Access
Immunoassay Systems

Hybritech p2PSA
REF B03704

Caution **For U.S.A. only, Federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and use is restricted to by or on the order of a physician.**

Warning Access Hybritech p2PSA should be used only with Access Hybritech PSA and Access Hybritech free PSA to calculate the Beckman Coulter *phi* (prostate health index). Use of another manufacturer's PSA and/or free PSA (fPSA) assays may result in:

- Selection of an inappropriate population of patients for follow-up testing.
- Significantly different cutoffs and cancer probabilities than those presented in the Expected Values section.

Expected values apply only to Beckman Coulter *phi* as measured by the Access Hybritech PSA, free PSA, and p2PSA assays.

The concentration of [-2]proPSA, fPSA, and PSA in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must specify the manufacturer of the [-2]proPSA, fPSA, and PSA assays used. Values obtained with different manufacturers' assays cannot be used interchangeably.

PSA and fPSA concentrations are dependent on the standard used to calibrate the assays. PSA and fPSA concentrations based on calibration to the WHO 96/670 (PSA) or WHO 96/668 (fPSA) Reference Preparations will differ significantly from PSA and fPSA concentrations based on calibration to the original Hybritech Tandem-R assays. The concentrations are not interchangeable. If the calibration is changed, accepted laboratory practice is to establish a new baseline for patient monitoring.[1]

Intended 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.

Summary and Explanation Prostate cancer continues to be a leading cause of cancer mortality. The American Cancer Society estimates that approximately 217,730 new cases will be diagnosed and 32,050 will die from prostate cancer in 2010 in the United States.[2]

Prostate-specific antigen (PSA) was identified and purified by Wang and co-workers in 1979.[3] PSA, a serine protease, is produced by the epithelial cells of the prostate, and is produced by both benign and malignant cells. Abnormalities in the prostate gland architecture resulting from trauma or disease can lead to "leakage" of PSA into the bloodstream.

Serum PSA exists primarily in either the free "non-complexed form" (fPSA) or in a "complex" (cPSA) primarily with the serum protease inhibitor, alpha 1-antichymotrypsin.[4,5] Typically from 70–90% of the PSA in serum is cPSA, with the remainder being fPSA.[6] The %fPSA (ratio of fPSA to PSA) in serum has been demonstrated to significantly improve the discrimination of prostate cancer from benign prostatic conditions, especially in patients with PSA levels in the ≥ 4 to ≤ 10 ng/mL range. A higher %fPSA in serum is correlated with a lower probability of prostate cancer, while %fPSA values below 10% are more highly associated with cancer.[6,7,8]

ProPSA and BPSA represent distinct forms of fPSA that demonstrate greater disease-association than PSA, fPSA or cPSA alone.[5] Truncated forms of proPSA were found to be elevated in peripheral zone cancer tissue compared with BPH tissues.[9] The proPSA was elevated in prostate tumor tissue, while BPSA was elevated in nodular BPH transition zone tissue, compared to its concentration in peripheral zone tissue. ProPSA has been found as the native proPSA form containing a 7 amino acid pro leader peptide ([-7]proPSA),[10] as well as forms with truncated pro leader peptides. Truncated proPSA forms consist primarily of proPSA with a 5 amino acid pro leader peptide ([-5]proPSA), 4 amino acids ([-4]proPSA) and 2 amino acids ([-2]proPSA).[11,12] The [-2]proPSA has received the most attention since it was the primary form found in tumor extracts and shows higher immunostaining in prostate tumor than benign tissue.[6,13] Additionally, in vitro, the most stable of the five identified proPSA forms is [-2]proPSA.[14,15]

Access Hybritech p2PSA was developed by Beckman Coulter, Inc. to measure [-2]proPSA in serum. In studies of men with biopsy confirmed prostate cancer, [-2]proPSA in the ≥ 4.0 to ≤ 10.0 ng/mL PSA range was shown to improve the specificity for cancer detection relative to %fPSA alone.[6]

