Access®
Immunoassay Systems

BECKMAN COULTER®

Hybritech® PSA
REF 37200

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 The concentration of 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 include the identity of the PSA assay used. Values obtained with different assay methods cannot be used interchangeably. If, in the course of monitoring a patient, the assay method used for determining PSA levels serially is changed, additional sequential testing should be carried out to confirm baseline values.

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

Intended Use The Access Hybritech PSA assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of total prostate specific antigen (PSA) levels in human serum using the Access Immunoassay Systems. This device is indicated for the measurement of serum PSA in conjunction with digital rectal examination (DRE) as an aid in the detection of prostate cancer in men aged 50 years or older. Prostate biopsy is required for the diagnosis of cancer. This device is further indicated for the serial measurement of PSA to aid in the prognosis and management of patients with prostate cancer.

Summary and Explanation Prostate cancer is the most common type of cancer found in men in the United States, with an incidence of approximately one case for every ten men.[2] It is also the second leading cause of cancer deaths among American men.[2] A reliable test for detecting early stage prostate cancer, when the tumor is confined to the gland and effective treatment can be provided, can be of great value to the physician.[3] Historically, a majority of prostate cancers had advanced beyond the gland at the time of diagnosis.[4] The digital rectal examination (DRE) is a commonly used technique for prostate cancer detection; nevertheless DRE, as it is generally performed in medical practice, misses a significant number of cancers, including many organ-confined tumors.[4,5,6]

A multicenter, prospective clinical study of 6,374 men has provided additional information about the use of Hybritech PSA and DRE in the identification of men with prostate cancer.[7] A summary of the results of this study follows in the "Expected Values" section.

Other clinical applications have been clearly demonstrated for PSA. When employed for the management of prostate cancer patients, serial measurement of PSA is useful in detecting residual tumor and recurrent cancer after radical prostatectomy.[8] Moreover, PSA may serve as an accurate marker for monitoring advancing clinical stage in untreated patients,[9] as well as assessing response to therapy.[10,11,12,13] Therefore, serial measurement of PSA concentrations can be an important tool in monitoring patients with prostate cancer and in determining the potential and actual effectiveness of surgery or other therapies. Other biochemical markers

such as prostatic acid phosphatase (PAP) and carcinoembryonic antigen (CEA) lack sufficient specificity for monitoring disease, and are unsuited for detecting early stage prostate cancer.[14]

Prostate specific antigen (PSA) was identified and purified by Wang and co-workers in 1979.[15] PSA is a single chain glycoprotein with a molecular weight of approximately 34,000 daltons, containing 7% carbohydrate by weight.[15] PSA exists primarily as three forms in serum.[16] One form of PSA is believed to be enveloped by the protease inhibitor, alpha-2 macroglobulin[16] and has been shown to lack immunoreactivity. A second form is complexed to another protease inhibitor, alpha-1 antichymotrypsin (ACT).[16,17,18] The third form of PSA is not complexed to a protease inhibitor, and is termed free PSA.[16,17,18] The latter two forms are immunologically detectable in commercially available PSA assays and are referred to collectively as total PSA. The relative concentrations of the two detectable forms within and between patient samples is variable and unknown.[19] However, it has been reported that the concentration of free PSA usually ranges from 5 to 50% of the total PSA in serum.[20] Additional studies have also shown that various immunoassays react differently to these two forms in serum.[19,20] Specifically, there are two distinct types of immunoassays, based upon their relative response to PSA forms. Equimolar-response assays detect the free and complexed forms of PSA equally; non-equimolar or skewed-response assays have been shown to produce two to three times more signal per free PSA molecule than with PSA-ACT. The Access Hybritech PSA assay is an equimolar assay in which sample recovery is unaffected by the ratio of PSA forms in serum. Therefore, the reported result is not changed by the relative concentrations of free PSA and PSA-ACT in the sample. Results generated by the Access Hybritech PSA assay cannot be applied to other manufacturers' assays.

Immunohistochemical studies have shown that PSA is found predominantly in the cytoplasm of prostatic acinar cells and ductal epithelium.[21] PSA is present in normal, benign hyperplastic, and malignant prostatic tissue, and also in prostatic fluid and seminal plasma.[22] PSA has not been detected in cancers of the lung, colon, rectum, stomach, pancreas or thyroid.[23] Purified PSA lacks any acid phosphatase activity and does not react with antibodies against PAP and vice versa.[24] Therefore, it is biochemically and immunologically distinct from PAP.

Elevated serum PSA concentration can only suggest the presence of prostate cancer until a biopsy is performed. Serum PSA concentrations can also be elevated in benign prostatic hypertrophy or inflammatory conditions of the prostate and other adjacent tissues. PSA is generally not elevated in apparently healthy men or men with non-prostatic carcinoma. Physicians should discuss the risks and benefits of PSA testing with their patients.

A PSA standard (90% PSA-ACT and 10% free PSA) was proposed in the mid-1990s, with the intent to mitigate the non-equimolar response of some PSA assays. This material is prepared from human seminal plasma that is assigned using a molar extinction coefficient different from the original Hybritech Tandem PSA standard. Over time, the original intent to establish an "Equimolarity-Standard" evolved into adoption of WHO 96/670 as a new "Mass-Standard" for PSA.[25] Calibration to the First International Standard for PSA, (WHO 96/670), results in a ~ 20% dose shift across the curve relative to the Hybritech calibration. The clinical PSA cutoff (4.0 ng/mL) is based on the Hybritech calibration. Calibration to the WHO 96/670, using an adjusted cutoff of 3.1 ng/mL correlates results to the original Hybritech Tandem assay clinical performance.

PSA values from 0.00 to 20.0 ng/mL obtained with the Hybritech calibration and the corresponding expected values for the WHO 96/670 calibration are provided in the following conversion table.

