SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Quantitative test for determination of [-2]proPSA levels

Device Trade Name: Access® Hybritech® p2PSA on the Access Immunoassay Systems

Device Procode: OYA

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: P090026

Date of FDA Notice of Approval: June 14, 2012

Expedited: Not applicable

II. INDICATIONS FOR USE

The Access® Hybritech® p2PSA assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of [-2]proPSA antigen, an isoform of free PSA, in human serum using the Access Immunoassay Systems. Access® Hybritech® p2PSA is intended to be used in combination with Access® Hybritech® (total) PSA and Access® Hybritech® free PSA to calculate the Beckman Coulter Prostate Health Index (*phi*), an In Vitro Diagnostic Multivariate Index Assay (IVDMIA).

Beckman Coulter *phi* as calculated using the Access® Hybritech® assays is indicated for use as an aid in distinguishing prostate cancer from benign prostatic conditions, for prostate cancer detection in men aged 50 years and older with total PSA ≥ 4.0 to ≤ 10.0 ng/mL, and with digital rectal examination findings that are not suspicious for cancer. Prostatic biopsy is required for diagnosis of cancer.

III. CONTRAINDICATIONS

None

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the Access® Hybritech® p2PSA on the Access Immunoassay Systems labeling.

V. DEVICE DESCRIPTION

Access® Hybritech® p2PSA is a two-site immunoenzymatic "sandwich" assay. A sample is added to a reaction vessel with mouse monoclonal anti-PSA-alkaline phosphatase conjugate, paramagnetic particles coated with a mouse monoclonal anti-[-2]proPSA antibody, and a blocking reagent. The [-2]proPSA in the sample binds to the immobilized monoclonal anti-[-2]proPSA on the solid phase while, at the same time, the monoclonal anti-PSA-alkaline phosphatase conjugate reacts with different antigenic sites on the [-2]proPSA molecule. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos® 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of [-2]proPSA in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

Access® Hybritech® p2PSA reagents and the Access Immunoassay Analyzers comprise the Access Immunoassay Systems for the quantitative determination of [-2]proPSA levels in human serum using paramagnetic particle solid phase technology and chemiluminescent signal detection. Calibrators, quality controls, and samples containing [-2]proPSA are analyzed in a two-site immunoenzymatic ("sandwich") assay, using alkaline phosphatase conjugated anti-PSA mouse monoclonal antibody and paramagnetic particles coated with mouse monoclonal anti-[-2]proPSA antibody.

Access® Hybritech® p2PSA consists of reagent packs, calibrators, quality control material, substrate, and wash buffer.

a) Reagent Kit

The Access® Hybritech® p2PSA Reagent Kit (item number B03704) consists of two reagent packs. Each reagent pack contains:

- paramagnetic streptavidin particles coated with mouse monoclonal anti-[-2]proPSA antibodies in TRIS buffered saline with surfactant, bovine serum albumin (BSA), and preservatives
- blocking reagent with citrate, surfactants, BSA, alkaline phosphatase, and proteins (mouse, goat, and bovine)
- mouse monoclonal anti-PSA antibody alkaline phosphatase (bovine) conjugate in phosphate buffered saline with surfactant, BSA, protein (normal mouse IgG), PolyMak, and preservatives.

b) Calibrator Kit

The Access® Hybritech® p2PSA Calibrator Kit (item number B03705) consists of ready-to-use, liquid, multi-point calibrators for use with Access® Hybritech® p2PSA. Vials contain zero and approximately 10, 20, 50, 100, 500, and 5000 pg/mL [-2]proPSA in a citrate buffered bovine serum albumin (BSA) matrix with preservatives.

c) Quality Control Kit

The Access® Hybritech® p2PSA QC Kit (item number A56934) consists of ready-to-use, liquid, tri-level controls for use with Access® Hybritech® p2PSA. Vials

contain approximately 20, 175, and 1000 pg/mL [-2]proPSA in a citrate buffered bovine serum albumin (BSA) matrix with preservatives.

d) Substrate

The Access Substrate (item number 81906) is a dioxetane-based chemiluminescent compound. This chemiluminescent substrate, Lumi-Phos® 530, is added to the reaction vessel and light generated by the reaction is measured with a luminometer.

e) Wash Buffer

The Access Wash Buffer II (item number A16792 for Access 2 and UniCel DxC 600i or item number A16793 for UniCel DxI) is TRIS buffered saline containing surfactant and preservatives. The Wash Buffer is used:

- to clean the pipetting probe tip in the Access Immunoassay Analyzers,
- to wash paramagnetic particles to remove unbound analyte and excess reagents in each reaction, and as a diluent for making on-board pre-dilutions.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are several other alternatives for aiding in the detection of prostate cancer. Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his/her physician to select the method that best meets expectations and lifestyle.

The alternative practices or procedures used as an aid in the detection of prostate cancer include one or more of the following:

1. physical evaluation and digital rectal examination (DRE),
2. histological examination such as needle biopsy or transurethral resection,
3. lymphangiography
4. bone scan
5. serum total PSA and %free PSA

VII. MARKETING HISTORY

Access® Hybritech® p2PSA, used in combination with the Access Hybritech PSA and free PSA assays to calculate the Beckman Coulter *phi*, was made commercially available in the European Union in October 2009. The device has not been withdrawn from marketing for any reason related to its safety or effectiveness.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected may lead to incorrect p2PSA test results and the Beckman Coulter *phi* calculation. A falsely high Beckman Coulter *phi* result (higher Beckman Coulter *phi* is associated with a greater probability of cancer) may lead to a medical decision to perform an unnecessary prostate biopsy. A falsely low Beckman Coulter *phi* result (lower Beckman Coulter *phi* is associated with a lesser probability of cancer) may lead to a medical decision to not perform a prostate biopsy.

In addition, there are risks from venipuncture (e.g. hematoma) and complications from biopsy [e.g. infection, fever, rectal bleeding, hematuria and hematospermia (blood in the urine and semen), urinary retention, and hospitalization in some cases] if the Beckman Coulter *phi* result indicated biopsy.

IX. SUMMARY OF NONCLINICAL STUDIES

Analytical performance was assessed to evaluate the safety and effectiveness of the Access® Hybritech® p2PSA on the Access Immunoassay Systems. The studies included

A. Laboratory Studies:

Characterization of Antigen and Antibody

The [-2]pro PSA antigen (clone VAV12-PSA008.6) was characterized using SDS-PAGE and N-terminal amino acid sequencing. The antigen was functionally tested with the Access® Hybritech® free PSA and p2PSA assays to assess purity. A summary of the antigen characterization study results is provided in Table 1.

Table 1 [-2]proPSA Antigen Characterization Study Results

	Antigen
Clone Designation	VAV12-PSA008.6
SDS-PAGE (MW)	36 kD
N-terminal Sequencing	8 amino acid sequence of : **SRIVGGWE**
Functional Testing (p2PSA /free PSA dose ratio)	≥587

The [-2]proPSA monoclonal antibodies (clones PS2X373.3 and PSM773.3.3) were characterized using isoelectric focusing and heavy and light chain determination. Clone PS2X373.3 was also characterized using SDS-PAGE. A summary of the antibody characterization study results is provided in Table 2.

Table 2 [-2]proPSA Monoclonal Antibody Characterization Study Results

	Capture Antibody	Conjugate Antibody
Clone Designation	PS2X373.3	PSM773.3.3
SDS-Page (MW)	150 kD	Not Applicable
Isoelectric Focusing (Isoelectric Point)	6.5 – 6.9	7.5 – 7.8
Heavy Chain	IgG_1	IgG_1
Light Chain	K	K

Assay Standardization

Access® Hybritech® p2PSA is standardized to an internal reference preparation of purified [-2]proPSA. Commercial calibrators are prepared and tested versus internal reference calibrators. The Access® Hybritech® p2PSA Calibrator Kit consists of ready-to-use, liquid, multi-point calibrators (S0 – S6) for use with Access® Hybritech® p2PSA. Vials contain zero and approximately 10, 20, 50, 100, 500, and 5000 pg/mL [-2]proPSA in a citrate buffered bovine serum albumin (BSA) matrix with preservatives. A calibration card listing the assigned calibrator values is provided with each calibrator kit.

Access® Hybritech® p2PSA Imprecision

Imprecision of Access® Hybritech® p2PSA was evaluated at an internal site using a protocol based on CLSI Guideline EP5-A2 (Evaluation of Precision Performance of Clinical Chemistry Devices). Commercial controls and serum patient samples at various [-2]proPSA concentrations were tested on a UniCel DxI 800 analyzer. Each replicate run over each run was used to calculate within run, between run, and total %CV. The results are summarized in Table 3.

Table 3 Access Hybritech p2PSA Imprecision

Sample	Mean (pg/mL)	Within Run SD (pg/mL)	Within Run CV (%)	Total SD (pg/mL)	Total CV (%)
QC1	2.661	0.173	6.51	0.288	10.83
QC2	22.745	0.699	3.08	1.091	4.80
QC3	106.641	4.084	3.83	6.349	5.95
PT1	8.632	0.423	4.90	0.527	6.11
PT2	38.455	1.403	3.65	1.822	4.74
PT3	108.208	3.069	2.84	5.042	4.66
PT4	1179.705	36.528	3.09	52.575	4.46
PT5	2899.484	64.082	2.21	105.081	3.62
PT6	4748.600	112.059	2.36	139.782	2.94

In addition to the internal site, imprecision was assessed at three external sites on two lots of reagents using the Access 2 analyzer. For this study, three patient serum samples ([-2]proPSA concentrations ~10, 34 and 66 pg/mL) and three quality controls ([-2]proPSA concentrations ~21, 182 and 1037 pg/mL) were tested at each site. Each patient and control sample was run in duplicate. Testing was performed over 20 days (2 runs per day) for a total of 40 runs and 80 replicates on one Access analyzer using two lots of reagents at two sites and at the third site, quality control sample #2 was run on an additional day with each reagent lot resulting in a total of 42 runs and 84 replicates per lot. Within-run, between-run and total %CV for each site, reagent lot imprecision by site, instrument imprecision by lot and combined imprecision for sites and lots were determined.

