BECKMAN COULTER

CE 2797

ACCESS
Immunoassay Systems

Instructions For Use

Access Hybritech p2PSA [-2]proPSA

REF A49752

FOR PROFESSIONAL USE ONLY

ANNUAL REVIEW

Reviewed by	Date	Reviewed by	Date

PRINCIPLE

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

Access Hybritech p2PSA is a paramagnetic particle, chemiluminescent assay used in combination with the Access Hybritech PSA and free PSA assays to calculate the Beckman Coulter *phi*, a multivariate index, intended to aid the determination of the risk of prostate cancer using human serum on the Access Immunoassay Systems. Beckman Coulter *phi* is used as an aid in distinguishing prostate cancer from benign prostatic conditions in men aged 50 years and older

with total PSA ≥ 2.0 to ≤ 10.0 ng/mL, 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, accounting for approximately 85,000 deaths annually in Europe and the United States.[2] Prostate cancer accounts for approximately 55,000 deaths annually in Europe and 30,000 deaths annually in the United States.

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 risk 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]

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 ≥ 2.0 to ≤ 10.0 ng/mL PSA range was shown to improve the specificity for cancer detection relative to %fPSA alone.[6] The usefulness of [-2]proPSA in men with PSA below 4.0 ng/mL is of particular interest since many cancers exist in this range.[15]

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 ≥ 2.0 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]

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. At 95% clinical sensitivity the clinical specificity for Beckman Coulter *phi* was 18.2% compared to 6.6% for %fPSA for PSA ranging from ≥ 2 ng/mL to ≤ 10 ng/mL. The improvement in clinical specificity for Beckman Coulter *phi* relative to %fPSA represents a substantial advance in testing intended to aid in distinguishing prostate cancer from benign prostatic conditions in men aged 50 years and older with total PSA ≥ 2.0 to ≤ 10.0 ng/mL, with digital rectal examination findings that are not suspicious for cancer.

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

METHODOLOGY

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 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.

SPECIMEN

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.[16]
3. Transrectal needle biopsy has also been shown to cause transient increases in [-2]proPSA, fPSA and PSA elevations,[16,17] thus a six-week waiting period between needle biopsy and [-2]proPSA, fPSA, and PSA sampling has been 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.**[18]
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.[18,19] Specimens to be held for longer than 5 months should be frozen at -70°C.[18,19,20] Repeated freeze-thaw cycles have no effect on free PSA or total PSA,[18] 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.

REAGENTS

PRODUCT INFORMATION

Access Hybritech p2PSA Reagent Pack

Cat. No. A49752: 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 2 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.

*ProClin™ is a trademark of The Dow Chemical Company (“Dow”) or an affiliated company of Dow.

WARNING 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.
- For hazards presented by the product refer to the following sections: REACTIVE INGREDIENTS and GHS HAZARD CLASSIFICATION.

REACTIVE INGREDIENTS

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

p2PSA PMP (Compartment R1a)	WARNING	
		
	H317	May cause an allergic skin reaction.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
p2PSA Blocker Buffer (Compartment R1b)	WARNING	
		
	H317	May cause an allergic skin reaction.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
p2PSA Conjugate (Compartment R1c)	WARNING	
		
	H317	May cause an allergic skin reaction.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

SDS	Safety Data Sheet is available at techdocs.beckmancoulter.com

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

1. Access Hybritech p2PSA Calibrators
   Provided at zero and approximately 10, 20, 50, 100, 500 and 5,000 pg/mL.
   Cat. No. A49753
2. Access Hybritech p2PSA Quality Control (QC) or other commercially available control material.
   Cat. No. A56934
3. Access Substrate
   Cat. No. 81906
4. Access Wash Buffer II, Cat. No. A16792
   UniCel DxI Wash Buffer II, Cat. No. A16793

EQUIPMENT AND MATERIALS

R1	Access Hybritech p2PSA Reagent Packs

CALIBRATION

CALIBRATION INFORMATION

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 commercially available quality control materials that cover at least two levels of analyte.[21] 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.

TESTING PROCEDURE(S)

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.

CALCULATIONS

CALCULATION OF BECKMAN COULTER *phi*

[-2]proPSA values alone 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*.

RESULTS INTERPRETATION

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.

REPORTING RESULTS

EXPECTED RESULTS

Beckman Coulter *phi* is a multifactorial mathematical combination of PSA, fPSA, and [-2]proPSA concentrations designed to optimize clinical sensitivity and specificity to aid in the determination of the risk of prostate cancer.