Hybritech Calibration and WHO Calibration PSA Values

Hybritech Calibration PSA Value (ng/mL)	WHO Calibration PSA Value (ng/mL)	Description
0.00	0.00	Not Applicable
0.35	0.30	PSA velocity to trigger biopsy if PSA < 4.0 ng/mL[26,27]
0.75	0.64	PSA velocity suspicious for prostate cancer if PSA 4.0–10.0 ng/mL[26,27]
2.0	1.6	PSA velocity for aggressive prostate cancer[26,28]
2.5	2.0	Total PSA value to trigger biopsy[28,29,30]
4.0	3.1	Total PSA value to trigger biopsy[7]
10.0	7.8	Upper end of threshold for biopsy[7]
20.0	15.6	Prostate cancer risk stratification[31,32]

Principles of the Procedure

The Access Hybritech PSA assay is a two-site immunoenzymatic ("sandwich") assay. A sample is added to a reaction vessel with mouse monoclonal anti-PSA alkaline phosphatase conjugate, and paramagnetic particles coated with a second mouse monoclonal anti-PSA antibody. The PSA in the sample binds to the immobilized monoclonal anti-PSA on the solid phase while, at the same time, the monoclonal anti-PSA alkaline phosphatase conjugate reacts with a different antigenic site on the sample PSA. 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 PSA in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

Product Information

Access Hybritech PSA Reagent Pack

Cat. No. 37200: 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.

R1a:	Paramagnetic particles coated with mouse monoclonal anti-PSA suspended in TRIS buffered saline, with surfactant, bovine serum albumin (BSA), < 0.1% sodium azide, and 0.1% ProClin** 300.
R1b:	Mouse monoclonal anti-PSA alkaline phosphatase (bovine) conjugate diluted in phosphate buffered saline, with surfactant, BSA, protein (mouse), < 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.[33]
- 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. Serum is the recommended sample. Plasma samples should **not** be used.
2. Specimens for PSA testing should be drawn prior to such prostatic manipulations as digital rectal exam (DRE), prostatic massage, transrectal ultrasound (TRUS), and prostatic biopsy. DRE may cause a transient increase in serum PSA levels.[34] A repeat PSA measurement in the case of borderline elevation has been recommended.[35] Transrectal needle biopsy has also been shown to cause persisting PSA elevations.[35] Thus, a 6 week waiting period between needle biopsy and PSA sampling has been recommended.
3. 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.
4. The specimen should be allowed to clot fully and the serum separated by centrifugation.
5. **If the specimens will be potentially used for free PSA testing, it should be processed (centrifuged) and refrigerated within 3 hours of blood draw.**[36]
6. 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.[36,37] Specimens to be held for longer than 5 months should be frozen at -70°C.[36,37,38] Repeated freeze-thaw cycles have no effect on free PSA, total PSA, or percent free PSA.[36] However, prompt refreezing of the thawed samples is recommended.
7. Turbid serum samples or samples containing particulate matter should be centrifuged prior to assay.
8. 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.
9. 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 PSA Reagent Packs

Materials Required But Not Provided

1. Access Hybritech PSA Calibrators
   Cat. No. 37205
   Two options for calibration are provided with the Access Hybritech PSA Calibrators, Hybritech calibration or WHO calibration.
   Hybritech calibration: concentrations are zero and approximately 0.5, 2.0, 10, 75 and 150 ng/mL
   WHO calibration: concentrations are zero and approximately 0.4, 1.7, 8, 58 and 121 ng/mL

2. Access Hybritech PSA Quality Control (QC) or other commercially available control material.
   Cat. No. 37209
   Access Hybritech PSA QC is provided with two sets of ranges, a Hybritech calibration range and a WHO calibration range.
   Hybritech calibration: concentrations are approximately 1.0, 15 and 90 ng/mL
   WHO calibration: concentrations are approximately 0.8, 12 and 73 ng/mL
3. Access Hybritech PSA Sample Diluent
   Cat. No. 37206
4. Access Substrate
   Cat. No. 81906
5. Access Wash Buffer
   Cat. No. 81907 (Access, Access 2, SYNCHRON LX®i, UniCel® DxC 600i)
   Cat. No. 8547197 (UniCel DxI)

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 twenty five (25) 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 ng/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 PSA 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.

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

Quality Control

Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of immunochemical assays. Because samples can be processed at any time in a "random access" format rather than a "batch" format, quality control materials should be included in each 24-hour time period.[39] Include Access Hybritech PSA QC or other commercially available quality control materials that cover at least two levels of analyte. Access Hybritech PSA QC is provided with two sets of ranges, a Hybritech calibration range and a WHO Calibration range. The QC range must correspond to the calibration used. 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 test results are determined automatically by the system software using a weighted four parameter logistic curve (4PLC) math model. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient test 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.

Limitations of the Procedure

1. Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value (approximately 0.008–150 ng/mL Hybritech calibration or 0.008 – 121 ng/mL WHO calibration).
   - If a sample contains less than the lower limit of detection for the assay, report the results as less than that value (i.e., < 0.008 ng/mL for both Hybritech and WHO calibration).
   - If a sample contains more than the stated value of the highest Access Hybritech PSA Calibrator (S5), report the result as greater than that value (i.e., > 150 ng/mL Hybritech calibration or > 121 ng/mL WHO calibration). Alternatively, dilute one volume of sample with 4 or 9 volumes of Access Hybritech PSA Sample Diluent. Refer to the appropriate system manuals and/or Help system for instructions on entering a sample dilution in a test request. The system reports the results adjusted for the dilution.
2. 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.[40,41]
   Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
3. The Access Hybritech PSA 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. Serum PSA concentrations should not be interpreted as absolute evidence for the presence or absence of prostate cancer. Elevated concentrations may be observed in the serum of patients with benign prostatic hyperplasia or other non-malignant disorders, as well as in prostate cancer. Furthermore, low concentrations are not necessarily indicative of the absence of cancer. Serum PSA values should be used in conjunction with information available from the clinical evaluation of the patient and other diagnostic procedures such as 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. In monitoring previously-diagnosed prostate cancer patients, predictions of disease recurrence should not be based solely on values obtained from serial PSA serum values.
4. The Access Hybritech PSA assay does not demonstrate any "hook" effect up to 50,000 ng/mL with both Hybritech calibration and WHO calibration.
5. The safety and effectiveness of using a cutoff value other than 4.0 ng/mL with Hybritech calibration or 3.1 ng/mL with WHO calibration has not been established.
6. The 5 alpha-reductase inhibitor drugs may affect PSA levels in some 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.
7. PSA concentrations are dependent on the standard used to calibrate the assay. PSA concentrations based on calibration to the WHO 96/670 Reference Preparation will differ significantly from PSA concentrations based on calibration to the original Hybritech Tandem-R assay. The concentrations are not interchangeable. If the calibration is changed, accepted laboratory practice is to establish a new baseline for patient monitoring.[1]

Expected Values

Expected Values for Detection of Prostate Cancer

A multicenter, prospective clinical trial was conducted to test the effectiveness of PSA along with digital rectal examination (DRE) as an aid in the detection of prostate cancer.[7] A total of 6,374 men 50 years of age and older participated in the study. Although the PSA results in this trial were generated with the Hybritech Tandem PSA assay, the Access Hybritech PSA assay has been developed using the same monoclonal antibodies employed in the Hybritech Tandem PSA assay and has been standardized to provide the same clinical performance. The WHO calibration was established based on the First International Standard for PSA (WHO 96/670), and is matched to the Hybritech Tandem standardization (Hybritech calibration) by proportional adjustments to provide the same clinical performance as the Hybritech calibration in the Access Hybritech PSA assay.