For reagent lot imprecision analyzed by site, the total imprecision ranged from 2.88% to 7.33%. For instrument imprecision analyzed by reagent lot, the total imprecision ranged from 4.38% to 10.07% for reagent lot 1 and 4.19% to 8.87% for reagent lot 2. For combined imprecision for the three sites and the two reagent lots pooled, the total imprecision ranged from 5.53% to 9.39%. In conclusion, external site imprecision results demonstrated acceptable performance.

Reagent and Calibrator Lot Reproducibility

Access® Hybritech® p2PSA reagent and calibrator lot-to-lot reproducibility were evaluated at an internal site in a methods comparison study based on CLSI EP9-A2 Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline, 2nd Edition. Serum patient samples with PSA concentrations between 2 and 10 ng/mL ([-2]proPSA concentrations approximately 5 to 123 pg/mL) were tested on Access 2, UniCel DxI 800, and UniCel DxI 600 analyzers. Passing-Bablok linear regression was used to estimate the bias by reagent and calibrator lots. The reagent results are summarized in Table 4 and the calibrator results are summarized in Table 5.

Table 4 Access Hybritech p2PSA Reagent Reproducibility

System	Reagent Lot Comparison	Intercept	Slope	95% CI of Slope	R^2
Access 2	2 vs. 1	0.12	1.00	1.00 to 1.01	1.00
	3 vs. 1	0.30	1.01	1.00 to 1.01	1.00
	3 vs. 2	0.16	1.00	0.99 to 1.01	1.00
UniCel DxI 800	2 vs. 1	0.10	0.99	0.98 to 1.00	1.00
	3 vs. 1	0.13	1.02	1.01 to 1.03	1.00
	3 vs. 2	0.05	1.01	1.00 to 1.02	1.00
UniCel DxI 600	2 vs. 1	0.22	0.99	0.98 to 1.00	1.00
	3 vs. 1	0.21	1.01	1.00 to 1.02	1.00
	3 vs. 2	-0.07	1.03	1.01 to 1.04	0.99

Table 5 Access Hybritech p2PSA Calibrator Reproducibility

System	Calibrator Lot Comparison	Intercept	Slope	95% CI of Slope	R^2
Access 2	2 vs. 1	-0.04	0.98	0.97 to 0.99	1.00
	3 vs. 1	-0.05	1.00	0.99 to 1.01	1.00
	3 vs. 2	0.00	1.01	1.01 to 1.02	1.00
UniCel DxI 800	2 vs. 1	-0.13	1.03	1.02 to 1.03	1.00
	3 vs. 1	-0.04	1.03	1.03 to 1.04	1.00
	3 vs. 2	0.09	1.01	1.00 to 1.01	1.00
UniCel DxI 600	2 vs. 1	0.11	1.02	1.01 to 1.03	1.00
	3 vs. 1	0.07	1.02	1.01 to 1.03	1.00
	3 vs. 2	-0.08	1.02	1.01 to 1.03	1.00

Beckman Coulter *phi* Imprecision

In one study, imprecision of Beckman Coulter *phi* was evaluated at one internal and two external sites using a protocol based on CLSI Guideline EP5-A2 (Evaluation of Precision Performance of Clinical Chemistry Devices). Serum patient samples at various Beckman Coulter *phi* values were tested with the Access® Hybritech® PSA, free PSA, and p2PSA assays on Access 2 instruments. The testing results were used to calculate Beckman Coulter *phi* ([-2]proPSA/free PSA) x $PSA^{1/2}$). Each Beckman Coulter *phi* value was used to calculate within run, between run, and total %CV for each site. Inter-site and combined imprecision for the three sites was determined. The results are summarized in Tables 6 and 7.

Table 6 Imprecision of Beckman Coulter *phi* - Three Sites

Site 1						
Sample	**N**	**Mean *Phi***	**Within Run %CV**	**Between Day %CV**	**Between Run %CV**	**Total %CV**
1	84	66.6	3.9	4.0	4.5	6.0
2	82	47.4	2.4	3.0	3.5	4.2
3	84	19.2	2.6	1.8	3.7	4.5
4	84	27.1	3.6	3.8	4.1	5.4
5	84	50.6	1.7	1.5	3.7	4.1
6	84	65.3	2.7	2.8	4.0	4.8
7	84	28.3	2.9	1.9	3.6	4.7
8	84	64.2	3.0	0.6	2.9	4.2
9	84	97.9	2.3	0.8	2.9	3.7
10	84	122.2	2.4	3.2	4.5	5.1
Site 2						

Sample	N	Mean *Phi*	Within Run %CV	Between Day %CV	Between Run %CV	Total %CV
1	80	67.3	3.5	3.4	4.2	5.5
2	80	46.9	1.9	2.8	3.5	4.0
3	78	20.1	2.9	3.6	3.6	4.6
4	78	28.4	2.1	3.4	4.3	4.8
5	80	50.5	1.8	3.7	7.0	7.2
6	80	65.7	2.3	3.5	3.9	4.5
7	80	30.0	2.7	2.2	4.1	4.9
8	80	64.4	2.2	3.5	4.4	4.9
9	80	97.5	2.9	1.5	2.7	4.0
10	76	121.0	2.2	3.3	4.0	4.6
Site 3						
Sample	**N**	**Mean *Phi***	**Within Run %CV**	**Between Day %CV**	**Between Run %CV**	**Total %CV**
1	80	62.8	4.1	5.1	6.6	7.8
2	80	44.2	2.2	3.0	5.4	5.9
3	80	18.8	4.4	4.0	7.0	8.2
4	80	26.4	2.8	3.9	6.4	7.0
5	80	49.3	2.5	1.6	4.1	4.8
6	80	62.8	2.2	4.3	6.2	6.5
7	80	27.7	3.3	2.8	5.9	6.7
8	80	61.2	2.3	3.1	5.0	5.6
9	80	93.9	2.1	1.9	4.9	5.3
10	80	114.4	4.3	4.7	7.3	8.4

Table 7 Imprecision of Beckman Coulter *phi* - Three Sites Combined

Combined Sites								
Sample	**N**	**Mean *Phi***	**Within Run %CV**	**Between Day %CV**	**Between Run %CV**	**Intra-Site (Total) %CV**	**Inter-Site %CV**	**Combined %CV**
1	244	65.6	3.8	4.2	5.1	6.4	3.5	7.3
2	242	46.2	2.2	2.9	4.2	4.7	3.6	5.9
3	242	19.3	3.3	3.2	4.9	6.0	3.3	6.8
4	242	27.3	2.9	3.7	5.0	5.8	3.4	6.7
5	244	50.1	2.1	1.9	5.2	5.6	1.2	5.7
6	244	64.6	2.4	3.6	4.7	5.3	2.2	5.8
7	244	28.7	3.0	2.3	4.6	5.5	4.0	6.7
8	244	63.3	2.5	2.7	4.2	4.9	2.7	5.6
9	244	96.4	2.5	1.4	3.6	4.4	2.2	4.9
10	240	119.2	3.1	3.7	5.3	6.2	3.3	7.0

In a second study, imprecision of Beckman Coulter *phi* was evaluated at one internal site using a protocol based on CLSI Guideline EP5-A2 (Evaluation of Precision Performance of Clinical Chemistry Devices). A commercial control and serum patient samples at various Beckman Coulter *phi* values were tested with the Access® Hybritech® PSA, free PSA, and p2PSA assays on Access 2, UniCel DxI 800, and UniCel DxI 600 instruments. The testing results were used to calculate Beckman Coulter *phi* ([-2]proPSA/free PSA) X $PSA^{1/2}$).

Each Beckman Coulter *phi* value was used to calculate within run, between run, and total %CV for each instrument (platform) and reagent lot. Reagent lot-to-lot and platform-to-platform imprecision was determined. The results are summarized in Tables 8, 9, and 10.

Table 8 Imprecision of Beckman Coulter *phi*

Sample	Mean *Phi*	Total %CV	Between Platform %CV	Between Lot %CV	Between Run (Day) %CV	Within Run %CV
1	8.4	8.5	3.0	4.2	3.1	5.9
2	70.8	11.8	7.4	5.6	3.7	6.3
3	81.3	12.0	8.5	5.5	2.6	5.7
4	88.8	9.9	6.4	4.3	2.8	5.6
5	754.8	11.6	8.7	4.2	2.8	5.8

Table 9 Imprecision of Beckman Coulter *phi* by Platform (Instrument)

Platform	Sample	Mean *phi*	Total %CV	Between Lot %CV	Between Run (Day) %CV	Within Run %CV
Access 2	1	8.4	9.8%	7.8%	3.9%	4.5%
	2	66.1	9.7%	8.5%	2.4%	4.1%
	3	74.8	9.5%	8.5%	1.5%	4.0%
	4	83.2	6.6%	5.3%	2.0%	3.4%
	5	690.9	6.9%	5.9%	1.7%	3.2%
DxI 600	1	8.2	6.7%	3.1%	3.8%	4.6%
	2	70.2	8.3%	4.6%	4.3%	5.4%
	3	80.6	8.7%	6.0%	4.1%	4.8%
	4	88.8	8.5%	6.1%	3.5%	4.8%
	5	753.2	8.5%	6.5%	3.6%	4.0%
DxI 800	1	8.7	7.4%	1.8%	5.4%	4.7%
	2	76.6	9.7%	3.9%	7.0%	5.4%
	3	88.7	7.4%	2.4%	4.7%	5.2%
	4	94.7	7.6%	1.4%	5.4%	5.2%
	5	823.7	8.0%	0.2%	6.2%	5.1%

Table 10 Imprecision of Beckman Coulter *phi* by Reagent Lot

Reagent Lot	Sample	Mean *phi*	Total %CV	Between Platform %CV	Between Run (Day) %CV	Within Run %CV
1	1	8.2	7.6%	4.5%	3.4%	5.1%
	2	67.9	11.3%	8.9%	3.8%	5.8%
	3	77.9	12.4%	10.7%	2.7%	5.6%
	4	86.0	10.0%	7.9%	2.9%	5.3%
	5	730.5	12.3%	10.8%	2.9%	5.2%
2	1	8.7	7.2%	2.8%	3.2%	5.8%
	2	73.7	9.5%	6.0%	3.5%	6.5%
	3	84.5	8.8%	6.4%	2.5%	5.5%
	4	91.6	8.0%	5.1%	2.4%	5.6%
	5	778.2	9.4%	6.9%	2.7%	5.7%

Limit of Blank (LOB) and Limit of Detection (LOD)

LOB and LOD of Access® Hybritech® p2PSA were tested at an internal site on UniCel DxI 800 analyzers using a protocol based on CLSI EP17-A (Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline). Patient serum samples with [-2]proPSA concentrations close to the approximate LOD were used in the determination of the LOD for serum samples. The Access® Hybritech® p2PSA S0 calibrator was used for the LOB determination.