Reports from the literature are consistent with the intended use for the Access Hybritech p2PSA assay, used in conjunction with Access Hybritech PSA and free PSA assays to calculate Beckman Coulter *phi*, in the further evaluation of patients with PSA levels in the ≥ 4.0 to ≤ 10.0 ng/mL range. Literature reports support the conclusion that precursor forms of PSA are emerging as potentially important diagnostic serum markers to augment PSA and improve prostate cancer detection.[6,16,17,18]

Results of the Beckman Coulter, Inc. multi-center pivotal clinical trial found that Beckman Coulter *phi* values significantly enhanced the clinical specificity relative to PSA and %fPSA for prostate cancer detection in the ≥ 4.0 ng/mL to ≤ 10.0 ng/mL PSA range. At 95% clinical sensitivity the clinical specificity for Beckman Coulter *phi* was 14.1% compared to 9.9% for %fPSA. At 90% clinical sensitivity the clinical specificity for Beckman Coulter *phi* was 31.1%. At 80% clinical sensitivity the clinical specificity for Beckman Coulter *phi* was 46.1%. The improvement in clinical specificity for Beckman Coulter *phi* relative to %fPSA represents a substantial improvement in testing intended as an aid in distinguishing prostate cancer from benign prostatic conditions in men aged 50 years and older with total PSA ≥ 4.0 to ≤ 10.0 ng/mL, with digital rectal examination findings that are not suspicious for cancer.

Beckman Coulter *phi* may also be used to determine the probability of prostate cancer on biopsy for an individual patient. Higher Beckman Coulter *phi* values are associated with higher probability of prostate cancer.

Principles of the Procedure

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.

Product Information

Access Hybritech p2PSA Reagent Pack

Cat. No. B03704: 100 determinations, 2 packs, 50 tests/pack

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Stable at 2 to 10°C for 28 days after initial use.
- Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.
- If the reagent pack is damaged (i.e., broken elastomer), discard the pack.
- All antisera are polyclonal unless otherwise indicated.

R1a:	Paramagnetic streptavidin particles coated with mouse monoclonal anti-[-2]proPSA antibodies in TRIS buffered saline with surfactant, bovine serum albumin (BSA), < 0.1% sodium azide, and 0.1% ProClin** 300.
R1b:	Blocking reagent with citrate, surfactants, BSA, alkaline phosphatase, proteins (mouse, goat, and bovine), < 0.1% sodium azide, and 0.1% ProClin 300.
R1c:	Mouse monoclonal anti-PSA antibody alkaline phosphatase (bovine) conjugate in phosphate buffered saline with surfactant, BSA, mouse proteins, < 0.1% sodium azide, and 0.25% ProClin 300.

Warnings and Precautions

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[19]
- Xi. Irritant: 0.25% ProClin 300.

R 43: May cause sensitization by skin contact.
S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- The Material Safety Data Sheet (MSDS) is available upon request.

Specimen Collection and Preparation

1. No special preparation of the patient sample is necessary.
2. Specimens for [-2]proPSA testing should be drawn prior to such prostatic manipulations as digital rectal examination (DRE), prostatic massage, transrectal ultrasound (TRUS), and prostatic biopsy. DRE may cause a transient increase in [-2]proPSA, fPSA, and PSA.[20]
3. Transrectal needle biopsy has also been shown to cause transient increases in [-2]proPSA, fPSA and PSA elevations,[20,21] thus a six-week waiting period between needle biopsy and [-2]proPSA, fPSA, and PSA sampling is recommended.

4. Serum is the recommended sample for the Access Hybritech p2PSA, free PSA and PSA assays. Plasma samples should not be used.
5. Only blood drawn by an acceptable medical technique into a collection tube with no anticoagulants should be used. Specimens should be collected in such a way as to avoid hemolysis.
6. 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.**[22]
7. 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.[22,23] Specimens to be held for longer than 5 months should be frozen at -70°C.[22,23,24] Repeated freeze-thaw cycles have no effect on free PSA or total PSA,[22] or [-2]proPSA. However, prompt refreezing of the thawed samples is recommended.
8. Turbid serum samples or samples containing particulate matter should be centrifuged prior to assay.
9. Use the following guidelines when preparing specimens:
   - Ensure residual fibrin and cellular matter have been removed prior to analysis.
   - Follow blood collection tube manufacturer's recommendations for centrifugation.
10. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.