A multi-center (6 clinical sites) clinical trial with a combination of prospective and retrospective 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, when used in conjunction with Access Hybritech PSA, free PSA, and p2PSA assays as an aid in prostate cancer detection. 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 2 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. Comparable prostate cancer risks of approximately 25% were shown for the 2 to 4 ng/mL and the 4 to 10 ng/mL PSA ranges.[22,23,24,25,26,27]

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 ≥ 2.0 and ≤ 10 ng/mL, ≥ 6 cores TRUS guided needle biopsy and diagnosis was histologically confirmed.

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, PSA ≤ 2.0 or ≥ 10.0 ng/mL.

A total of 519 men participated in the study (233 with prostate cancer and 286 without prostate cancer). Median age for cancer and benign disease subjects was 63 and 62 years, respectively. Table 1.0 and Table 2.0 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 ≥ 2 to ≤ 10 ng/mL with the Hybritech calibration corresponds to a PSA range of ≥ 1.6 to ≤ 7.8 ng/mL with the WHO calibration.

Table 1.0 PSA, fPSA, [-2]proPSA, %fPSA, and Beckman Coulter *phi*
Expected Values by Diagnosis
(Hybritech Calibration of PSA and free PSA)

		Benign	Cancer	Total
PSA (ng/mL)	**Median**	5.09	5.28	5.15
Hybritech	**Mean ± SD**	5.29 ± 1.95	5.35 ± 1.87	5.31 ± 1.91
Calibration	**Range**	1.99-10.04	2.02-9.68	1.99-10.04
fPSA (ng/mL)	**Median**	0.98	0.80	0.90
Hybritech	**Mean ± SD**	1.04 ± 0.51	0.92 ± 0.55	0.99 ± 0.53
Calibration	**Range**	0.26-4.34	0.18-3.91	0.18-4.34
[-2]proPSA	**Median**	12.44	13.41	12.94
(pg/mL)†	**Mean ± SD**	13.84 ± 6.79	16.08 ± 10.30	14.85 ± 8.61
	Range	2.86-43.54	3.98-90.78	2.86-90.78
%fPSA	**Median**	19.38	16.15	17.80
	Mean ± SD	20.33 ± 7.94	17.51 ± 8.05	19.06 ± 8.11
	Range	3.51-53.22	5.37-51.07	3.51-53.22
phi	**Median**	29.42	37.63	32.59
	Mean ± SD	31.81 ± 13.25	43.69 ± 26.64	37.14 ± 21.20
	Range	13.67-94.44	14.03-325.80	13.67-325.80

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

Table 2.0 PSA, fPSA, [-2]proPSA, %fPSA, and Beckman Coulter *phi*
Expected Values by Diagnosis (WHO Calibration of PSA and free PSA)

		Benign	Cancer	Total
PSA (ng/mL)	**Median**	3.99	4.20	4.09
WHO	**Mean ± SD**	4.19 ± 1.52	4.24 ± 1.45	4.21 ± 1.49
Calibration	**Range**	1.57-7.94	1.60-7.63	1.57-7.94
fPSA (ng/mL)	**Median**	0.77	0.63	0.70
WHO	**Mean ± SD**	0.82 ± 0.40	0.73 ± 0.44	0.78 ± 0.42
Calibration	**Range**	0.20-3.51	0.14-3.15	0.14-3.51
[-2]proPSA	**Median**	12.44	13.41	12.94
(pg/mL)†	**Mean ± SD**	13.84 ± 6.79	16.08 ± 10.30	14.85 ± 8.61
	Range	2.86-43.54	3.98-90.78	2.86-90.78

Table 2.0 PSA, fPSA, [-2]proPSA, %fPSA, and Beckman Coulter *phi* Expected Values by Diagnosis (WHO Calibration of PSA and free PSA), Continued

		Benign	Cancer	Total
%fPSA	**Median**	18.96	15.79	17.71
	Mean ± SD	20.07 ± 7.71	17.31 ± 7.94	18.83 ± 7.93
	Range	3.50-49.51	5.06-51.39	3.50-51.39
phi	**Median**	33.18	42.76	37.27
	Mean ± SD	36.12 ± 14.98	49.51 ± 30.53	42.13 ± 24.19
	Range	15.57-106.03	15.52-377.29	15.52-377.29

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

Table 3.0 and Table 4.0 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 2 to 10 ng/mL range for the Hybritech calibration and in the 1.6 to 7.8 ng/mL range for the WHO calibration. Subject age ranged from 50 to 84 years.