This study demonstrated that the majority (72% or 93/130) of cancers detected by PSA and DRE were organ-confined (Stages A or B). This study also demonstrated that PSA testing, when used in conjunction with DRE, was more effective in detecting prostate cancer than DRE alone. Cancer was present in 21% (126/588) of symptomatic subjects with an elevated PSA and /or suspicious DRE, and in 23% (104/452) of asymptomatic subjects with an elevated PSA and/or suspicious DRE. PSA determinations detected 41% (94/230) of cancers that DRE did not; PSA elevations greater than 4[h] ng/mL may warrant additional testing even if the DRE is negative. However, the converse is also true; a subject with a suspicious DRE and a normal PSA may also require additional testing since DRE detected 21% (48/230) of cancers that PSA determinations did not. The study also demonstrated that the majority (68% or 69/102) of cancers detected by PSA when the concentration was above 4[h] ng/mL were organ-confined (Stages A or B). A summary of the study results is provided in Table 1.

[h] Data are based on Hybritech Tandem calibration with a cutoff of 4.0 ng/mL. The corresponding cutoff based on WHO calibration is 3.1 ng/mL.

Table 1: Summary Table of Clinical Trial Results[h] (Number of Subjects Tested = 6,374)

	No. of Subjects n (%)	No. of Biopsies n	No. of Cancers n	% Positive Biopsies (95% CI)*	No. of Prostatec-tomies n	No. of Pathologic Stage Reports n	No. of Organ-confined (Stage A or B) Cancers n (%)	No. of Advanced (Stage C or D) Cancers n (%)
All Subjects	6,374 (100%)	1,040	230	22 (19.6–24.6)	135	130	93 (72%)	37 (28%)
PSA > 4.0	923 (14%)	594	182	31 (26.9–34.4)	104	102	69 (68%)	33 (32%)
DRE +	946 (15%)	626	136	22 (18.5–25.0)	83	78	53 (68%)	25 (32%)
PSA ≤ 4.0DRE-	4,750 (75%)	0	N/A	N/A	N/A	N/A	N/A	N/A
PSA > 4.0DRE-	678 (11%)	414	94	23 (18.7–26.8)	52	52	40 (77%)	12 (23%)
PSA ≤ 4.0DRE+	701 (11%)	446	48	11 (7.9–13.6)	31	28	24 (86%)	4 (14%)
PSA > 4.0DRE+	245 (4%)	180	88	49 (41.6–56.2)	52	50	29 (58%)	21 (42%)

Key: PSA measured in (ng/mL)
+ Suspicious for Cancer
– Not suspicious for Cancer
DRE: Digital Rectal Examination
N/A: Not Available – Not Part of Study Protocol
* 95% Confidence Interval (Lower limit – Upper Limit)

[h] Data are based on Hybritech Tandem calibration with a cutoff of 4.0 ng/mL. The corresponding cutoff based on WHO calibration is 3.1 ng/mL.

Table 2 contains the distribution of PSA values by age for those asymptomatic subjects in the clinical study who had both a negative PSA and a non-suspicious DRE and therefore were not biopsied, as well as for those subjects who were negative for cancer at biopsy. There is no

certainty that all of these subjects were indeed free of prostate disease. Therefore, these data should be interpreted with caution since it is questionable whether these subjects represent a truly normal population. There are presently no data proving that the use of age-specific reference ranges is safe or effective.

Table 2: % Distribution of PSA (ng/mL) by Age for Apparently Healthy, Asymptomatic Subjects[h]

Age (years)	Number of Subjects	PSA Concentration (ng/mL)			
		0–4.0		> 4.0	
		%	(n)	%	(n)
50–59	1,273	97	(1,240)	3	(33)
60–69	1,120	92	(1,032)	8	(88)
70–79	298	90	(268)	10	(30)
> 80	30	90	(27)	10	(3)
TOTAL	2,721	94	(2,567)	6	(154)

[h] Data are based on Hybritech Tandem calibration with a cutoff of 4.0 ng/mL. The corresponding cutoff based on WHO calibration is 3.1 ng/mL.

Of the 6,374 subjects studied, 1,040 were biopsied based on elevated PSA (> 4.0[h] ng/mL) or a suspicious DRE. The percentage of biopsied subjects with cancer corresponding to PSA and DRE results are shown in Table 3.

Table 3: Percent of Biopsied Subjects with Cancer Corresponding to Test Results[h]

Results Category		Percent of Biopsied Subjects with Cancer %	(95% CI)*	Number of Biopsied Subjects with Cancer
PSA > 4.0		31	(26.9–34.4)	182/594
DRE +		22	(18.5–25.0)	136/626
PSA ≤ 4.0	DRE +	11	(7.9–13.6)	48/446
PSA > 4.0	DRE +	49	(41.6–56.2)	88/180
PSA ≤ 4.0	DRE –	N/A		N/A
PSA > 4.0	DRE –	23	(18.7–26.8)	94/414

*95% Confidence Interval (Lower Limit – Upper Limit)

[h] Data are based on Hybritech Tandem calibration with a cutoff of 4.0 ng/mL. The corresponding cutoff based on WHO calibration is 3.1 ng/mL.

The effectiveness of PSA and DRE in detecting organ-confined cancers (Stage A or B) is demonstrated in Table 4.

Table 4: Detection of Organ-Confined Cancer[h]

		PSA		
		POSITIVE (> 4.0 ng/mL)	NEGATIVE (0–4.0 ng/mL)	TOTAL
DRE	POSITIVE	29 (31.2%)	24 (25.8%)	53 (57%)
	NEGATIVE	40 (43.0%)	0 (0%)	40 (43%)
	TOTAL	69 (74%)	24 (26%)	93 (100%)

[h] Data are based on Hybritech Tandem calibration with a cutoff of 4.0 ng/mL. The corresponding cutoff based on WHO calibration is 3.1 ng/mL.