Materials Provided

R1 Access Hybritech p2PSA Reagent Packs

Materials Required But Not Provided

1. Access Hybritech p2PSA Calibrators
   Provided at zero and approximately 10, 20, 50, 100, 500 and 5000 pg/mL.
   Cat. No. B03705
2. Access Hybritech p2PSA Quality Control (QC) or other FDA cleared commercially available control material.
   Cat. No. A56934
3. Access Substrate
   Cat. No. 81906
4. **Access 2, UniCel DxC 600i:**
   Access Wash Buffer II, Cat. No. A16792
   UniCel DxI:
   UniCel DxI Wash Buffer II, Cat. No. A16793

Procedural Comments

1. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.
2. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.
3. Use fifty (50) µL of sample for each determination in addition to the sample container and system dead volumes. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.
4. The system default unit of measure for sample results is pg/mL.

Procedure

Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

Calibration Details

An active calibration curve is required for all tests. For the Access Hybritech p2PSA assay, calibration is required every 28 days. Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

Quality Control

Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of immunochemical assays. Quality control materials should be included with all patient sample testing. Laboratories that may experience temperature changes of more than six degrees Celsius within a calibration cycle should include QC materials closely associated with all patient samples. Include Access Hybritech p2PSA QC or other FDA cleared commercially available quality control materials that cover at least two levels of analyte.[25] More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish mean values and acceptable ranges to assure proper performance. Quality control results that do not fall within acceptable ranges may indicate invalid test results. Examine all test results generated since obtaining the last acceptable quality control test point for this analyte. Refer to the appropriate system manuals and/or Help system for information about reviewing quality control results.

Results

Patient Access Hybritech p2PSA test results are determined automatically by the system software. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient Access Hybritech p2PSA test results and Beckman Coulter *phi* results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

Calculation of Beckman Coulter *phi*

[-2]proPSA values alone, as measured by the Access Hybritech p2PSA assay, have not been shown to be effective in patient management. PSA, fPSA, and [-2]proPSA concentrations should be determined from the same serum specimen on the same analyzer and used to calculate Beckman Coulter *phi*. Beckman Coulter *phi* results are then used for patient management. Beckman Coulter *phi* is automatically calculated by the Access Immunoassay Systems.

Important: Beckman Coulter *phi* can only be calculated if the PSA and fPSA results were derived from the same type of calibration (Hybritech or WHO). Never mix Hybritech and WHO calibrations when calculating Beckman Coulter *phi*.

Limitations of the Procedure

1. For Access 2, UniCel DxC 600i, UniCel DxI 800, UniCel DxI 600, UniCel DxC 880i, UniCel DxC 860i, UniCel DxC 680i, and UniCel DxC 660i Immunoassay Systems:
   - For optimal Access Hybritech p2PSA results, assay calibration and patient sample testing should be conducted under similar room temperature conditions. If ambient laboratory temperature varies by more than ±6°C from the temperature of calibration, review quality control results and recalibrate as necessary.
   - Quality control materials should be included with all patient sample testing. Include Access Hybritech p2PSA QC or other commercially available quality control materials that cover at least two levels of analyte.
2. The Beckman Coulter *phi* results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests, and other appropriate information. Beckman Coulter *phi* should not be interpreted as absolute evidence for the presence or absence of prostate cancer. Elevated PSA concentrations, increased Beckman Coulter *phi*, or decreased %fPSA may be observed in the serum of patients with non-malignant disorders, as well as those with prostate cancer. Furthermore, low PSA concentrations, low Beckman Coulter *phi*, or elevated %fPSA are not

necessarily indicative of the absence of cancer. Serum p2PSA, fPSA, and PSA values should be used in conjunction with information available from the clinical evaluation of the patient and other diagnostic procedures such as digital rectal examination (DRE). Some cases of early prostate cancer will not be detected by PSA testing; the same is true for DRE. Biopsy of the prostate is the standard method used to confirm the presence or absence of prostate cancer.