Table 3.0 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)

	Hybritech Calibration	
%Clinical Sensitivity	***phi* Cutoff**	**%Clinical Specificity**
99	**17.78**	8.7
98	**18.44**	10.5
95	**21.13**	18.2
90	**23.82**	30.4
88	**25.00**	33.6
85	**26.34**	38.8
80	**27.58**	45.1
75	**29.25**	49.3
70	**30.44**	54.2
65	**31.69**	58.0
60	**33.98**	66.1
55	**36.22**	72.7
50	**37.63**	75.2
45	**39.34**	80.1
40	**42.14**	84.6
35	**45.11**	88.1
30	**47.64**	90.2
25	**50.01**	92.0
20	**55.08**	94.4
15	**59.20**	95.5

Table 3.0 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), Continued

	Hybritech Calibration	
%Clinical Sensitivity	***phi* Cutoff**	**%Clinical Specificity**
10	**68.00**	96.9
5	**87.23**	99.3

Table 4.0 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)

	WHO Calibration	
%Clinical Sensitivity	***phi* Cutoff**	**%Clinical Specificity**
99	**19.96**	8.4
98	**20.57**	9.8
95	**23.45**	16.1
90	**26.93**	28.3
88	**28.09**	31.8
85	**29.98**	40.2
80	**31.57**	45.1
75	**33.34**	50.7
70	**35.01**	55.6
65	**36.90**	59.8
60	**38.79**	66.1
55	**40.63**	71.3
50	**42.76**	76.6
45	**45.03**	80.8
40	**46.97**	82.5
35	**50.94**	88.1
30	**53.84**	90.6
25	**56.30**	92.0
20	**61.85**	93.7
15	**66.51**	95.5
10	**78.42**	97.6
5	**97.73**	99.0

INDIVIDUAL PATIENT RISK ASSESSMENT

Beckman Coulter *phi* may be used to determine the relative risk (probability) of prostate cancer 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 2.0 to 10.0 ng/mL range and a non-suspicious DRE, a 25% positive biopsy rate has been previously reported.[22,23,24,25,26,27] The multi-site study population consisted of approximately 45% (233/519) cancer subjects and 55% (286/519) non-cancer subjects. Cancer probabilities based on the 45% proportion of cancer subjects would inflate the risk 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's population.[28] Each sampling consisted of 286 (75%) benign subjects and 95 (25%) cancer subjects, for a total of 381 subjects. This random sampling process was repeated 1000 times. We calculated mean cancer probabilities (risk estimates) and nonparametric 95% confidence intervals (2.5th and 97.5th percentiles). This repetitive sampling method increases the reliability of the risk estimates. Table 5.0 (based on the Hybritech calibration) and Table 6.0 (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 can be seen, with high Beckman Coulter *phi* values associated with high cancer risk.

Table 5.0 Probability (Risk Assessment) of Prostate Cancer for Beckman Coulter *phi* in Patients with PSA between 2 and 10 ng/mL (Hybritech Calibration of PSA and free PSA)

Beckman Coulter *phi* Range (Hybritech Calibration)	Probability of Cancer	95% Confidence Interval
0-21	8.4%	1.9-16.1%
21-40	21.0%	17.3-24.6%
40+	44.0%	36.0-52.9%

Table 6.0 Probability (Risk Assessment) of Prostate Cancer for Beckman Coulter *phi* in Patients with PSA between 1.6 and 7.8 ng/mL (WHO Calibration of PSA and free PSA)

Beckman Coulter *phi* Range (WHO Calibration)	Probability of Cancer	95% Confidence Interval
0-23	8.7%	2.0-17.0%
23-45	20.6%	17.1-24.1%
45+	43.8%	35.8-52.2%

Interpretation of Beckman Coulter *phi*

Beckman Coulter *phi* is a multifactorial mathematical combination of PSA, fPSA, and [-2]proPSA concentrations designed to optimize clinical sensitivity and specificity to aid in the determination of the risk of prostate cancer. 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 = 0.010) in the PSA range of 2 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 3.0 and Table 4.0.

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 25 corresponds to 88% clinical sensitivity and 33.6% clinical specificity. Therefore, approximately 1 in 3 men may avoid prostate biopsy while detecting 88% of cancers if their Beckman Coulter *phi* value is less than 25. For men with a Beckman Coulter *phi* value above 25 the risk of cancer increases and may affect the clinical management of each patient.