Serum PSA concentrations, regardless of value, should not be interpreted as definitive evidence for the presence or absence of prostate cancer. In addition, PSA testing should be done in

conjunction with DRE, because PSA and DRE together detected the greatest number of cancers. Other clinically acceptable tests and procedures should also be considered in the diagnosis of cancer and good patient management. Prostatic biopsy is required for diagnosis of cancer.

Expected Values for Prognosis and Management

The relative distribution of PSA concentrations in healthy subjects, patients with prostatic carcinoma, and patients with non-malignant diseases is presented in Table 5. In this study, 99% of the healthy men had PSA concentrations of 4.0[h] ng/mL or less. The "Other" classification in the Cancerous category consists of leukemia, bone carcinoma, liver carcinoma, skin carcinoma, and a variety of other cancerous diseases. The "Misc. Genitourinary" classification in the Non-Cancerous category includes patients with the following diseases: renal, orchitis, prostatitis, urethritis, and other genitourinary diseases.

Table 5: % Distribution of PSA (ng/mL)[h]

Clinical Category	n	0–4.00 (ng/mL)	4.01–10.0 (ng/mL)	10.01–20.0 (ng/mL)	20.01–40 (ng/mL)	> 40 (ng/mL)
Healthy Subjects						
Men < 40 yrs.	265	100	0	0	0	0
Men ≥ 40 yrs.	207	97	3	0	0	0
Total Men	472	99	1	0	0	0
Women	388	100	0	0	0	0
TOTAL	860	99	1	0	0	0
Cancerous Subjects						
Prostate						
Stage A	70	37	33	13	6	11
Stage B	90	29	21	12	8	30
Stage C	128	19	9	10	13	49
Stage D	265	12	9	11	9	59
Total Prostate	553	19	14	11	10	46
Gastrointestinal	187	95	5	0	0	0
Genitourinary	323	98	2	0	0	0
Mammary	91	99	1	0	0	0
Pulmonary	147	95	5	0	0	0
Renal	54	96	4	0	0	0
Other	114	95	5	0	0	0
TOTAL	1469	68	7	4	4	17
Non Cancerous Diseases						
Benign Prostate Hypertrophy	352	80	18	2	< 1	0
Misc. Genitourinary	408	93	7	0	0	0
Other	394	98	2	0	0	0
TOTAL	1154	91	8	< 1	< 1	0

[h] Data are based on Hybritech Tandem calibration with a cutoff of 4.0 ng/mL. The corresponding cutoff based on WHO calibration is 3.1 ng/mL.

Specific Performance Characteristics

Dilution Recovery (Linearity)

Ten serum samples containing elevated PSA concentrations were diluted with the Access Hybritech PSA Sample Diluent and assayed in quadruplicate at multiple dilutions. Observed PSA concentrations versus expected concentrations were analyzed by linear regression. The correlation coefficients (r) varied between 0.9996 and 1.000.

Spiking Recovery

Concentrations of PSA spanning the range of the assay were spiked into each of five normal male sera to obtain four spiked levels for each serum. The PSA concentrations were measured in the spiked sera. The percent recovery was calculated as (observed concentration/expected concentration) x 100%. The mean recoveries of the five sera ranged from 96.9% to 101.7% with an average mean recovery of 98.5% for the Hybritech calibration. The mean recoveries of the five sera ranged from 96.6% to 101.6% with an average mean recovery of 98.2% for the WHO calibration.

Imprecision

This assay exhibits total imprecision of less than or equal to 7% at concentrations greater than 1.4 ng/mL, and SD less than or equal to 0.1 ng/mL at concentrations less than or equal to 1.4 ng/mL for Hybritech and WHO calibration. Reproducibility of the Access Hybritech PSA assay was determined in one study by assaying three human based PSA controls in triplicate across 40 runs using the UniCel DxI Access Immunoassay System. The data presented were calculated based on NCCLS EP5-A guidelines.

Table 6: Imprecision[h] with the Hybritech Calibration

Sample	Grand Mean (n=132) (ng/mL[h])	Within Run (SD)	Within Run (%CV)	Total Imprecision (%CV)
1	0.98	0.04	4.53	5.17
2	5.04	0.21	4.10	4.41
3	37.67	1.46	3.89	4.20

[h] Data are based on Hybritech Tandem calibration with a cutoff of 4.0 ng/mL. The corresponding cutoff based on WHO calibration is 3.1 ng/mL.

Table 7: Imprecision with the WHO Calibration

Sample	Grand Mean (n=132) (ng/mL)	Within Run (SD)	Within Run (%CV)	Total Imprecision (%CV)
1	0.79	0.04	4.44	5.07
2	3.95	0.16	4.04	4.34
3	28.99	1.13	3.91	4.22

Analytical Specificity / Interferences

Samples containing up to 500 mg/dL (5 g/L) hemoglobin, 20 mg/dL (0.2 g/L) bilirubin, 1500 mg/dL (15 g/L) triglycerides, and total protein concentrations of 4.2–12.1 g/dL (42–121 g/L) do not affect the concentration of Access Hybritech PSA assayed.

Various concentrations of drugs were added to serum samples containing PSA and assayed in quadruplicate. The drugs and the highest concentrations tested are listed below. At the concentrations listed, these drugs did not interfere with the recovery of PSA from the serum samples.

Table 8: Drug Interference Testing (Commonly Used Drugs)

Drug	Concentration	Drug	Concentration
acetaminophen	0.2 mg/mL	goserelin acetate	2.5 ng/mL
aspirin	0.5 mg/mL	hydrocodone bitartrate	240 ng/mL
biotin	50 ng/mL	ibuprofen	0.4 mg/mL
captopril	4 µg/mL	leuprolide acetate	8 ng/mL
cimetidine	0.1 mg/mL	lovastatin	270 ng/mL
ciprofloxacin	46 µg/mL	megesterol acetate	39.6 µg/mL
clemastine fumarate	2.7 µg/mL	methotrexate	13.2 µg/mL
clomipramine hydrochloride	2.7 µg/mL	metoprolol tartrate	2.7 µg/mL
cyclophosphamide	0.33 mg/mL	naproxen sodium	1 mg/mL

Table 8: Drug Interference Testing (Commonly Used Drugs)

Drug	Concentration	Drug	Concentration
doxorubicin hydrochloride	6.6 µg/mL	nifedipine	270 ng/mL
doxycycline hyclate	2.6 µg/mL	paclitaxel	0.85 mg/mL
estramustine phosphate solution	81.7 µg/mL	prednisone	1.65 µg/mL
finasteride	370 ng/mL	sildenafil	0.2 mg/mL
fluoxetine hydrochloride	0.55 µg/mL	sulfamethoxazole	117 µg/mL
flutamide	78 ng/mL	(in combination with) trimethoprim	23.4 µg/mL
furosemide	20 µg/mL	terazosin hydrochloride	1.45 mg/mL

Analytical Sensitivity

The lowest detectable level of PSA distinguishable from zero (Access Hybritech PSA Calibrator S0) with 95% confidence is < 0.008 ng/mL for both Hybritech and WHO calibration. This value is determined by processing a complete six point calibration curve, controls, and 20 replicates of the zero calibrator in multiple assays. The analytical sensitivity value is calculated from the curve at the point that is two standard deviations from the mean measured zero calibrator signal.