3. Routine use of 5 alpha-reductase inhibitor drugs typically lower PSA, fPSA, and [-2]proPSA levels in patients. Other drugs used to treat benign prostatic hyperplasia (BPH) may also affect PSA levels. Care should be taken in interpreting results from patients taking these drugs.
4. Samples can be accurately measured within the analytic range as defined by the limit of blank and the highest Access Hybritech p2PSA Calibrator (S6) (approximately 0.50 and 5000 pg/mL, respectively):
   - If a sample contains less than the assay limit of blank (0.50 pg/mL), report the [-2]proPSA result as less than limit of blank (e.g., "< 0.50 pg/mL") and do not report Beckman Coulter *phi*.
   - If a sample contains more than the stated value of the S6 calibrator, report the [-2]proPSA result as greater than S6 calibrator concentration (e.g., "> 5000 pg/mL") and do not report Beckman Coulter *phi*.

   Note: Dilution of samples with a value greater than the stated value of the highest Access Hybritech p2PSA Calibrator (S6) is not recommended.
5. For assays employing antibodies, the possibility exists for interference by Heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples.[26,27]
   Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
6. Normal physiological total protein levels range from 6 to 8 g/dL.[28] 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.
7. The Access Hybritech p2PSA assay does not demonstrate any "hook" effect up to 15,000 pg/mL [-2]proPSA.

Expected Values

Beckman Coulter *phi* is an In Vitro Diagnostic Multivariate Index Assay (IVDMIA), a combination of the results from the Access Hybritech PSA, free PSA, and p2PSA assays designed to optimize clinical sensitivity and specificity as an aid in distinguishing prostate cancer from benign prostatic conditions.

A multi-center (7 clinical sites) clinical trial with a combination of prospective (97%) and retrospective (3%) subjects was conducted to test the effectiveness of Beckman Coulter *phi*. Beckman Coulter *phi* is used as an aid in distinguishing prostate cancer from benign prostatic conditions. Subjects included men who were being evaluated to determine their prostate status.

All subjects were between 50 and 84 years of age, with serum PSA values between 4 and 10 ng/mL (Hybritech calibration) and digital rectal examination (DRE) findings that were not suspicious for cancer. These men represent the "diagnostic gray zone," in which PSA has identified the men as high risk (25% cancer rate in men over 50 years of age), but where clinical specificity could be improved. [29,30,31] The study was blinded; clinicians did not have access to Beckman Coulter *phi* values, and laboratory technicians did not have access to diagnoses. Inclusion criteria included: subjects signed informed consent, men ≥ 50 years of age, subjects were untreated for prostate disease at the time of their blood draw, Hybritech PSA ≥ 4.0 and ≤ 10 ng/mL, ≥ 6 core biopsy; TRUS guided needle biopsy and diagnosis was histologically

confirmed. Of the 658 total evaluable subjects, 652 (99.1%) had ≥ 8 core biopsies; 644 (97.9%) of the subjects had ≥ 10 core biopsies. Exclusion criteria included: prior history of prostate cancer, use of Avodart*** or Proscar**** at any time prior to blood draw, use of other drugs or therapies, or recent prostatic manipulation which might have affected PSA values in the three months preceding the blood draw (including Propecia****, and androgen therapy including testosterone or Androgel*****), acute prostatitis, urinary tract infection, prior transurethral resection of the prostate (TURP), equivocal biopsy results, DRE with discrete nodules suspicious for cancer, or PSA < 4.0 or > 10.0 ng/mL.

A total of 658 men participated in the study (324 with prostate cancer and 334 without prostate cancer). Median age for both cancer and benign disease subjects was 63 years. Table 1 and Table 2 show the expected values, based on the Hybritech and WHO calibrations, respectively for PSA (ng/mL), fPSA (ng/mL), [-2]proPSA (pg/mL), %fPSA [(fPSA/PSA) x 100%], and Beckman Coulter *phi* for this population of men.