The LOB was determined with a non-parametric estimate of the 95th percentile of the replicates of a zero analyte sample run on the UniCel DxI 800 analyzers. The LOD was determined as a value that is 1.645 standard deviations (SD) higher than LOB.

The LOB for Access® Hybritech® p2PSA was determined to be 0.495 pg/mL. The LOD for the Access® Hybritech® p2PSA was determined to be 0.692 pg/mL.

Limit of Quantitation (LOQ)

LOQ of Access® Hybritech® p2PSA was tested at an internal site on a UniCel DxI 800 analyzer using a protocol based on CLSI EP17-A (Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline). Samples were prepared by gravimetric dilution of Access® Hybritech® p2PSA S5 or S6 calibrator to obtain [-2]proPSA concentrations ranging from approximately seven times the Limit of Detection (LOD) to the approximate LOD.

The mean concentration and total percent CV were calculated for each sample on each day. The mean concentration and total percent CV underwent a log transformation. Polynomial regression was used to determine the best line fit. The concentration that provided the 20% CV was calculated from the quadratic model for the line. The 95% CI for that concentration was determined from the regression plot. The LOQ for Access® Hybritech® p2PSA was determined to be 3.226 pg/mL (upper 95% CI concentration).

Dilution Recovery and Linearity

Dilution recovery and linearity of Access® Hybritech® p2PSA was tested at an internal site on an Access 2 analyzer to evaluate the recovery and linearity of Access® Hybritech® p2PSA when diluting known concentrations of [-2]proPSA in serum samples. Linearity was evaluated using a study design based on CLSI Guideline EP6-A (Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach).

For each dilution, an observed value was calculated by computing the mean of the replicates, then the percent recovery was calculated. Each dilution study was analyzed using Deming regression. Observed [-2]proPSA concentration was plotted against expected [-2]proPSA concentration. For samples meeting the acceptance criterion, standard CLSI Guideline EP6-A procedure was used to create a linear fit and a quadratic or cubic fit to the data. The predicted dose value for each of the diluted levels was calculated for both models. The percent difference was computed and presented as percent non-linearity.

Eleven out of twelve samples (92%) had a slope of 1.0 ± 0.15. The overall mean sample percent recovery of the serum samples is 123% with individual mean sample recoveries ranging from 103% to 180%. The neat ranges of the samples ranged from approximately 58 to 4922 pg/mL [-2]proPSA. The resultant lowest dilution level was 13 pg/mL. The maximum non-linearity was 17%.

Access® Hybritech® p2PSA is intended to be used at total PSA concentrations ≥4.0 to ≤10.0 ng/mL. Total PSA concentrations within this range typically correspond to [-2]proPSA concentrations within the dynamic range of Access® Hybritech® p2PSA (0 to 5000 pg/mL). Therefore, dilution of patient samples should not be required and will not be recommended in the Access® Hybritech® p2PSA directional insert.

Spiking Recovery

Spiking recovery of Access® Hybritech® p2PSA was tested at an internal site on an Access 2 analyzer to evaluate the accuracy of Access® Hybritech® p2PSA when measuring known concentrations of [-2]proPSA in serum samples. Test samples were prepared by adding purified [-2]proPSA into male normal human serum patient samples to obtain multiple final [-2]proPSA concentrations for each sample. The percent recovery was calculated as a ratio of the average observed (measured) dose and the expected dose. Overall, 100% (6 of 6) sample means fell within 100±15%. The overall mean sample recovery of the serum samples is 93% with individual mean sample recoveries ranging from 90%-96%.

Assay Hook

Assay hook (high dose hook) of Access® Hybritech® p2PSA was tested at internal site on an Access 2 analyzer. Hook samples were prepared by spiking purified [-2]proPSA into Access® Hybritech® S0 calibrator to multiple concentrations including 3 times the highest calibrator value, which represents a value that is significantly higher than the highest expected value. The results were plotted graphically (dose vs. RLU) and the graph and data were visually evaluated for the presence of a high dose hook.

The relative light units (RLU) at approximately 15,000 pg/mL do not fall below the Access® Hybritech® S6 calibrator RLU. Therefore, there is no high dose hook up to approximately 15,000 pg/mL.

Interference

Potential interference in Access® Hybritech® p2PSA by normal human blood constituents, commonly encountered medications, and therapeutic drugs was evaluated at an internal site on an Access 2 analyzer using a paired differences approach as described in CLSI EP7-A2, Interference Testing in Clinical Chemistry – Approved Guideline, 2nd Edition. The potential interferents were spiked individually into patient serum samples containing low, medium, or high concentrations of [-2]proPSA (approximately 15, 100, and 1600 pg/mL, respectively). Samples were measured using Access® Hybritech® p2PSA and the dose of each spiked (test) sample was compared to the dose of the unspiked (control) sample. For each substance analyzed at each [-2]proPSA concentration, an observed value was calculated by computing the mean of the replicate

measurements of the spiked sample. The expected value was equal to the mean of the replicates of the neat sample prior to addition of the substance. The interference was calculated as follows:

[mean Interferent test value (observed) ÷ mean Dilutional Control value (expected)] X 100 = Mean % Interference

The mean recovery PSA (ng/mL) of the control samples was subtracted from the mean recovery of the test samples, and the 95% confidence intervals around the difference (the true interference effect) were calculated using the equation:

$$(\bar{x}_{test} - \bar{x}_{control}) \pm 2.78\sqrt{\left(s^2_{test} + s^2_{control}\right)/n}$$

where s is the average within-run standard deviation and n is the number of replicates.

The results met the acceptance criteria and indicate that normal human blood constituents, commonly encountered medications, and therapeutic drugs do not cause significant interference in Access Hybritech p2PSA on the Access Immunoassay Systems analyzers.

Substances were considered to have no interference effect if:

1. the 95% confidence intervals included zero, indicating that the recovery of the test sample was not statistically different from the control sample, and/or
2. the test sample mean was within 90%-110% of the control sample mean, (percent recovery = (test/control) x 100%) indicating that the recovery of the test sample was not clinically different from the control sample.

Access® Hybritech® p2PSA exhibits no significant interference when samples were spiked with normal human blood constituents, commonly encountered medications, and therapeutic drugs.

Normal physiological total protein levels range from 6 to 8 g/dL. For patient samples containing elevated levels of total protein (> 8 g/dL), the possibility exists for interference by total protein. Carefully evaluate the results of patients suspected of having elevated total protein levels.

The normal human blood constituents, commonly encountered medications, and therapeutic drugs, along with the corresponding concentrations tested, are summarized in Table 11.

Table 11 Interference by Normal Human Blood Constituents, Commonly Encountered Medications and Therapeutic Drugs

Normal Human Blood Constituents (Concentration Tested)				
Unconjugated Bilirubin (20 mg/dL)	Conjugated Bilirubin (20 mg/dL)	Hemoglobin (500 mg/dL)	Triglycerides (1500 mg/dL)	Human Serum Albumin (6000 mg/dL)
Total Protein (6.2 g/dL)	Total Protein (8.4 g/dL)			

Commonly Encountered Medications and Therapeutic Drugs (Concentration Tested)				
Acetylsalicylic acid (0.5 mg/mL)	Captopril (5 µg/mL)	Cimetidine (0.1 mg/mL)	Clomipramine (2.7 µg/mL)	Cyclophosphamide (0.33 mg/mL)
Doxorubicin Hydrochloride (6.6 µg/mL)	Hydrocodone bitartrate (240 ng/mL)	Ibuprofen (0.4 mg/mL)	Metoprolol tartrate (2.7 µg/mL)	Naproxen Sodium (1 mg/mL)
Sildenafil citrate (0.2 mg/mL)	Terazosin (Hytrin) (1.45 mg/mL)	Finasteride (370 ng/mL)	Nifedipine (270 ng/mL)	Lovastatin (270 ng/mL)
Nilutamide (8 µg/mL)	Ketoconazole (Nizoral) (6.2 µg/mL)	Prednisone (1.65 µg/mL)	Cisplatin (10 µg/mL)	Paclitaxel (0.85 mg/mL)
Furosemide (20 µg/mL)	Flutamide (Eulexin) (78 µg/mL)	Ibuprofen (0.4 mg/mL)	Dutasteride (Avodart) (40 ng/mL)	Etoposide (14 µg/mL)
Zometa (667 ng/mL)	Megestrol acetate (Megace) (39.6 µg/mL)	Ciprofloxacin (46 µg/mL)	Docetaxel (Taxotere) (5.5 µg/mL)	Sulphamethoxazole (117 µg/mL)
Alfuzosin (Uroxatral) (19 ng/mL)	Doxazosin (Cardura) (40 ng/mL)	Vinblastin (Velban) (2 µg/mL)	Biotin (50 ng/mL)	Doxycyline hyclate (2.6 µg/mL)
Estramustine phosphate sodium (Emcyt, Estracyte) (81.7 µg/mL)	Fluoxetine hydrochloride (300 ng/mL)	Leuprolide acetate (Lupron, Viadur, Eligard) (8 ng/mL)	Methotrexate (13.2 µg/mL)	Prazosin (85 ng/mL)
Tamsulosin (Flomax) (55 ng/mL)	Triptorelin (Trelstar) (28 ng/mL)	Bicalutamide (Casodex) (35 µg/mL)	Nilutamide (Nilandron) (8 µg/mL)	Trimethoprim (23.4 µg/mL)
Goserelin acetate (Zoladex) (2.6 ng/mL)	Multivitamin (Centrum) (1:20 dilution)	Heparin (8000 units/dL)	Acetaminophen (0.2 mg/mL)	

Potential interference by human anti-mouse antibody (HAMA) and rheumatoid factor (RF) was also evaluated. Samples were tested with two formulations of Access Hybritech p2PSA reagents. Control reagents contained the complete complement of blocking agents. Test reagents contained bovine serum albumin substituted for the blocking agents (mock blocker). Each sample was tested with the control and test reagents in duplicate in the HAMA study or triplicate in the RF study on Access 2 analyzers. The percent difference between results of the test and control reagent for each sample was calculated and compared to the mean difference observed between the quality controls tested with the test and control reagents. All of the tested samples characterized as having HAMA or positive for RF were blocked by greater than 10% with the control reagents containing the complete complement of blocking agents.

