. The safety and effectiveness of using a cut-off value other than 4.0 ng/mL with Hybritech calibration or 3.1 ng/mL with WHO calibration has not been established.
  - c. The results from the Hybritech and WHO calibrations are not interchangeable. Care should be taken to determine which calibration is appropriate for the laboratory and to specify which calibration the results were generated on.
2. Calibrator primary and secondary package labeling has been revised to include both and distinguish between Hybritech (“Hyb”) and WHO (“WHO”) calibrator values.
  3. Two different colored calibrator cards are provided with each calibrator kit (a white card titled “Hybritech® PSA” contains Hybritech calibration information and a yellow card titled “PSA-WHO” contains WHO calibration information).
  4. Two assay protocol files with distinct numbers and test names (“PSA-Hyb” vs. “PSA-WHO”) are provided and require the user to actively enable one or both options.
  5. Results listed on the instrument printout are identified as originating from either the Hybritech (“PSA-Hyb”) or WHO (“PSA-WHO”) calibration
  6. User must manually enter the appropriate LIS information into the system to report results
  7. Educational documents created for laboratory personnel and physicians to address potential questions on technical information, materials management, and physician notification:
    - a. Customer Letter
    - b. Physician Letter
    - c. Materials Management Letter
    - d. External Quality Assurance Program Letter
    - e. Hybritech and WHO Calibration Option Technical Bulletin
    - f. WHO Cut-off Technical Bulletin
    - g. QC Values Technical Bulletin
  8. Physicians letter created for laboratory to notify physicians of a change to the calibration and associated cut-off (letter instructs that laboratories report results to physicians with calibration and applicable cut-off identified).

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### 9. Proactive customer education initiative to clearly communicate calibration options and associated changes

The controls implemented to mitigate the risk identified including different assay protocols, proposed labeling changes and end user education plan provide reasonable assurance of the safety and effectiveness of the Access Hybritech PSA Assay when used according to the current labeling and indications for use with both the Hybritech and the WHO calibration. The benefits associated with adding the WHO calibration outweigh the risks associated with providing the second calibration.

The results of validation of the relationship between the original Hybritech PSA calibration and WHO calibration in general support conclusion that the use of the WHO calibration does not introduce any new concerns of safety or effectiveness for the Access Hybritech PSA assay. Based on the information contained in this supplement, it is concluded that the Access PSA assay with the Hybritech and WHO calibrations performs as intended and the data presented provides reasonable assurance of the continued safety and effectiveness of the Access Hybritech PSA assay when used according to the labeling and indications for use.

Therefore, it is reasonable to conclude that the benefits of use of the device for the target population outweigh the risk of illness or injury when used as indicated in accordance with the directions for use.

#### Conclusion

The data presented in this supplement demonstrate that the Access WHO calibration 3.1 ng/mL cut-off is equivalent to the Access Hybritech calibration 4.0 ng/mL cut-off when used for the clinical interpretation of PSA results.

Potential risks introduced by addition of the WHO calibration were evaluated, and appropriate controls were implemented to adequately mitigate potential risks.

### XII. PANEL RECOMMENDATION

Advisory panel homework assignments were sent to three FDA Immunology Device Panel members to solicit panel input on safety and effectiveness of offering of dual Access® Hybritech total PSA calibration methods by the manufacturer.

No specific recommendations were provided on the approval of the Beckman's PMA supplement on WHO standardization for the Hybritech total PSA assay.

### XIII. CDRH DECISION

CDRH concurred with the Immunology Device Panel members' recommendation, and issued a letter to Beckman Coulter, on March 10, 2008, advising that its PMA supplement was approvable subject to compliance of the changes as recommended by the Panel and required by FDA.

FDA issued an approval order on May 9, 2008. The applicant's manufacturing facility was inspected and was found to be in compliance with the Quality System Regulation

SUMMARY OF SAFETY AND EFFECTIVENESS DATA

(21 CFR 820).

XIV. APPROVAL SPECIFICATIONS

Directions for use: See the labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions and Adverse Events in the labeling.

Postapproval Requirements and Restrictions: See approval order.

XV. REFERENCES

1. Stamey TA, Chen Z, and Prestigiacomo AF. Reference Material for PSA: The IFCC Standardization Study. Clin Biochem 1998, 31(6) pp475-481.



## U.S. FOOD AND DRUG ADMINISTRATION

# New Device Approval

Access® Hybritech® PSA WHO Standardization

[Picture of Device]

This is a brief overview of information related to FDA's approval to market this product. See the links below to the Summary of Safety and Effectiveness Data (SSED) and product labeling for more complete information on this product, its indications for use, and the basis for FDA's approval.

**Product Name:** Access® Hybritech® PSA WHO Standardization

**PMA Applicant:** Beckman Coulter, Inc.

**Address:** 1000 Lake Hazeltine Drive, Chaska, MN 55318

**Approval Date:** May XX, 2008

**Approval Letter:** A link to web for the approval letter

**What is it?** Addition of assay calibrators with PSA values connected to the PSA reference material provided by World Health Organization (WHO) to the existing set of PSA calibrators as an alternative method for calculating PSA concentrations in patient blood samples.

**How does it work?** The calibrators are used to generate a curve for calculating PSA concentrations. The six levels of the original PSA assay calibrators are assigned values linked to the WHO PSA standard using three conversion factors. The WHO PSA calibration gives 20% lower values than the original calibration. As the result of this realignment, the clinical cut-off for detection of prostate cancer is adjusted from 4.0 ng/mL to 3.1 ng/mL.

**When is it used?** The clinical laboratories performing the test decide which calibration method to use for the Access® Hybritech® PSA assay.

**What will it accomplish?** PSA assay results from different manufacturers may be comparable through the standardization process by using the same PSA reference standard.

**When should it not be used?** PSA results should not be converted directly between different assay methods using the calibration factors. The calibration conversion factors are only applicable during the assay calibration on Beckman Coulter Access® analyzer.

**Additional information:** Summary of Safety and Effectiveness and labeling are available at SSED and product insert.

**Other:** Links to other web sites, as appropriate. For example, a link to FDA's Office of Women's Health web site for products of particular interest to women, such as Ultrasonic Bone Sonometry system or Oxifirst Fetal Oxygen Saturation Monitor. Or, a link to the Jama web site if the product is the subject of one of the Commissioner's articles or to the agency's FDA news and Publications web site if the product is the subject of a press release or Talk Paper.

**Prepared by:** Dai J. Li  
**Project Manager:** Maria M. Chan

**Telephone:** 240-276-0997  
**Telephone:**

**Concurrence:** (ODE Program Operations Staff (POS) person)

**PMA Number:** P850048 S021

**N.B.** The “Prepared by,” “PMA Number,” and “Concurrence” information will be deleted before posting to the CDRH Internet.

**Rev. 07/23/02**