Low Beckman Coulter *phi* scores are associated with a lower risk of having cancer and higher scores are associated with an increased risk of having cancer. 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 5.0 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.

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

A PSA range of 2 to 10 ng/mL using Hybritech calibration corresponds to a PSA range of 1.6 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. Therefore, approximately 1 in 3 men may avoid biopsy if their Beckman Coulter *phi* score is 28.1 or less. Table 6.0 shows the probability of finding prostate cancer on biopsy based on categories of Beckman Coulter *phi* scores using the WHO calibration for PSA and free PSA.

Important: A PSA range of 2 to 10 ng/mL with the Hybritech calibration corresponds to a PSA range of 1.6 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.

PROCEDURAL NOTES

LIMITATIONS

1. 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 5,000 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”).
   - 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., "> 5,000 pg/mL").

     Note: Dilution of samples with a value greater than the stated value of the highest Access Hybritech p2PSA Calibrator (S6) is not recommended.
2. 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 material should be included with all patient sample testing. Include Access Hybritech p2PSA QC or other commercially available quality control materials that cover at least 2 levels of analyte.
3. 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.[29,30] Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.

4. Normal physiological total protein levels range from 6 to 8 g/dL.[31] 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.
5. 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.
6. The Access Hybritech p2PSA assay does not demonstrate any “hook” effect up to 15,000 pg/mL [-2]proPSA.
7. 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.

PERFORMANCE CHARACTERISTICS

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 4,200, 2,000, 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., > 5,000 pg/mL). Dilution of samples with a value greater than the stated value of the highest Access Hybritech p2PSA Calibrator (S6) is not recommended.

IMPRECISION

Access Hybritech p2PSA exhibits total imprecision of < 20% at [-2]proPSA concentrations between the Limit of Quantitation (LOQ) of 3.23 pg/mL and 10 pg/mL, and ≤ 10% at [-2]proPSA concentrations ≥ 10 pg/mL.

In one study listed in Table 7.0, reproducibility of Access Hybritech p2PSA was determined by assaying eight controls containing [-2]proPSA (six of which were serum based). In this study, data were collected over 20 days and analyzed based on CLSI EP5-A2[32] guidelines.

Table 7.0 Imprecision of Access Hybritech p2PSA

Sample	Mean (pg/mL)	Within Run SD (pg/mL)	Within Run CV (%)	Total SD (pg/mL)	Total CV (%)
1	22.75	0.70	3.08	1.09	4.80
2	106.64	4.08	3.83	6.35	5.95
3	8.63	0.42	4.90	0.53	6.11
4	38.46	1.40	3.65	1.82	4.74
5	108.21	3.07	2.84	5.04	4.66
6	1,179.71	36.53	3.10	52.58	4.46
7	2,899.48	64.08	2.21	105.08	3.62
8	4,748.60	112.06	2.36	139.78	2.94

ANALYTICAL SPECIFICITY / INTERFERENCES

Cross-reactivity with a mixture of PSA isoforms (PSA-ACT, fPSA, [-4]proPSA,[-5/-7]proPSA, and BPSA) at proportions reported in the literature[33] was determined to be less than or equal to 5%.

Serum samples containing up to 500 mg/dL (5 g/L) hemoglobin, 20 mg/dL (0.2 g/L) bilirubin, 1,500 mg/dL (15 g/L) triglycerides, and a total protein concentration of 6.2 g/dL (62 g/L) do not interfere with the Access Hybritech p2PSA assay.

Note: Normal physiological total protein levels range from 6 to 8 g/dL.[31] 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.

Various concentrations of drugs were added to serum samples containing [-2]proPSA and assayed in replicates of five. The drugs and the concentrations tested are provided in Table 8.0. At the concentrations listed, these drugs did not interfere with the recovery of [-2]proPSA from the serum samples.