Cross-Reactivity

Potential cross-reactivity in Access® Hybritech® p2PSA by similar PSA isoforms was evaluated at an internal site on an Access 2 analyzer using a protocol based on CLSI EP7-A2, Interference Testing in Clinical Chemistry – Approved Guideline, 2nd Edition.

A mixture of PSA isoforms was prepared in synthetic matrix. The mixture of PSA isoforms was used to spike patient serum samples containing different endogenous [-2]proPSA concentrations to multiple total PSA concentrations (Test). The patient serum samples were also spiked with synthetic matrix only and were used as the baseline determination for each of the patient serum samples (Expected).

The endogenous level of [-2]proPSA (Endogenous p2PSA) in the PSA isoforms mixture was determined by spiking a normal human serum sample with the PSA isoforms mixture and comparing the amount of [-2]proPSA detected to that detected when an equal volume of synthetic matrix was added. The difference of the two measurements equaled the amount of endogenous [-2]proPSA in the PSA isoforms mixture. The level of cross-reactivity was determined by calculating the percent difference between Test p2PSA and Expected p2PSA concentrations.

The results indicate that similar PSA isoforms (PSA-ACT, free PSA, [-4]proPSA, [-5/-7]proPSA, and BPSA) do not cause significant cross-reactivity, defined as recovered [-2]proPSA dose ≤5% of expected [-2]proPSA dose for each PSA isoform tested, in Access Hybritech p2PSA.

Sample Carryover

Sample carryover in Access® Hybritech® p2PSA was assessed at an internal site on Access 2 and UniCel DxI 800 analyzers by running replicates of the Access® Hybritech® p2PSA S0 calibrator, followed by replicates of a patient serum sample containing a high concentration of [-2]proPSA, followed by replicates of the Access® Hybritech® p2PSA S0 calibrator. The mean of the first replicates of S0 calibrator was used as the baseline for the study. The mean of the replicates of S0 calibrator that immediately follow the patient sample (Test Replicates) was used to determine the occurrence of "carryover" into sample using the following equation:

[(Mean of Test Replicates) – baseline]*100 ÷ baseline = % carryover

Another potential type of carryover is contamination of the reagents with antigen. This "carry into" is identified by analyzing the S0 calibrator replicates that immediately follow the patient sample by linear regression for the presences of signal drift. The results indicate that sample carryover and antigen carry into with Access® Hybritech® p2PSA on the Access Immunoassay Systems analyzers is not significant.

Reagent Shelf-Life Stability

Access® Hybritech® p2PSA reagent shelf-life stability was tested at an internal site on Access, Access 2, and UniCel DxI 800 analyzers. To verify the stability of the reagents at the recommended storage conditions (2-10°C), patient serum samples and commercial controls were assayed at multiple time points throughout the expected reagent shelf-life expiration interval.

Commercial control and patient serum sample dose values were converted to percent of baseline value (dose at time zero). A linear regression line was fitted through the data, and a 95% confidence interval determined.

The percent change at 12 months was 2.16% (95% CI: -0.79%, 5.11%). The results indicate that the Access® Hybritech® p2PSA reagents are stable in sealed packs through the 12 month reagent shelf-life expiration interval.

Open Reagent Pack Stability

Access® Hybritech® p2PSA open reagent pack stability was tested at an internal site on an Access 2 analyzer. To verify the stability for the open pack at the recommended opened storage conditions (2-10°C), commercial control samples and patient serum samples were assayed using Access® Hybritech® p2PSA at multiple time points throughout the expected open reagent pack expiration interval.

Commercial control and patient serum sample dose values were converted to percent of baseline value (dose at time zero). A linear regression line was fitted through the data, and a 95% confidence interval determined.

The percent change at 28 days is 2.44% (95% CI: 1.0%, 3.87%). The results indicate that the Access® Hybritech® p2PSA reagents on the Access Immunoassay Systems analyzers are stable in an open state through the 28 day open reagent pack expiration interval.

Reagent Shipping Stability

Access® Hybritech® p2PSA reagent shipping stability was tested at an internal site on an Access 2 analyzer. Stability of the Access® Hybritech® p2PSA reagents was assessed at multiple time points following simulated summer and winter shipping cycles. Reagents were kitted and packaged according to general shipping operation procedures. Packaged reagent kits were subjected to simulated summer conditions and simulated winter conditions. A non-cycled control group of packaged reagent kits were stored at the recommended storage condition of 2-10°C.

The differences between control and patient samples using non-cycled calibrators were analyzed by calculating

1. the mean dose responses for each control and patient sample using the calibration curves established with the summer and winter cycled reagents,
2. the percent difference of the controls' and patient samples' doses obtained on the summer and winter cycled reagent pack versus the non-cycled reagent pack,
3. an overall dose average percent of controls and patient samples for both summer and winter cycled reagent packs.

The grand mean recovery for commercial controls and patient samples at baseline, 30 days, and 60 days was within ±10%. The results indicate that the Access® Hybritech® p2PSA reagents are stable following shipping.

Calibrator Shelf-Life Stability

Access® Hybritech® p2PSA calibrator shelf-life stability was tested at an internal site on an Access 2 and analyzer. To verify the stability of the calibrators at the recommended unopened storage conditions (≤-20°C), patient serum samples and commercial controls were assayed at baseline and multiple time points throughout the expected calibrator shelf-life expiration interval.

Commercial control and patient serum sample dose values were converted to percent of baseline value (dose at time zero). A linear regression line was fitted through the data, and a 95% confidence interval determined.

The percent change at 12 months is 1.13% (95% CI: -1.11%, 3.37%). The results indicate that the Access® Hybritech® p2PSA calibrators are stable in an unopened state through the 12 month calibrator shelf-life expiration interval.

Open Calibrator Vial Stability

Access® Hybritech® p2PSA calibrator open vial stability was tested at an internal site on an Access 2 analyzer. To verify the stability of the calibrators at the recommended opened storage conditions (2-10°C), patient serum samples and commercial controls were assayed at baseline and multiple time points throughout the expected open calibrator vial expiration interval.

Commercial control and patient serum sample dose values were converted to percent of baseline value (dose at time zero). A linear regression line was fitted through the data, and a 95% confidence interval determined.

The percent change at 60 days is 6.14% (95% CI: 5.47%, 6.82%). The results indicate that the Access® Hybritech® p2PSA calibrators are stable in an open state through the 60 day open calibrator vial expiration interval.

Calibration Curve Stability

Access® Hybritech® p2PSA calibration curve stability was tested at an internal site on an Access 2 analyzer. To verify the 28 day stability for the calibration curve, commercial control samples and patient serum samples were assayed using Access® Hybritech® p2PSA at multiple time points throughout the expected calibration curve expiration interval.

Commercial control and patient serum sample dose values were converted to percent of baseline value (dose at time zero). A linear regression line was fitted through the data, and a 95% confidence interval determined.

The percent change at 28 days is 6.94% (95% CI: 5.37%, 8.52%). The results indicate that the Access® Hybritech® p2PSA calibration curve is stable in a stored state through the 28 day calibration curve expiration interval.

Calibrator Shipping Stability

Access® Hybritech® p2PSA calibrator shipping stability was tested at an internal site on an Access 2 analyzer. Stability of the Access® Hybritech® p2PSA calibrators was assessed at multiple time points following simulated summer and winter shipping cycles. Calibrators were kitted and packaged according to general shipping operation procedures. Packaged calibrator kits were subjected to simulated summer conditions and simulated winter conditions. A non-cycled control group of packaged calibrator kits were stored at the recommended storage condition of 2-10°C.

The differences between control and patient samples using non-cycled calibrators were analyzed by calculating

1. the mean dose responses for each control and patient sample using the calibration curves established with the summer and winter cycled reagents,
2. the percent difference of the controls' and patient samples' doses obtained on the summer and winter cycled reagent pack versus the non-cycled reagent pack,
3. an overall dose average percent of controls and patient samples for both summer and winter cycled reagent packs.

The grand mean recovery for commercial controls and patient samples at baseline, 30 days, and 60 days was within ±10%. The results indicate that the Access® Hybritech® p2PSA calibrators are stable following shipping.

Sample Handling and Storage

The *in vitro* stability of [-2]proPSA antigen in serum using Access® Hybritech® p2PSA was characterized at an external site on an Access 2 analyzer to determine sample collection and storage conditions. Parameters evaluated in the study include:

1. Stability as a function of clotting time
2. Short-term stability at ambient temperature and 4°C
3. Long-term stability at -20° C and -70°C
4. Freeze-thaw stability at -20° C and -70°C

Blood samples from twenty-two subjects with total PSA concentrations ≥4.0 and ≤25.0 ng/mL were collected and allowed to clot at ambient temperature. Serum was prepared by centrifugation and aliquoted. The serum aliquots were stored and tested according to the collection and storage parameter of interest.