A PSA range of ≥ 4 to ≤ 10 ng/mL with the Hybritech calibration corresponds to a PSA range of ≥ 3.1 to ≤ 7.8 ng/mL with the WHO calibration.

Table 1: PSA, fPSA, [-2]proPSA, %fPSA, and Beckman Coulter *phi*
Expected Values by Diagnosis
(Hybritech Calibration of 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
	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 2: 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

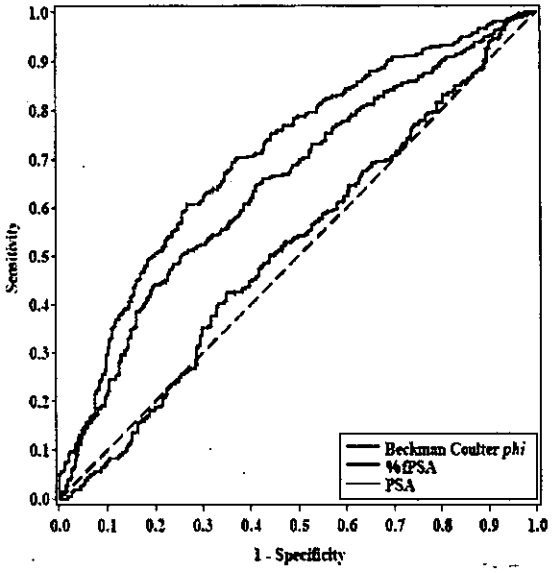
†No WHO standard available for [-2]proPSA – Hybritech Calibration only.

PSA, %fPSA, and Beckman Coulter *phi* Cutoffs, Clinical Sensitivity, Clinical Specificity, and Area Under the Curve (AUC)

Two measures have traditionally been used to describe the validity of cancer detection tests: clinical sensitivity and clinical specificity. Clinical sensitivity is defined as the ability of a test to detect all persons with cancer in the tested population. Clinical specificity is defined as the ability of a test to correctly identify those persons free of cancer in the tested population. Clinical sensitivity and clinical specificity have a reciprocal relationship: increases in clinical sensitivity result in decreases in clinical specificity and vice versa. This relationship may be expressed by plotting clinical sensitivity versus 1 minus the clinical specificity; these plots are called Receiver Operating Characteristic (ROC) curves.

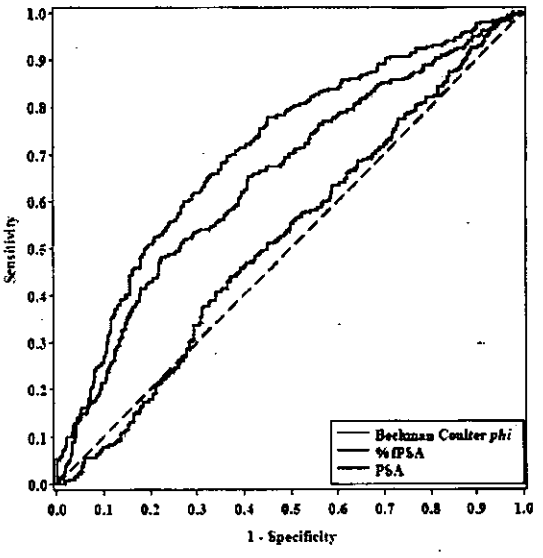
Clinical sensitivity and clinical specificity values were calculated for various PSA, %fPSA, and Beckman Coulter *phi* cutoffs in the 4 to 10 ng/mL PSA range for the Hybritech calibration. These values are plotted on the ROC curve shown in Figure 1.

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)



Clinical sensitivity and clinical specificity values were calculated for various PSA, %fPSA, and Beckman Coulter *phi* cutoffs in 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 2.