Table 8.0 Drug Interference Testing

Drug	Concentration
Acetaminophen	0.2 mg/mL
Acetylsalicylic acid	0.5 mg/mL
Alfuzosin (Uroxatral)	19 ng/mL
Bicalutamide (Casodex)	35 µg/mL
Biotin	50 ng/mL
Captopril	5 µg/mL
Cimetidine	0.1 mg/mL
Ciprofloxacin	46 µg/mL
Cisplatin	10 µg/mL
Clomipramine	2.7 µg/mL
Cyclophosphamide	0.33 mg/mL
Docetaxel (Taxotere)	5.5 µg/mL
Doxazosin (Cardura)	40 ng/mL
Doxorubicin Hydrochloride	6.6 µg/mL

Table 8.0 Drug Interference Testing, Continued

Drug	Concentration
Doxycyline hyclate	2.6 µg/mL
Dutasteride (Avodart)	40 ng/mL
Estramustine phosphate sodium (Emcyt, Estracyte)	81.7 µg/mL
Etoposide	14 µg/mL
Finasteride	370 ng/mL
Fluoxetine hydrochloride	300 ng/mL
Flutamide (Eulexin)	78 µg/mL
Furosemide	20 µg/mL
Goserelin acetate (Zoladex)	2.6 ng/mL
Heparin	8,000 units/dL
Hydrocodone bitartrate	240 ng/mL
Ibuprofen	0.4 mg/mL
Ketoconazole (Nizoral)	6.2 µg/mL
Leuprolide acetate (Lupron, Viadur, Eligard)	8 ng/mL
Lovastatin	270 ng/mL
Megestrol acetate (Megace)	39.6 µg/mL
Methotrexate	13.2 µg/mL
Metoprolol tartrate	2.7 µg/mL
Multivitamin (Centrum)	1:20 dilution
Naproxen Sodium	1 mg/mL
Nifedipine	270 ng/mL
Nilutamide	8 µg/mL
Nilutamide (Nilandron)	8 µg/mL
Paclitaxel	0.85 mg/mL
Prazosin	85 ng/mL
Prednisone	1.65 µg/mL
Sildenafil citrate	0.2 mg/mL
Sulphamethoxazole	117 µg/mL
Tamsulosin (Flomax)	55 ng/mL
Terazosin (Hytrin)	1.45 mg/mL
Trimethoprim	23.4 µg/mL
Triptorelin (Trelstar)	28 ng/mL
Vinblastin (Velban)	2 µg/mL
Zometa	667 ng/mL

ANALYTICAL SENSITIVITY

Limit of Blank (Analytical Sensitivity)

Limit of Blank (LOB) for Access Hybritech p2PSA was determined to be 0.50 pg/mL. LOB was tested using a protocol based on CLSI EP17-A.[34] A total number of 148 replicates of a zero analyte sample (Access Hybritech p2PSA Calibrator S0) were measured in 12 runs. The 95th percentile of the 148 replicates was estimated using a non-parametric approach. The 95% upper confidence limit of this estimate was determined as LOB.

FUNCTIONAL SENSITIVITY

Limit of Quantitation (Functional Sensitivity)

Limit of Quantitation (LOQ) for Access Hybritech p2PSA was determined to be 3.23 pg/mL (upper 95% CI concentration). LOQ was tested using a protocol based on CLSI EP17-A.[34] In total, 60 replicates of each of seven samples (420 total) were measured for the LOQ determination.

LOQ was determined by calculating the mean and total percent CV of each sample. These results underwent a log transformation and polynomial regression was used to determine the best line fit. The lowest concentration of [-2]proPSA with a 20% CV and 95% CI were determined from that regression plot.

COMPARISON OF ACCESS IMMUNOASSAY SYSTEMS

The Deming regression statistics for the Access Hybritech p2PSA assay on the Access Immunoassay Systems are provided in Table 9.0.

Table 9.0 Deming Regression Statistics for Access Hybritech p2PSA on the Access Immunoassay Systems

Access Systems	n	Range of Observations (pg/mL)	Intercept	Slope (95% CI)	Correlation Coefficient r^2
Access 2 v. UniCel DxI 800	166	5-5,000	10.72	1.02 (1.01 to 1.03)	1.00
Access 2 v. UniCel DxI 600	166	5-5,000	7.09	1.03 (1.02 to 1.04)	1.00
UniCel DxI 800 v. UniCel DxI 600	166	5-5,000	-3.71	1.01 (1.00 to 1.01)	1.00

ADDITIONAL INFORMATION

U.S. Patent No. 7,288,636, U.K. Patent No. 0981629, U.K. Pat. 1392719, AU Patent No. 739546.

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****Androgel is a trademark of Unimed Pharmaceuticals.

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

May be covered by one or more pat. -see www.beckmancoulter.com/patents.

REVISION HISTORY

Revision N

IFU updated to add Dutch, Finnish, Macedonian, Traditional Chinese, and Estonian

SYMBOLS KEY

Glossary of Symbols is available at techdocs.beckmancoulter.com (document number C02724)

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