The p2PSA values were followed over time to assess stability. The first replicate data of each of the 22 individual samples at the different time points were transformed to a percentage of recovery from their respective baseline values. At each time point, the recoveries in all individual samples were averaged and the 95% confidence intervals of the mean recoveries were determined. To assess stability as a function of clotting time, linear least squares regression was used to estimate the relationship between the mean recoveries and clotting time.

The *in vitro* stability of [-2]proPSA antigen in serum using Access® Hybritech® p2PSA was characterized to determine sample collection and storage conditions. The data collected in this study support the following sample handling recommendations:

1. The specimen should be allowed to clot fully and the serum separated by centrifugation. Specimens should be processed (centrifuged) and refrigerated within 3 hours of blood draw.
2. If the serum sample is to be assayed within 24 hours after collection, the specimen should be stored in a refrigerator at 2 to 8°C. Specimens held for longer times (up to 5 months) should be frozen at -20°C or colder. Specimens to be held for longer than 5 months should be frozen at -70°C. Two freeze-thaw cycles have no effect on

[-2]proPSA recovery. However, prompt refreezing of the thawed samples is recommended.

These sample handling recommendations are consistent with those for the Access® Hybritech® PSA assay and the Access Hybritech free PSA assay.

Access Immunoassay Systems Equivalence

The functional equivalence of the Access Immunoassay Systems analyzers with respect to Access® Hybritech® p2PSA was evaluated via method comparison and imprecision studies.

In the methods comparison study, a total of 166 patient samples with PSA levels >2 ng/mL ([-2]proPSA levels from >5 to <5000 pg/mL) were tested at an internal site on Access 2, UniCel DxI 800, and UniCel DxI 600 analyzers. Following CLSI recommendations in EP9-A2 Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline, 2nd Edition, a Deming linear regression model was used to evaluate analyzer equivalence. The results are summarized in Table 11.

Table 11 Access Immunoassay Systems Analyzer Methods Comparison

System Comparison	Intercept	Slope	95% CI of Slope	R^2
Access 2 vs. UniCel DxI 800	10.719	1.024	1.014 to 1.034	1.00
Access 2 vs. UniCel DxI 600	7.090	1.030	1.023 to 1.036	1.00
UniCel DxI 800 vs. UniCel DxI 600	-3.707	1.005	0.999 to 1.012	1.00

In the imprecision study, patient serum samples with various [-2]proPSA concentrations were tested at an internal site on Access 2, UniCel DxI 800, and UniCel DxI 600 analyzers. The mean of the replicates of each sample and percent CV for within run, between run, total imprecision (system) and overall imprecision (including all components of variability studied) were determined, by analysis of variance (ANOVA), following the methods described in CLSI EP5-A2, Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline, 2nd Edition. The results are summarized in Table 12.

Table 12 Access Analyzer Imprecision

System	Within Run (%CV)	Between Run %CV	Total (%CV)
Access 2	3.98	0.42	4.00
UniCel DxI 800	5.26	1.74	5.54
UniCel DxI 600	5.65	2.42	6.14

Closed Tube Aliquoter (CTA) Carryover

Sample carryover in Access® Hybritech® p2PSA on the closed tube aliquoter (CTA) was assessed at an internal site on the UniCel DxC 600i integrated workstation by running Access® Hybritech® p2PSA S0 calibrator (baseline), followed by a patient serum sample containing a high concentration of [-2]proPSA, followed by Access® Hybritech® p2PSA S0 calibrator (test).

The mean of the replicates of Access® Hybritech® p2PSA S0 calibrator (baseline) was calculated. Each S0 test replicate was compared to the corresponding mean baseline S0 value. The results are summarized in Table 13.

Table 13 Access Hybritech p2PSA CTA Carryover

Parameter	Set Number				
	1	2	3	4	5
Baseline Relative Light Unit (RLU)	7318	7460	7293	7265	7377
Test S0 RLU	7745	7665	7540	7447	7633
Percent Difference (%)	3.88	4.21	3.22	0.70	3.62

Autogloss Interference

Autogloss is a lubricant used by the integrated workstations (UniCel DxC 600i, DxC 880i, DxC 860i, DxC 660i, and DxC 680i) in the Closed Tube Aliquoter (UniCel DxC 600i) and the UniCel Closed Tube Aliquoter (UniCel DxC 880i, DxC 860i, DxC 660i, and DxC 680i) to lubricate the sample pipettor to facilitate puncturing of the rubber enclosure on sample tubes. Potential interference in Access® Hybritech® p2PSA by Autogloss lubricant was evaluated at an internal site on Access 2 and UniCel DxI 800 analyzers using a protocol based on CLSI EP7-A2, Interference Testing in Clinical Chemistry – Approved Guideline, 2nd Edition.

Autogloss was spiked into patient serum samples (Test) containing various concentrations of [-2]proPSA. Access® Hybritech® p2PSA S0 calibrator was spiked into patient serum samples (Control) containing various concentrations of [-2]proPSA. Test and Control patient samples were measured using Access® Hybritech® p2PSA and the dose of each Test sample was compared to the dose of the Control sample. For the Test and Control patient sample sets at each [-2]proPSA concentration, Test mean and Control mean values were calculated from the corresponding sets of replicate measurements. The percent recovery was calculated. The Access 2 results are summarized in Table 14 and the UniCel DxI 800 results are summarized in Table 15.

Table 14 Autogloss Interference in the Access Hybritech p2PSA on Access 2

Sample	Control Mean (pg/mL)	Test Mean (pg/mL)	Percent Recovery (pg/mL)
Patient 1	7.59	7.52	99
Patient 2	16.41	17.26	105
Patient 3	104.76	106.94	102
Patient 4	1638.18	1629.87	99

Table 15 Autogloss Interference in the Access Hybritech p2PSA on DxI 800

Sample	Control Mean (pg/mL)	Test Mean (pg/mL)	Percent Recovery (pg/mL)
Patient 1	8.31	8.14	98
Patient 2	18.64	19.92	107
Patient 3	70.64	69.88	99
Patient 4	1446.72	1408.63	97

Thermal sensitivity

Thermal sensitivity of the Access® Hybritech® p2PSA assay was tested at an internal site on one lot of reagents using three analyzers (Access 2, DxI 800, and DxI 600). Four serum patient samples at [-2]proPSA concentrations approximating 17, 38, 111, and 1,340 pg/mL, and three commercial controls at [-2]proPSA concentrations approximating 22, 38, and 97 pg/mL were tested. Each patient and control sample was assayed in replicates of 5. An Access® Hybritech® p2PSA assay calibration curve was performed at approximately 23°C ambient temperature 24 hours prior to testing. Testing of the serum patient samples and commercial controls was performed at ambient temperatures 18°C, 23°C and 31°C. Access® Hybritech® p2PSA assay calibration curves were performed at approximately 18°C, 23°C, and 31°C. The [-2]proPSA dose values were determined using the calibration curves at various ambient temperatures. The [-2]proPSA dose value of each sample was standardized by the dose value at the center point ambient temperature and calibration temperature. All the sample results were pooled to estimate the ambient temperature effect on dose percent change.

Once the ambient temperature effect on dose percent change was determined, the corresponding effect on Beckman Coulter *phi* and the probability of prostate cancer estimates was evaluated. The original p2PSA dose value of all the subjects in the external, multi-center feasibility study was adjusted to reflect the thermal effect. It should be noted that the feasibility data provided in the PMA were not adjusted fo thermal effect.

There were a total of 645 subjects (330 cancer and 345 non-cancer) in the external, multi-center feasibility study. 115 cancer subjects were randomly sampled from 330 cancer subjects using the bootstrap method to reflect the 25% prostate cancer prevalence in the 4-10 ng/mL PSA range. The sampling procedure was repeated 1000 times, creating 1000 bootstrap samples each containing 115 cancer subjects and 345 benign subjects. Probability of cancer was calculated for each bootstrap sample at different Beckman Coulter *phi* ranges as number of cancer subjects within the range divided by the total number of subjects within the same range. The calculation of probability of cancer was repeated on five different thermal conditions:

1. Assayed ambient temperature = calibration temperature
2. Assayed ambient temperature = calibration temperature +3°C
3. Assayed ambient temperature = calibration temperature +6°C
4. Assayed ambient temperature = calibration temperature -3°C
5. Assayed ambient temperature = calibration temperature -6°C

The thermal effect on p2PSA dose relative to calibration temperature was determined to be

1. 1.84% p2PSA dose change per 1°C ambient temperature change on the Access 2,
2. 2.82% p2PSA dose change per 1°C ambient temperature change on the DxI 800, and
3. 2.31% p2PSA dose change per 1°C ambient temperature change on the DxI 600.

The maximum Access® Hybritech® p2PSA thermal effect is 16.9% (2.82% ÷ 1°C x 6°C = 16.9%) p2PSA dose difference on the DxI 800 over an ambient temperature range of ±6°C from the ambient temperature at which the calibration is performed.

To evaluate the thermal effect on Beckman Coulter *phi* and the probability of cancer estimates, the original p2PSA dose value of all the subjects in the external, multi-center feasibility study was adjusted to reflect a thermal effect of 2.82% p2PSA dose change per 1°C ambient temperature change. For example, if the ambient temperature at test is running 3°C higher than the instrument calibration temperature, the p2PSA dose value of all subjects were adjusted by a 8.46% decrease (2.82% ÷ 1°C x 3°C = 8.46% p2PSA dose decrease). Table 16 shows the thermal effect on Beckman Coulter *phi* and the probability of cancer estimates Beckman Coulter *phi* value at < and ≥ 20.