Functional Sensitivity (Limit of Quantitation)

The literature suggests functional (clinical) sensitivity for PSA assays is defined in terms of precision.[42] A study was conducted using Access Hybritech PSA Calibrator antigen in Access Hybritech PSA Calibrator matrix. The study was performed with two instruments (one calibration curve per instrument) and two reagent pack lots, generating six replicates per assay over 11 assays. One data set from this study resulted in a functional sensitivity of < 0.019 ng/mL (95% confidence interval upper limit dose) at 20% between run CV for both the Hybritech and WHO calibration.

Comparison of Access Immunoassay Systems[h]

The following table provides the Deming regression statistics for the Access Hybritech PSA assay on the Access Immunoassay Systems.

Access Systems	N	Range of Observations (ng/mL)	Intercept (95% CI)	Slope (95% CI)	Correlation Coefficient r^2
Access 2 v. Access	122	0.008–136.5	-0.12 (-0.29 to 0.042)	0.999 (0.995 to 1.002)	0.999
Synchron LXi 725 v. Access 2	64	0.1 – 146.5	-0.05 (-0.51 to 0.41)	0.912 (0.904 to 0.920)	0.998
UniCel DxI 800 v. Access 2	111	0.29– 147.8	0.05 (-0.61 to 0.71)	0.959 (0.946 to 0.972)	0.990
UniCel DxC 600i v. Access 2	107	0.18–136.8	-0.18 (-0.382 to 0.021)	0.966 (0.960 to 0.974)	0.998

[h] Data are based on Hybritech Tandem calibration with a cutoff of 4.0 ng/mL. The corresponding cutoff based on WHO calibration is 3.1 ng/mL.

Access®
Immunoassay Systems

BECKMAN COULTER®

Hybritech® PSA CALIBRATORS

REF 37205

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.**

Intended Use The Access Hybritech PSA Calibrators are intended to calibrate the Access Hybritech PSA assay for the quantitative determination of total prostate specific antigen (PSA) levels in human serum using the Access Immunoassay Systems.

Summary and Explanation Quantitative assay calibration is the process by which samples with known analyte concentrations (i.e., assay calibrators) are tested like patient samples to measure the response. The mathematical relationship between the measured responses and the known analyte concentrations establishes the calibration curve. This mathematical relationship, or calibration curve, is used to convert RLU (Relative Light Unit) measurements of patient samples to specific quantitative analyte concentrations.

Traceability Two options for calibration are provided with the Access Hybritech PSA Calibrators, Hybritech calibration or WHO calibration.

Hybritech calibration: The measurand (analyte) in the Access Hybritech PSA Calibrators is traceable to the manufacturer's working calibrators. Traceability process is based on EN ISO 17511.

WHO calibration: The measurand (analyte) in the Access Hybritech PSA Calibrators is traceable by comparison with a set of primary reference calibrators standardized to the WHO First International Standard (1st IS) for PSA (WHO 96/670).

The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the Access reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Product Information

Access Hybritech PSA Calibrators
Cat. No. 37205: S0–S5, 2.5 mL/vial

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Mix contents by gently inverting before use. Avoid bubble formation.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Signs of possible deterioration are control values out of range.
- Refer to calibration cards for exact concentrations.
- Calibration Cards: One calibration card is provided for the Hybritech calibration and a separate calibration card is provided for the WHO calibration.
- Each calibrator card has a unique lot number specific to each calibration.

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S0:	Buffered bovine serum albumin (BSA), < 0.1% sodium azide and 0.5% ProClin** 300.
S1, S2, S3, S4, S5:	Human PSA at levels of approximately 0.5, 2.0, 10, 75, and 150 ng/mL for Hybritech calibration (or 0.4, 1.7, 8, 58, and 121 ng/mL for WHO calibration) in buffered BSA, < 0.1% sodium azide and 0.5% ProClin 300.
Calibration Cards:	2

Warnings and Precautions

- For *in vitro* diagnostic use.
- Human source material used in the preparation of the reagent has been tested and found negative or non-reactive for Hepatitis B, Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.[43]
- 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.[33]
- The results from the Hybritech and WHO calibrations are not interchangeable. Care should be taken to determine which calibration is appropriate for the laboratory and to specify which calibration the results were generated on.
- Hybritech values and WHO values are assigned individual lot numbers to be used for the same calibrator vials provided, allowing calibration with Hybritech values and WHO values simultaneously.
- Xi. Irritant: 0.5% 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.

Procedure

Refer to the appropriate system manuals for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

Calibration Details

The Access Hybritech PSA Calibrators are provided at six levels:

- For Hybritech calibration: zero and approximately 0.5, 2.0, 10, 75, and 150 ng/mL.
- For WHO calibration: zero and approximately 0.4, 1.7, 8, 58, and 121 ng/mL.

Assay calibration data are valid up to 28 days.

Calibrators run in duplicate.

Limitations of the Procedure

If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.

Access®
Immunoassay Systems

BECKMAN COULTER®

Hybritech® PSA SAMPLE DILUENT

REF 37206

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.**

Intended Use The Access Hybritech PSA Sample Diluent is intended for use with the Access Hybritech PSA assay to dilute patient samples containing total prostate specific antigen (PSA) concentrations greater than the S5 calibrator.

Summary and Explanation The PSA level in patient samples may exceed the levels of the Access Hybritech PSA Calibrator S5. If a quantitative value is required, it will be necessary to dilute the samples in order to determine the PSA concentration.