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)



Theoretically, a perfect test with 100% clinical sensitivity and 100% clinical specificity would appear at the top left-hand corner on a ROC curve, and the closer a test is to this point, the better clinical sensitivity and clinical specificity it has. It can be seen in Figure 1 and Figure 2 that Beckman Coulter *phi* is 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 to 7.8 ng/mL for the WHO calibration. The ROC curve for Beckman Coulter *phi* is closer to the upper left-hand corner than the curves for PSA and %fPSA for both the 4 to 10 ng/mL PSA range (Hybritech calibration) and for the 3.1 to 7.8 ng/mL PSA range (WHO calibration).

Table 3 and Table 4 show 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. PSA was in the 4 to 10 ng/mL range for the Hybritech calibration and in the 3.1 to 7.8 ng/mL range for the WHO calibration. Subject age ranged from 50 to 84 years.

Table 3: Clinical Sensitivity and Specificity of Prostate Cancer Cutoffs for Beckman Coulter *phi* in Men with Non-Suspicious DRE (Hybritech Calibration of PSA and free PSA)

%Clinical Sensitivity	Hybritech Calibration	
	phi Cutoff	%Clinical Specificity
99	17.2	4.2
98	19.4	8.4
95	22.1	14.1
90	27.0	31.1
85	28.9	37.7
80	31.3	46.1
75	34.0	55.7
70	36.2	63.2
65	38.1	65.9
60	40.9	73.4
55	42.8	76.3
50	44.4	80.5
45	47.6	83.8
40	49.3	85.3
35	51.7	88.9
30	54.8	89.8
25	58.2	91.0
20	62.7	92.5
15	68.1	94.3
10	77.1	96.7
5	99.9	100

Table 4: Clinical Sensitivity and Specificity of Prostate Cancer Cutoffs for Beckman Coulter *phi* in Men with Non-Suspicious DRE (WHO Calibration of PSA and free PSA)

%Clinical Sensitivity	WHO Calibration	
	phi Cutoff	%Clinical Specificity
99	19.7	4.2
98	22.0	9.0
95	24.6	13.5
90	30.2	29.6
85	33.3	39.2
80	36.4	49.4
75	38.8	56.6
70	41.2	62.6
65	43.3	67.7
60	45.9	72.8
55	48.4	76.0
50	50.6	81.1
45	53.6	83.8
40	55.7	85.0
35	58.9	88.3
30	62.0	88.9
25	65.7	91.0
20	71.9	92.8
15	78.4	94.6
10	87.9	96.7
5	114.1	100

In addition, the test with the greatest area under the curve (AUC) has higher clinical sensitivities and clinical specificities, and is generally the better test. The AUC was significantly greater for Beckman Coulter *phi* (0.708) than for PSA (0.516) and %fPSA (0.648) in this cohort of men with PSA between 4 to 10 ng/mL (Hybritech calibration) and a non-suspicious DRE. These data are shown in Table 5 along with the p-values for the AUC relative to the line of chance (AUC = 0.5). The AUC for Beckman Coulter *phi* was statistically different relative to the line of chance with a p-value < 0.001.

Table 5: Comparison of PSA, %fPSA and Beckman Coulter *phi* Area Under the ROC Curve (AUC) (Hybritech Calibration of PSA and free PSA)

	PSA 4–10 ng/mL (n=324 Cancer, n=334 Benign)		
	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	–

The AUC was significantly greater for Beckman Coulter *phi* (0.709) than for PSA (0.519) and %fPSA (0.649) in this cohort of men with PSA between 3.1 to 7.8 ng/mL (WHO calibration) and a non-suspicious DRE. These data are shown in Table 6 along with the p-values for the AUC relative to the line of chance (AUC = 0.5). The AUC for Beckman Coulter *phi* was statistically different relative to the line of chance with a p-value < 0.001.

Table 6: Comparison of PSA, %fPSA and Beckman Coulter *phi*
Area Under the ROC Curve (AUC)
(WHO Calibration of PSA and free PSA)

	PSA 3.10–7.80 ng/mL (n=324 Cancer, n=334 Benign)		
	AUC	95% Confidence Interval	P-value
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	–

Individual Patient Probability of Prostate Cancer on Biopsy

Beckman Coulter *phi* may be used to determine the probability of prostate cancer on biopsy in individual men. Family and patient history can be used in combination with Beckman Coulter *phi* results to determine the best individualized patient management decisions.