Table 16 Thermal Effect on Beckman Coulter *phi* and the Probability of Cancer Estimates

Thermal Effect	Median Probability of Cancer (%)	95% CI for Probability of Cancer (%)
Beckman Coulter *phi* <20		
Calibration Temperature	6	2-12
Calibration Temperature +3°C	11	6-17
Calibration Temperature +6°C	11	7-17
Calibration Temperature -3°C	5	1-13
Calibration Temperature -6°C	5	1-14
Beckman Coulter *phi* ≥20		
Calibration Temperature	30	26-36
Calibration Temperature +3°C	31	26-36
Calibration Temperature +6°C	33	28-39
Calibration Temperature -3°C	29	24-34
Calibration Temperature -6°C	28	24-34

The results of the analysis show overlapping 95% confidence intervals of the Beckman Coulter *phi* probability of cancer estimates over an ambient temperature range of ±6°C from the temperature at which the calibration is performed. Therefore, the Beckman Coulter *phi* probability of cancer estimates are statistically and clinically the same within the ±6°C ambient temperature range.

The results of this study show that the maximum Access® Hybritech® p2PSA thermal effect is 16.9% p2PSA dose difference on the DxI 800 over an ambient temperature range of ±6°C from the temperature at which the calibration is performed. The p2PSA thermal sensitivity does not affect the Beckman Coulter *phi* probability of cancer estimates within this temperature range.

The maximum Access® Hybritech® p2PSA thermal effect of 13.9% p2PSA dose difference on the DxI 600 over an ambient temperature range of ±6°C from the ambient temperature at which the calibration is performed is less than the maximum thermal effect of 16.9% p2PSA dose difference on DxI 800. Therefore, the Beckman Coulter *phi* probability of cancer estimates would not be affected by the maximum thermal effect of

13.9% p2PSA dose difference on DxI 600. The effect of room temperature shifts is the same over the entire range of p2PSA concentrations (10 - 91 pg/mL). Dose percent change per 1°C ambient temperature change versus mean sample concentrations showed no trend.

B. Animal Studies

None.

C. Additional Studies

None.

X. SUMMARY OF CLINICAL STUDY

A. Study Design

Study Objective

A multi-center pivotal trial was conducted in men ≥ 50 years of age, with non-suspicious digital rectal exam (DRE) and PSA values ranging from ≥4 to ≤10 ng/mL with the objectives of:

1. Validating that [-2]proPSA in combination with PSA and fPSA provided improved specificity relative to %fPSA alone to differentiate between benign prostatic conditions and prostate cancer, and potentially reduce the number of unnecessary biopsies;
2. Evaluating the mathematical combination of [-2]proPSA, PSA, and fPSA (Beckman Coulter *phi*) in order to enhance clinical specificity relative to %fPSA alone;
3. Establishing the safety and efficacy of the Access Hybritech p2PSA assay to support the proposed claims.

Subject Inclusion and Exclusion Criteria

Enrollment in the Access® Hybritech® p2PSA study was limited to patients who met the following inclusion criteria:

- Subject signed Informed Consent.
- Men ≥ 50 years of age at the time of the blood draw.
- The subject was untreated for prostate disease at the time of the blood draw with any treatment that may affect PSA levels (use of alpha blockers was allowed).
- Digital rectal exam (DRE) and blood draw for PSA testing performed within 6 months of each other.
- Hybritech total PSA ≥ 4.0 and ≤ 10.0 ng/mL or Total PSA ≥ 3.5 and ≤ 11.0 ng/mL if determined using an FDA approved assay from a different manufacturer. Note: the PSA level was used for subject screening purposes.
- Systematic transrectal ultrasound (TRUS)-guided needle biopsy performed (≥ 6 cores biopsy).
- Prostatic biopsy performed within 6 months of the blood draw.

- Diagnosis was histologically confirmed.
- Specimens processed (centrifuged and refrigerated) within 8 hours of blood draw.
- Serum required: ≥ 1.0 mL.
- Serum stored frozen at -20°C or colder within 24 hours of venipuncture.
- Serum stored frozen at -70°C or colder if held for longer than 5 months.

Patients were not permitted to enroll in the study if they met any of the following exclusion criteria:

- Prior history of prostate cancer.
- Use of investigational drugs, approved drugs or other therapies which might have affected PSA values in the 3 months preceding the blood draw including Propecia®, and androgen therapy including testosterone or Androgel®.
- Use of Avodart® or Proscar® at any time prior to the blood draw.
- Prostatic biopsy, or other procedures which might elevate PSA, performed within 3 months prior to the blood draw.
- Equivocal biopsy results (i.e. unable to determine if cancer was present or not).
- Open prostatectomy or transurethral resection of the prostate (TURP) for BPH performed any time prior to blood draw.
- Digital rectal exam (DRE) with discrete nodules suspicious for cancer.
- Acute prostatitis at the time of the blood draw or biopsy.
- Urinary tract infection at the time of the blood draw or biopsy.
- Sample stored frozen for > 5 years.
- Final Access Hybritech total PSA ng/mL < 4.0 or > 10.0.

Study Hypothesis

Primary Null Hypothesis - The clinical specificity of Access p2PSA results is no greater than %fPSA for the defined population.

This null hypothesis will be rejected and the alternative hypothesis of p2PSA having greater clinical specificity within the defined population will be accepted if, using the specificity derived from the ROC analysis (achieved with each arithmetic combination) at a sensitivity of 95%, the specificity of p2PSA results are statistically significantly ($p<.05$) greater than %fPSA.

The comparison of the specificities of p2PSA and %fPSA at 95% sensitivity was performed. Briefly, 1000 bootstrapped samples were generated separately for cancer and benign patients. For each pair of bootstrap datasets, specificities at 95% sensitivities were estimated for both analytes. The bootstrap variance of the differences in specificities between p2PSA and %fPSA was estimated with adjustment for covariance. The bootstrap-estimated variance was then used to test the null hypothesis with statistical significance at $p < 0.05$. This method accounts for the

sampling variability involved in estimating the cutoff at 95% sensitivity and the correlation between the two analytes for paired sample analysis.

Secondary Null Hypothesis - For the defined population, p2PSA results do not have improved area under the curve (AUC) compared to %fPSA.

This null hypothesis will be rejected and the alternative hypothesis of demonstrating a larger AUC for p2PSA results, within the defined population, will be accepted if the AUC of the Receiver Operating Characteristic (ROC) curves for p2PSA are greater than for %fPSA.

Statistical Analyses
The primary hypothesis tests will be performed at other clinical sensitivity levels to assess improvement in clinical specificity compared to %fPSA for each clinical sensitivity level. The underlying goal of the statistical analyses is to validate the mathematical combination of [-2]proPSA, PSA, and fPSA (Beckman Coulter *phi*), derived from the feasibility study, in order to enhance clinical specificity relative to %fPSA alone.

B. Study Population Demographics and Baseline Parameters

Beckman Coulter subject enrollment occurred from April 2008 through June 2009 for subjects who received their medical care (blood draw, DRE, biopsy) from April 2004 through June 2009. Enrollment was by 7 clinical sites in the United States and testing of the clinical samples were performed at three clinical laboratories in the United States.

The patient cohort was a combination of prospectively (97%) and retrospectively (3%) enrolled subjects. The 658 men enrolled consisted of 324 with prostate cancer and 334 without prostate cancer. All subjects were between 50 and 84 years of age with the median age of 63 years for both cancer and benign disease groups. Of the 658 subjects, 652 (99.1%) had $\geq$ 8 core biopsies; 644 (97.9%) of the subjects had $\geq$ 10 core biopsies. Caucasians accounted for 81.4% of the subjects, 5.2% African Americans, 1.1% Asians and 12.3% others. The mean and median age of the different racial groups were comparable.

Covariates including age, site and PSA were evaluated to determine whether they have an effect on the discriminatory ability of %fPSA, %[-2]proPSA, and Beckman Coulter *phi* by regression analysis. Results showed little or no effect.

C. Safety and Effectiveness Results

1. Safety Results

   There was no adverse effect due to the device in the PMA clinical study given that the device results were not used to make biopsy recommendations. There was the potential risk of hematoma from venipuncture.

2. Effectiveness Results

Analysis of effectiveness was based on 658 subjects enrolled in the clinical study. Key effectiveness outcome is improved clinical specificity relative to %fPSA alone in differentiation between benign prostatic conditions and prostate cancer in men with total PSA values of 4 to 10 ng/mL and with DRE results not suspicious of cancer. Analyses of the clinical study results are summarized below.

Expected Values Determination

The expected values for PSA, fPSA, [-2]proPSA, %fPSA, and Beckman Coulter *phi* for this cohort were determined using the Hybritech and the WHO calibration method for determination of Access® Hybritech® total PSA and fPSA. The difference between the two calibration methods is the reference material used for assigning values to the PSA and fPSA calibrators. PSA values derived using the Hybritech calibrators are different from those generated using the WHO calibrators. As a result, a PSA range of ≥ 3.1 to ≤ 7.8 ng/mL with the WHO calibration corresponds to a PSA range of ≥ 4 to ≤ 10 ng/mL with the Hybritech calibration. Consequently, Hybritech calibration and WHO calibration is not interchangeable. For the Access® Hybritech® p2PSA device, one can only use PSA and fPSA values generated from the same type of calibration (Hybritech or WHO) in the calculation of Beckman Coulter *phi*.

The expected values for PSA, fPSA, [-2]proPSA, %fPSA, and Beckman Coulter *phi* for this cohort were determined using the Hybritech or WHO calibration method for total PSA and fPSA. Results of the Hybritech calibration and the WHO calibration are summarized in Table 17 and 18 respectively.