Product Information

Access Hybritech PSA Sample Diluent
Cat. No. 37206: 14 mL/vial

- Provided ready to use.
- Allow the contents to stand for 10 minutes at room temperature.
- Mix gently by inverting before use. Avoid bubble formation.
- Stable until the expiration date stated on the vial label when stored at 2 to 10°C.

Diluent:	Buffered bovine serum albumin, < 0.1% sodium azide, 0.5% 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.[33]
- Xi. Irritant: 0.5% 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.

Procedure Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value (approximately 0.008 to 150 ng/mL for Hybritech calibration or 0.008 to 121 ng/mL for WHO calibration). If a sample contains more PSA than the stated value of the S5 calibrator, dilute one volume of sample with 4 or 9 volumes of Access Hybritech PSA

Sample Diluent. Refer to the appropriate system manuals and/or Help system for instructions on entering a sample dilution in a test request. The system reports the results adjusted for the dilution.

Limitations of the Procedure

If there is evidence of microbial contamination or excessive turbidity in the reagent, discard the vial.

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Printed in U.S.A.
Made in U.S.A.
Issued March 2008

CE 0050

Access® Hybritech® PSA Assay

Lower Total PSA Cutoff Required When Calibrating to the WHO Standard

Background

Prostate-specific antigen (PSA) is the serum biomarker most widely used to screen for prostate cancer and monitor patients with disease. PSA has been in use for a long time and has a rich history. In 1986, the Hybritech Tandem™-R assay became the first PSA assay to be approved by the FDA.* Manufacturers who wanted to obtain FDA approval standardized their assays to the Hybritech method. In 1994, 4.0 ng/mL was identified as the clinical decision point using the Hybritech PSA assay.[1] In 2000, the Hybritech Tandem-R assay migrated to the Access Immunoassay System.
In the late 1990s, an international standard for total PSA (the WHO 1st International Reference Preparation 96/670) became available. An increasing number of manufacturers began calibrating to the WHO (World Health Organization) standard, which increased the acceptance of the standard in the clinical setting.

Origin of the WHO Standard

Significant variation in PSA results among early non-equimolar PSA assays became a major factor in the desire for assay standardization. Equimolar recognition of free and complexed PSA forms is critical to accurate PSA testing, especially at decision limits, such as the 4.0 ng/mL cutoff. Inaccurate quantitation of PSA at this critical level can yield false positive or false negative results depending on the direction of the skew, and may lead to inappropriate management of the patient.

Thomas Stamey, M.D., a clinical urologist at Stanford University, proposed a PSA standard (90:10 ratio of complexed to free PSA) in the mid-1990s with the intent to mitigate the non-equimolar response of some PSA assays.[2] This preparation became the basis for the material adopted by the WHO in 1999 (WHO 96/670), a PSA standard that has a mass assigned using a molar extinction coefficient different from the original standard. Over time, the original intent to establish an "Equimolarity-Standard" evolved into adoption of WHO 96/670 as a new "Mass-Standard" for PSA.

Current Situation

In response to recent initiatives in countries that require laboratories to report PSA values calibrated to the WHO standard, Beckman Coulter will be offering the option of calibrating to the WHO standard.

Beckman Coulter will continue to provide the traditional Hybritech calibration for physicians and laboratories who prefer to continue with this previously established method.

Hybritech and WHO Calibration Values

The original Hybritech Tandem-R calibration (1986) was based on an internal reference preparation of purified human PSA. The clinical PSA cutoff of 4.0 ng/mL was established on samples from over 6,600 men tested with the Hybritech calibration.[3] Comparisons between Hybritech calibrated assays and assays calibrated to the absolute mass value of the WHO 96/670 show a negative bias in mass units for the WHO calibrated assays.[4]

Even though clinicians have recognized for many years that one man's PSA results differ significantly from one manufacturer to another, they may be unaware that restandardizing the PSA assay may result in a potential under-recovery of PSA values when transitioning from a Hybritech calibration to a WHO calibration. Beckman Coulter has validated the new clinical cutoff of 3.1 ng/mL to use with the Hybritech PSA WHO calibrated assay. We feel strongly that we must notify our customers about this difference and provide them with the information necessary to communicate to their clinicians and other clients if they choose to convert to the WHO calibration option.

Beckman Coulter Validation Process

The validation process had a two-part methodology:
1. To develop WHO primary calibrators to determine "offset" to the Hybritech Calibration.
2. To verify and validate WHO derived results.

By creating WHO primary calibrators, Beckman Coulter was able to compare directly to the original Hybritech calibrators in order to determine the proportional adjustments necessary to align the two assay methods. The adjustment was determined to be approximately 20% across the range of the assay. After a WHO calibration was established, an extensive validation was performed. Validation included a statistical analysis of how the WHO standardization impacts the original Hybritech data used to establish the 4.0 ng/mL cutoff.[3]

What Beckman Coulter Observed During Total PSA Validation

During the validation process, Beckman Coulter observed that different calibrations yielded different results, which indicated that the 4.0 ng/mL cutoff would not be appropriate for the WHO calibration.

Using the original data set that determined the Hybritech cutoff of 4.0 ng/mL, we evaluated various cutoffs for the WHO calibrated method. A cutoff of 3.1 ng/mL was selected based on the results below.

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

Calibration (ng/mL)	Hybritech ≤ 4.0	Hybritech > 4.0	Total Samples
WHO ≤ 3.1	5616	0	5616
WHO > 3.1	0	1014	1014
Total Samples	5616	1014	6630
Relative Agreement	100%	100%	

WHO Calibration Cutoff Questions and Answers (Q&A)

Did changing the cutoff for the WHO calibration change the sensitivity/specificity of the assay?

By establishing the WHO calibration cutoff of 3.1 ng/mL, we were able to retain the clinical sensitivity and specificity of the original Hybritech PSA assay, as shown in the table below.

Calibration Method	PSA Cutoff	Sensitivity	Specificity
Hybritech Calibration	4.0 ng/mL	81.6%	48.0%
WHO Calibration	3.1 ng/mL	81.6%	48.0%

Draft User Education Materials

Impact to Patient Results: Importance of Correct Cutoff

Using the original data that determined the 4.0 ng/mL cutoff for the Hybritech assay, results were evaluated among prostate cancer subjects using a 3.1 and 4.0 ng/mL cutoff for the WHO calibration. The importance of using the lower cutoff for the WHO calibrated assay is summarized in the following tables.