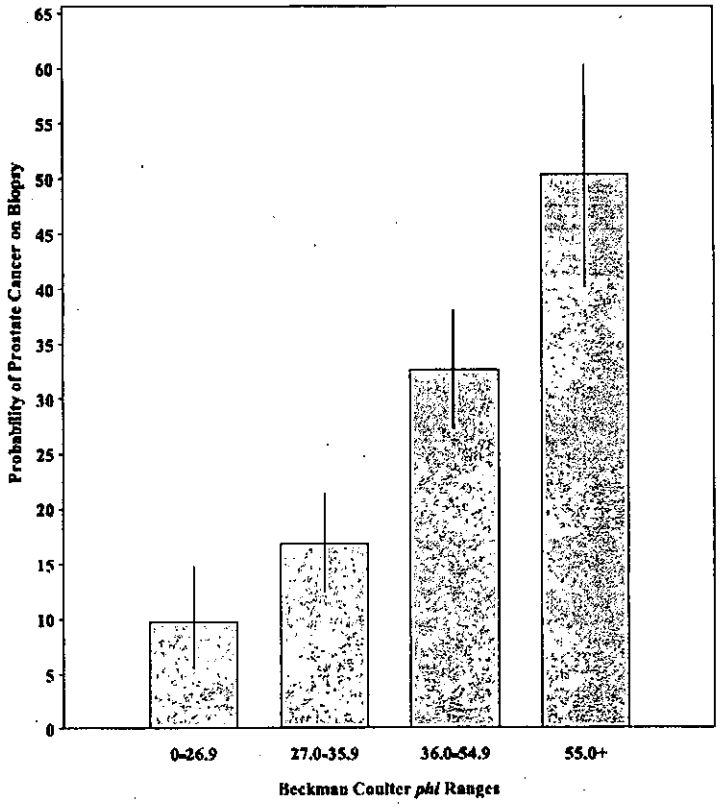
In addition to the sensitivity and specificity analyses of the multi-site study data, we estimated an individual's probability of having detectable cancer based on the Beckman Coulter *phi* values. In a population of men with PSA in the 4.0 to 10.0 ng/mL range and a non-suspicious DRE, a 25% positive biopsy rate has been previously reported.[29,30,31] 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 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 population.[32] 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. We calculated mean cancer probabilities on biopsy and nonparametric 95% confidence intervals (2.5^{th} and 97.5^{th} percentiles). This repetitive sampling method increases the reliability of the probability of prostate cancer on biopsy estimates. Table 7 and Figure 3 (based on the Hybritech calibration) and Table 8 and Figure 4 (based on the WHO calibration) show the probability of detecting prostate cancer on biopsy 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 7: 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%

FIGURE 3
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)