Table 17 PSA, fPSA, [-2]proPSA, %fPSA, and Beckman Coulter *phi* Expected Values by Diagnosis (Hybritech Calibration of total PSA and free PSA)

		Benign N=324	Cancer N=334	Total N=658
PSA (ng/mL) Hybritech Calibration	Median	5.7	5.9	5.8
	Mean ± SD	6.1 ± 1.6	6.2 ± 1.5	6.1 ± 1.6
	Range	4.0 – 10.0	4.0 – 9.8	4.0 – 10.0
fPSA (ng/mL) Hybritech Calibration	Median	1.1	0.8	1.0
	Mean ± SD	1.1 ± 0.5	1.0 ± 0.5	1.1 ± 0.5
	Range	0.1 – 4.3	0.2 – 3.9	0.1 – 4.3
[-2]proPSA (pg/mL)†	Median	14.0	15.2	14.7
	Mean ± SD	15.7 ± 7.4	18.1 ± 11.8	16.9 ± 9.9
	Range	3.6 – 43.5	5.3 – 93.5	3.6 – 93.5
%fPSA	Median	18.1	14.6	16.5
	Mean ± SD	19.0 ± 7.3	15.5 ± 6.7	17.3 ± 7.2

		Benign N=324	Cancer N=334	Total N=658
	Range	3.1 – 50.1	3.7 – 42.5	3.1 – 50.1
Beckman Coulter *phi*	Median	32.4	44.4	37.9
	Mean ± SD	35.9 ± 15.7	51.2 ± 31.5	43.5 ± 25.9
	Range	14.0 – 98.2	14.0 – 325.8	14.0 – 325.8

†No WHO standard available for [-2]proPSA – Hybritech Calibration only.

Table 18 PSA, fPSA, [-2]proPSA, %fPSA, and Beckman Coulter *phi* Expected Values by Diagnosis (WHO Calibration of PSA and free PSA)

		Benign N=324	Cancer N=334	Total N=658
PSA (ng/mL) WHO Calibration	Median	4.5	4.7	4.6
	Mean ± SD	4.9 ± 1.3	4.9 ± 1.2	4.9 ± 1.2
	Range	3.2 – 7.9	3.2 – 7.8	3.2 – 7.9
fPSA (ng/mL) WHO Calibration	Median	0.8	0.7	0.7
	Mean ± SD	0.9 ± 0.4	0.8 ± 0.4	0.8 ± 0.4
	Range	0.1 – 3.5	0.1 – 3.2	0.1 – 3.5
[-2]proPSA (pg/mL) †	Median	14.0	15.2	14.7
	Mean ± SD	15.7 ± 7.4	18.1 ± 11.8	16.9 ± 9.9
	Range	3.6 – 43.5	5.3 – 93.5	3.6 – 93.5
%fPSA	Median	18.0	14.4	16.1
	Mean ± SD	18.7 ± 7.2	15.3 ± 6.7	17.0 ± 7.2
	Range	3.0 – 47.0	3.6 – 43.2	3.0 – 47.0
Beckman Coulter *phi*	Median	36.8	50.6	42.5
	Mean ± SD	40.8 ± 17.9	58.2 ± 36.1	49.4 ± 29.7
	Range	15.6 – 112.7	15.5 – 377.3	15.5 – 377.3

†No WHO standard available for [-2]proPSA – Hybritech Calibration only.

Clinical Sensitivity and Clinical Specificity

Clinical sensitivity and clinical specificity values were calculated for various PSA, %fPSA, and Beckman Coulter *phi* cutoffs for the 4.0 to 10.0 ng/mL PSA range for the Hybritech calibration and the 3.1 to 7.8 ng/mL PSA range for the WHO calibration. These values are plotted on the ROC curve shown in Figure 1. and 2 respectively.

Figure 1 PSA, %fPSA, and Beckman Coulter *phi* ROC Curves in the 4 to 10 ng/mL PSA Range (Hybritech Calibration of PSA and free PSA)

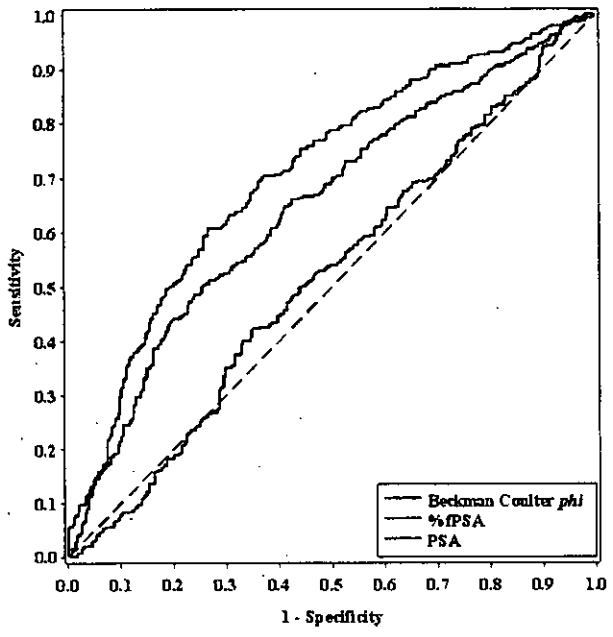
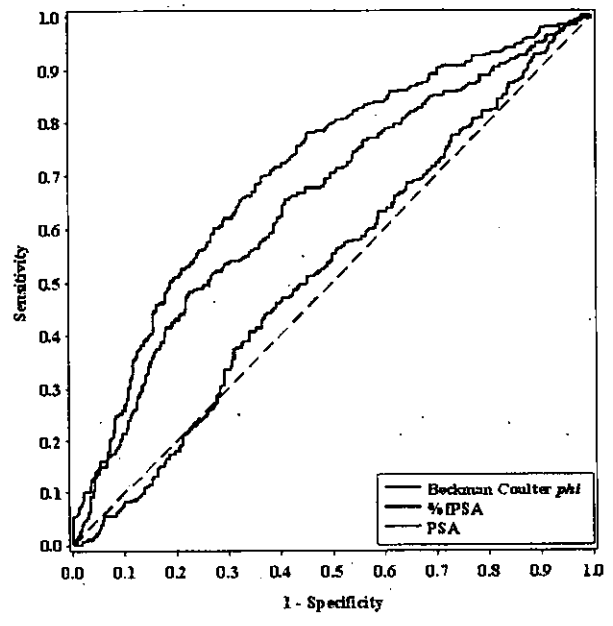


Figure 2 PSA, %fPSA, and Beckman Coulter *phi* ROC Curves in the 3.1 to 7.8 ng/mL PSA Range (WHO Calibration of PSA and free PSA)



The Beckman Coulter *phi* is shown to be significantly better than PSA and %fPSA for this study population of men with PSA between 4 to 10 ng/mL for the Hybritech calibration and PSA between 3.1 and 7.8 ng/mL for the WHO calibration.

Table 19 shows the multi-site study's clinical sensitivity and clinical specificity of detecting prostate cancer with prostate biopsy based on Beckman Coulter *phi* cutoffs using PSA and free PSA calibrated to the Hybritech and WHO standards.

Table 19 Clinical Sensitivity and Specificity of Prostate Cancer

Cutoffs for Beckman Coulter *phi* in Men with Non-Suspicious DRE

	Hybritech Calibration		WHO Calibration	
%Clinical Sensitivity	*phi* Cutoff	%Clinical Specificity	*phi* Cutoff	%Clinical Specificity
99	**17.2**	4.2	**19.7**	4.2
98	**19.4**	8.4	**22.0**	9.0
95	**22.1**	14.1	**24.6**	13.5
90	**27.0**	31.1	**30.2**	29.6
85	**28.9**	37.7	**33.3**	39.2
80	**31.3**	46.1	**36.4**	49.4
75	**34.0**	55.7	**38.8**	56.6
70	**36.2**	63.2	**41.2**	62.6
65	**38.1**	65.9	**43.3**	67.7
60	**40.9**	73.4	**45.9**	72.8
55	**42.8**	76.3	**48.4**	76.0
50	**44.4**	80.5	**50.6**	81.1
45	**47.6**	83.8	**53.6**	83.8
40	**49.3**	85.3	**55.7**	85.0
35	**51.7**	88.9	**58.9**	88.3
30	**54.8**	89.8	**62.0**	88.9
25	**58.2**	91.0	**65.7**	91.0
20	**62.7**	92.5	**71.9**	92.8
15	**68.1**	94.3	**78.4**	94.6
10	**77.1**	96.7	**87.9**	96.7
5	**99.9**	100	**114.1**	100

In addition, the AUC was significantly greater for Beckman Coulter *phi* than for PSA and %fPSA in this cohort of men with PSA. These data are shown in Table 20 along with the p-values for the AUC relative to the line of chance. The AUC for Beckman Coulter *phi* was statistically different relative to the line of chance with a p-value <0.001.

Table 20 Comparison of PSA, %fPSA and Beckman Coulter *phi* Area Under the ROC Curve (AUC) (324 Cancer, 334 Benign)

	Hybritech calibration PSA 4-10 ng/mL		
	AUC	95% Confidence Interval	P-value
PSA	0.516	0.472-0.560	<0.001
%fPSA	0.648	0.606-0.690	0.009
Beckman Coulter *phi*	0.708	0.668-0.747	-
	WHO calibration PSA 3.1-7.8 ng/mL		
PSA	0.519	0.475-0.564	<0.001
%fPSA	0.649	0.607-0.691	0.010
Beckman Coulter *phi*	0.709	0.669-0.748	-

Probability of Cancer on Biopsy

In addition to the sensitivity and specificity analyses of the multi-site study data, an individual's probability of having detectable cancer was estimated based on the Beckman Coulter *phi* values. In a population of men with PSA in the 4.0 to 10.0 ng/mL range and a non-suspicious DRE, a 25% positive biopsy rate has been previously reported[1,2,3]. The multi-site study population consisted of approximately 49.2% (324/658) cancer subjects and 50.8% (334/658) non-cancer subjects. Cancer probabilities based on the 49.2% proportion of cancer subjects would inflate the probability estimates for detecting cancer. Therefore, the proportion of cancer subjects was adjusted to 25% prior to calculating cancer probabilities on biopsy for various Beckman Coulter *phi* scores. This adjustment provides accurate probabilities for the group of men in whom this test will be used.