The first table provides PSA results for cancer patients using the 4.0 ng/mL cutoff with the Hybritech calibration and the 3.1 ng/mL cutoff with the WHO calibration. The second table provides PSA results for the same cancer patients using a 4.0 ng/mL cutoff with the Hybritech and the WHO calibrations. In 15% of the cases, cancer may have been missed using a 4.0 ng/mL cutoff with the WHO calibration.

Prostate Cancer Subjects: Distribution by PSA Cutoff

WHO Calibration	Hybritech Calibration		
	≤ 4.0 ng/mL	> 4.0 ng/mL	Total
≤ 3.1 ng/mL	47	0	47
> 3.1 ng/mL	0	208	208
Total	47	208	255

WHO Calibration	Hybritech Calibration		
	≤ 4.0 ng/mL	> 4.0 ng/mL	Total
≤ 4.0 ng/mL	47	38	85
> 4.0 ng/mL	0	170	170
Total	47	208	255

15% (38/255) of prostate cancers may be missed at a 4.0 ng/mL WHO calibration cutoff

What Should a Laboratory Do?

Communication is very important if the choice is made to convert to the WHO calibration. We recommend the following:

- Explain to clinicians that calibrations resulting from different standards or reference preparations will provide different results and therefore will require different cutoffs if the same clinical performance is to be achieved.

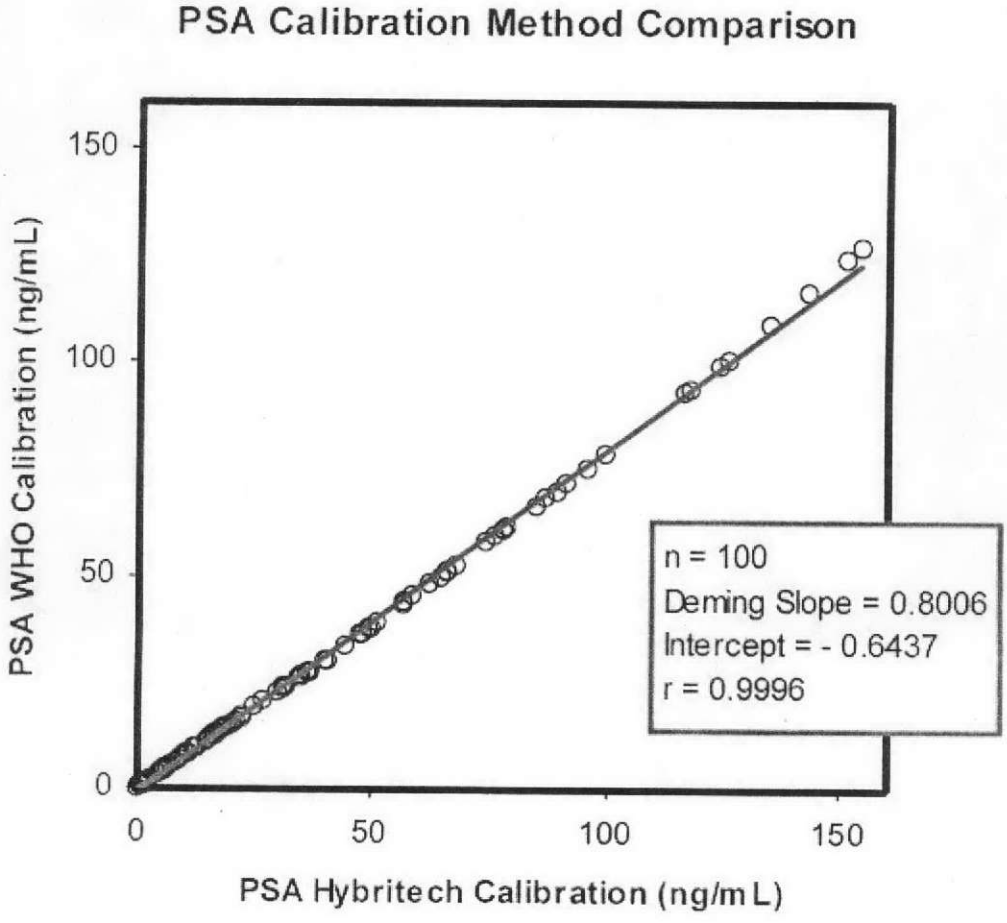
- Report to clinicians what calibration and methodology is being used.

• Alert physicians that WHO calibrated total PSA values for patients will be about 20% lower than Hybritech calibrated values across the assay range.

• Notify external Quality Assurance programs of additional WHO calibration reporting group requirements for the Access Hybritech PSA assay.

Method Correlation

During the validation process, many method correlation studies were performed on the Access Family of Immunoassay Systems comparing the Hybritech calibrated Access Hybritech PSA method to the WHO calibrated Access Hybritech PSA method. An example of one study, using 100 patient samples, is captured in the graph below. These results demonstrate an approximate 20% difference between the two calibrations.



Clinical PSA Cutoff for WHO Calibration

Using the original Hybritech PSA data that established the 4.0 ng/mL cutoff, it was determined that a different clinical cutoff for the WHO calibrated Access Hybritech PSA assay was necessary in order to provide the same clinical sensitivity and specificity that the traditional Hybritech calibration provides. The cutoffs for the respective assays are listed in the following table.

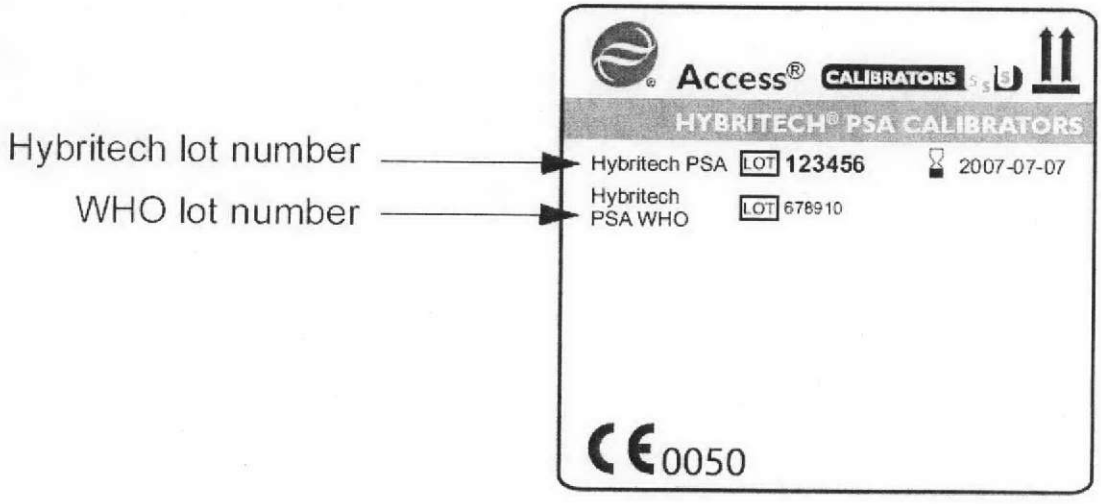
	Hybritech Calibration	WHO Calibration
PSA Cutoff	4.0 ng/mL	3.1 ng/mL