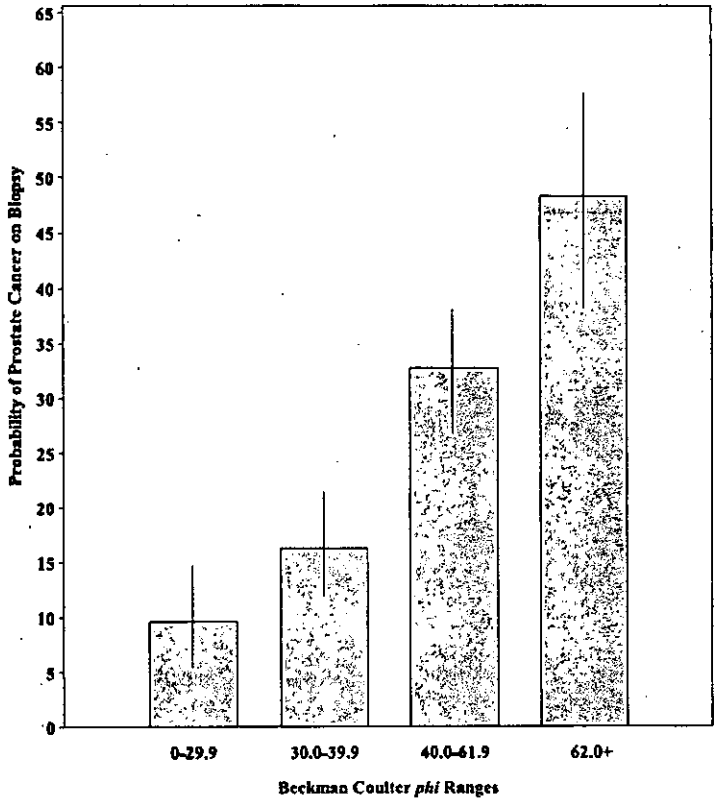


Error bars represent bootstrap estimates of the 95% confidence interval for the probability.

Table 8: 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%

FIGURE 4
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)



Error bars represent bootstrap estimates of the 95% confidence interval for the probability.

Interpretation of Beckman Coulter *phi*

Beckman Coulter *phi* is an In Vitro Diagnostic Multivariate Index Assay (IVDMIA) used in combination with the Access Hybritech PSA, free PSA, and p2PSA assays designed to optimize specificity relative to %fPSA and PSA to determine the probability of prostate cancer on biopsy. Beckman Coulter *phi* has been shown to significantly improve clinical specificity across the range of clinical sensitivity[†] and cancer detection relative to PSA (p-value < 0.001) and %fPSA (p-value = 0.009) in the PSA range of 4 to 10 ng/mL, in men ≥ 50 years of age with non-suspicious DRE. The results for Beckman Coulter *phi* for clinical sensitivity and specificity are summarized in Table 3 and Table 4.

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 prostate cancer on biopsy of cancer 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 7 and Figure 3 show the probability of 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 on biopsy at *phi* < 27.0 is 9.8%. Whereas, the probability of prostate cancer on biopsy at *phi* ≥ 55.0 is 50.1%.

WHO Calibration of PSA and free PSA tests modifies the Beckman Coulter *phi* score:

A PSA range of 4 to 10 ng/mL using Hybritech calibration corresponds to a PSA range of 3.1 to 7.8 ng/mL using WHO calibration. The Beckman Coulter *phi* scores will also be different if the PSA and free PSA tests used to derive the Beckman Coulter *phi* score were WHO calibrated.

Table 8 and Figure 4 show the probability of prostate cancer on biopsy based on categories of Beckman Coulter *phi* scores using the WHO calibration for PSA and free PSA. For example, the probability of prostate cancer on biopsy at *phi* < 30.0 is 9.8%. Whereas, the probability of prostate cancer on biopsy at *phi* ≥ 62.0 is 48.2%.

Important: A PSA range of 4 to 10 ng/mL with the Hybritech calibration corresponds to a PSA range of 3.1 to 7.8 ng/mL with the WHO calibration. PSA and fPSA can only be used in the calculation of Beckman Coulter *phi* if the results were derived from the same type of calibration (Hybritech or WHO). Therefore, never mix Hybritech and WHO PSA and free PSA calibrations when calculating Beckman Coulter *phi*.

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.

†As determined by comparing Areas Under the Curve (AUC) of the Receiver Operating Characteristics (ROC) curves.

Specific Performance Characteristics

Spiking Recovery

A spiking recovery study was performed 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 six male normal human serum patient samples to obtain final [-2]proPSA concentrations targeting approximately 4200, 2000, 750, 250, 75, and 10 pg/mL for each sample.

The percent recovery was calculated as a ratio of the average observed (measured) dose and the expected dose: [(Average Observed dose/Expected dose) x 100]. The overall mean sample recovery of the serum samples is 93% with individual mean sample recoveries ranging from 90% to 96%.

Note: If a sample contains more than the stated value of the highest Access Hybritech p2PSA Calibrator (S6), report the result as greater than that value (i.e., > 5000 pg/mL). Dilution of samples with a value greater than the stated value of the highest Access Hybritech p2PSA Calibrator (S6) is not recommended.