The bootstrap method was used to repetitively sample the multi-site study's population[4]. Each sampling consisted of 334 (75%) benign subjects and 111 (25%) cancer subjects, for a total of 445 subjects. This random sampling process was repeated 1000 times. The mean cancer probabilities on biopsy and nonparametric 95% confidence intervals (2.5^{th} and 97.5^{th} percentiles) were calculated. This repetitive sampling method increases the reliability of the probability of cancer on biopsy estimates. Table 21 (based on the Hybritech calibration) and Table 22 (based on the WHO calibration) show the probability of detecting prostate cancer based upon the adjusted 25% proportion of cancer subjects. A strong relationship between Beckman Coulter *phi* and probability of prostate cancer on biopsy can be seen, with higher Beckman Coulter *phi* values associated with higher probability of prostate cancer on biopsy.

Table 21 Probability of Prostate Cancer on Biopsy for Beckman Coulter *phi* in Patients with PSA between 4 and 10 ng/mL (Hybritech Calibration of PSA and free PSA)

Beckman Coulter *phi* Range (Hybritech Calibration)	Probability of Cancer	95% Confidence Interval
0 – 26.9	9.8%	5.2% – 15.4%
27.0 – 35.9	16.8%	11.3% – 22.2%
36.0 – 54.9	33.3%	26.8% – 39.9%
55.0+	50.1%	39.8% – 61.0%

Table 22 Probability of Prostate Cancer on Biopsy for Beckman Coulter *phi* in Patients with PSA between 3.1 and 7.8 ng/mL (WHO Calibration of PSA and free PSA)

Beckman Coulter *phi* Range (WHO Calibration)	Probability of Cancer	95% Confidence Interval
0 – 29.9	9.8%	5.0% – 15.8%
30.0 – 39.9	16.6%	11.1% – 22.4%
40.0 – 61.9	32.7%	26.4% – 38.6%
62.0+	48.2%	38.4% – 58.8%

The selection of an appropriate Beckman Coulter *phi* score that guides patient management considers the percentage of cancers detected (clinical sensitivity), and the percentage of men without cancer, in whom biopsy may be avoided (clinical specificity). For example, using the Hybritech calibration for PSA and free PSA, a Beckman Coulter *phi* value of 22.1 corresponds to 95% clinical sensitivity and 14.1% clinical specificity. Therefore, approximately 1 in 7 men may avoid prostate biopsy while detecting 95% of cancers if their Beckman Coulter *phi* value is less than 22.1.

A Beckman Coulter *phi* value of 27.0 corresponds to 90% clinical sensitivity and 31.1% clinical specificity. Therefore, nearly 1 in 3 men may avoid prostate biopsy while detecting 90% of cancers if their Beckman Coulter *phi* value is less than 27.0.

A Beckman Coulter *phi* value of 31.3 corresponds to 80% clinical sensitivity and 46.1% clinical specificity. Therefore, approximately 1 in 2 men may avoid prostate biopsy while detecting 80% of cancers if their Beckman Coulter *phi* value is less than 31.3. For men with a Beckman Coulter *phi* value above the cutoff the probability of cancer on biopsy increases and may affect the clinical management of each patient.

Low Beckman Coulter *phi* scores are associated with a lower probability of prostate cancer on biopsy and higher scores are associated with an increased probability of prostate cancer on biopsy. The choice of an appropriate Beckman Coulter *phi* score to be used in guiding clinical decision-making may vary for each patient and may depend in part on other clinically important factors or on family history of disease.

Table 21 shows the probability of finding prostate cancer on biopsy based on categories of Beckman Coulter *phi* scores using the Hybritech calibration for PSA and free PSA. For example, the probability of prostate cancer at *phi* < 27.0 is 9.8%. whereas, the probability of prostate cancer at *phi* ≥55.0 is 50.1%. Similarly, using the WHO calibration (Table 22), the probability of prostate cancer at *phi* < 30.0 is 9.8%, whereas, the probability of prostate cancer at *phi* ≥62.0 is 48.2%.

Beckman Coulter *phi* values should not be interpreted as definitive evidence for the presence or absence of prostate cancer. Prostatic biopsy is required for diagnosis of cancer.

XI. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Immunology Panel, an FDA advisory committee, for review and recommendation. Information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM NONCLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

According to the clinical study results, the use of [-2]proPSA and *phi* as determined by the Access® Hybritech® p2PSA assay can increase the specificity of Access® Hybritech® %fPSA and total PSA for prostate cancer detection in men with total PSA values of 4 to 10 ng/mL and with DRE results not suspicious of cancer. At 95% clinical sensitivity the clinical specificity for Beckman Coulter *phi* was 14.1% compared to 9.9% for %fPSA. The improvement in clinical specificity for Beckman Coulter *phi* relative to %fPSA represents a substantial decrease in the number of men who might undergo an unnecessary biopsy.

In addition, a significant relationship was found between Beckman Coulter *phi* and the probability of prostate cancer on biopsy in individual men. Higher Beckman Coulter *phi* values indicating higher probability of prostate cancer on biopsy. The probability of cancer ranged from 9.8% to 50.1% (Hybritech calibration of PSA and free PSA) depending upon the Beckman Coulter *phi* value. A physician may recommend biopsy for a patient with a Beckman Coulter *phi* value $\geq$ 55.0 (probability of cancer = 50.1%), whereas, may not recommend biopsy for a patient with a Beckman Coulter *phi* value < 27.0 (probability of cancer = 9.8%).

B. Safety Conclusions

The risks from venipuncture (e.g. hematoma) and complications from biopsy [e.g. infection, fever, rectal bleeding, hematuria and hematospermia (blood in the urine and semen), urinary retention, and hospitalization in some cases] if the Beckman Coulter *phi* result indicated biopsy.

C. Benefit-Risk Conclusions

The probable benefits of the device are also based on data collected in the studies described above.

Benefits of procedures for prostate cancer detection using PSA, free PSA, [-2]proPSA, and digital rectal examination include early diagnosis, decreased health care costs of advanced stage cancer, and decreased mortality. The benefit of Beckman Coulter *phi* as determined by the Access® Hybritech® p2PSA device is the increased specificity offered when used to determine whether patients with total PSA

≥ 4 to ≤ 10 ng/mL total PSA and DRE results not suspicious for cancer should receive biopsies. Results of the clinical study showed that Beckman Coulter *phi* significantly enhanced the clinical specificity relative to PSA and %fPSA for prostate cancer detection. At 95% clinical sensitivity the clinical specificity for Beckman Coulter *phi* was 14.1% compared to 9.9% for %fPSA. The improvement in clinical specificity for Beckman Coulter *phi* relative to %fPSA represents a substantial decrease in the number of men who might undergo an unnecessary biopsy.

The risk of a falsely high Beckman Coulter *phi* result (higher Beckman Coulter *phi* is associated with a greater probability of cancer) may lead to a medical decision to perform an unnecessary prostate biopsy. A falsely low Beckman Coulter *phi* result (lower Beckman Coulter *phi* is associated with a lesser probability of cancer) may lead to a medical decision to not perform a prostate biopsy. These risks could be mitigated when taken into consideration that Medical decisions are not based on [-2]proPSA or Beckman Coulter *phi*, alone but are in conjunction with digital rectal examination, ultrasonography, and other clinical signs and symptoms.

In addition, there are risks related to complications from venipuncture (e.g. hematoma) and biopsy (e.g. infection, fever, rectal bleeding, hematuria and hematospermia, urinary retention, and hospitalization in some cases). Therefore, the risks associated with Beckman Coulter *phi* are:

1. Complications resulting from venipuncture (e.g. hematoma).
2. Interpretation of Beckman Coulter *phi* resulting in unnecessary biopsy or delay in medical treatment.

In conclusion, given the available information above, the data support that for the Access® Hybritech® p2PSA on the Access Immunoassay Systems.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use.

According to the clinical study results, the use of [-2]proPSA and *phi* as determined by the Access® Hybritech® p2PSA assay can increase the specificity of Access Hybritech %fPSA and total PSA for prostate cancer detection in men with total PSA values of 4 to 10 ng/mL and with DRE results not suspicious of cancer. The clinical benefit is a reduction of unnecessary biopsies in men being evaluated for prostate cancer. At 95% clinical sensitivity the clinical specificity for Beckman Coulter *phi* was 14.1% compared to 9.9% for %fPSA. The improvement in clinical specificity for Beckman Coulter *phi* relative to %fPSA represents a substantial decrease in the number of men who might undergo an unnecessary biopsy. In addition, a significant relationship was found between *phi* and the probability of cancer in individual men. Lower *phi* values indicate lower probabilities. The calculated probability estimates based on [-2]proPSA, Total PSA and %fPSA maybe used by physicians to recommend treatment options.

XIII. CDRH DECISION

CDRH issued an approval order on June 14, 1012. The final conditions of approval cited in the approval order.

The applicant's manufacturing facility was inspected on December 30, 2009 and May 17, 2012 and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restriction: See device approval order

XV. REFERENCES

1. Catalona WJ, Smith DS, and Ornstein D. Prostate cancer detection in men with serum PSA concentrations of 2.6–4.0 ng/mL and benign prostate examination: enhancement of specificity with free PSA measurements. JAMA 1997; 277: 1452-1455.
2. Catalona WJ, Bartsch G, Rittenhouse HG, et al. Serum pro prostate specific antigen improves cancer detection compared to free and complexed prostate specific antigen in men with prostate specific antigen 2 to 4 ng/mL. J Urol 2003; 170: 2181-2185.
3. Khan MA, Partin AW, Ritttenhouse HG, et al. Evaluation of Proprostate specific antigen for early detection of prostate cancer in men with a total prostate specific antigen range of 4.0 to 10.0 ng/mL. J of Urology 2003; 170: 723-726.
4. Efron, B. and Tibshirani, R. J. (1993). An introduction to the bootstrap. Chapman and Hall/CRC Press, New York.