Calibrators

The Access Hybritech PSA calibrator kits will provide two calibration options: the traditional Hybritech calibration and the WHO calibration. These calibrations will use the same calibrators, but distinct calibration cards, differentiated by color:

- Hybritech calibration information White card
- WHO calibration information Yellow card

A WHO calibration card will be further differentiated with a unique calibrator lot number printed on the card. This lot number is also printed on the Calibration Data Report and on the outside surface of the calibrator kit box, as shown in the example below. The Hybritech PSA WHO lot numbers are used for tracking and reporting purposes.



APF/AAF and LIS Information

The assay information below is required for converting to the PSA WHO calibration. It is recommended that you install the most recent version of system software.

System Information	Access Hybritech PSA	Access Hybritech free PSA	Access Hybritech PSA WHO	Access Hybritech free PSA WHO
Access / Access 2 / UniCel® DxI Test Names and LIS Codes	PSA-Hyb	freePSA	PSA-WHO	fPSA-WHO
SYNCHRON LX®i / UniCel DxC 600i Test Names and LIS Codes	PSA-H	fPSA	PSA-W	fPSAW
DL2000 / DataLink™ Codes	A62	A63	A90	A89
Access APF	APF 1.1.**54**.1 or higher			
Access 2 APF	APF 1.9.**23**.1 or higher			
UniCel DxI APF	APF 1.10.**23**.1 or higher			
SYNCHRON LXi AAF	AAF L1.9.**23**.1 or higher			
UniCel DxC 600i AAF	AAF D1.9.**23**.1 or higher			

Quality Control Materials (QC)

The Access Hybritech PSA quality control material kits will contain two value cards. Each card will contain values specific for the respective calibration option.

- Hybritech QC values White card
- WHO QC values Yellow card

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Example Letter to Notify Physicians of the Access® Hybritech® PSA WHO Calibration

If you choose to convert to the WHO calibrated method using the Access Hybritech PSA assay, the following text is an example letter that you may use to notify physicians that the WHO calibrated method is now available in your laboratory. Fill in the blanks provided with the information applicable for your laboratory.

[Date]
[Physician's Name]
[Address]

Dear Dr. _____(name)_____ ,

On _____(date)_____, the laboratory will implement a PSA methodology that is calibrated to the WHO* reference preparations. The absolute (ng/mL) values obtained with the WHO calibrated method will be approximately 20% lower than the values obtained with the traditional Hybritech calibrated method.

Due to this difference, our previous cutoff of ____(4.0 ng/mL)___ will be ____(3.1 ng/mL)___. Please be sure to evaluate patient results accordingly. We will report results as follows:

"The PSA method used is the Access Hybritech PSA. This method has been calibrated to the WHO 96/670 (total PSA) reference preparations. Due to differences in reported values between the traditional Hybritech and WHO calibrated methods, a clinical total PSA cutoff of ____(3.1 ng/mL)___ is recommended."

If you have any questions about this methodology or your patient test results, please call _____(name and number)_____ in the clinical laboratory for further assistance.

Best Regards,

_____(signature)_____

* World Health Organization

Example External Quality Assurance Program Letter for Access® Hybritech® PSA WHO Calibration

If you choose to convert to the WHO calibrated method using the Access Hybritech PSA, notify your external Quality Assurance program of changes to your PSA reporting needs as soon as you complete the conversion. The following text is an example letter that you may use to inform your external Quality Assurance program of the conversion.

EXAMPLE

[Date]
[External Quality Assurance Program Name]
[Address]

Dear [External Quality Assurance Program Name],

The clinical laboratory at _____(insert institution name here)_____will be converting to the Access Hybritech PSA WHO* calibrated methods from Beckman Coulter, Inc. by (insert effective date here ___). Values reported will be approximately 20% lower than the traditional Hybritech PSA calibrated method and previously reported results.

Beckman Coulter will be offering both Hybritech and WHO calibration options for their total PSA assay on an ongoing basis. We therefore request that you institute separate reporting groups for the Hybritech and WHO calibrated methodologies on the Access systems. We recommend the following titles for the reporting groups:

- PSA (Hybritech)
- PSA (WHO)

Sincerely,

Signature

* World Health Organization

Draft User Education Materials

Dear Access® Hybritech® PSA Customer:

Recent initiatives in some countries require laboratories to report PSA values standardized to the WHO* 1st International Reference Preparations 96/670 and 96/668, respectively. In response, Beckman Coulter is providing the option of calibrating the Access Hybritech PSA to the WHO standard.

Beckman Coulter will continue to provide our traditional Hybritech calibration on the Access Family of Immunoassay Systems for customers who prefer to remain with the Hybritech calibrated method.

During our validation process, a difference of approximately 20% across the dynamic range of the assay was observed between the traditional Hybritech PSA calibrated method and the WHO calibrated method. This difference requires an adjustment of the 4.0 ng/mL cutoff that was originally established using the Hybritech calibration. Therefore, a cutoff of 3.1 ng/mL has been validated for the PSA WHO calibration and found to be clinically equivalent to the 4.0 ng/mL cutoff for the Hybritech calibration.

Enclosed are revised directional inserts for both the Hybritech and WHO calibration options.

Also enclosed are materials your laboratory will need if you choose to change to the Hybritech PSA WHO calibration. These documents will help reassure your physicians that the new PSA cutoff delivers the same clinical performance for the WHO calibrated method as the traditional PSA cutoff delivers for the Hybritech calibrated method.

Beckman Coulter is committed to providing the highest standard of clinical diagnostic products to aid in the diagnosis and monitoring of disease.

Should you have any questions or concerns, please contact Beckman Coulter Technical Support at 1-800-xxx- xxxx

Thank you for your continued partnership.