Access
Immunoassay Systems

Hybritech free PSA

REF 37210

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

Warning

Access Hybritech free PSA should be used only with Hybritech (total) PSA to calculate the ratio of free PSA to total PSA (percent free PSA). Use of another manufacturer's total PSA assay may result in:

1. an inappropriate population of patients selected for follow-up percent free PSA testing; and
2. significantly different percent free PSA values, cutoffs and cancer probabilities than those presented in the Expected Values section of this insert.

Expected values contained in this insert apply only to percent free PSA as measured by the Hybritech free PSA and (total) PSA Assays.

The concentration of free PSA and total 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 free and total PSA assays used. Values obtained with different manufacturers' assays cannot be used interchangeably.

Free PSA concentrations are dependent on the standard used to calibrate the assay. Free PSA concentrations based on calibration to the WHO 96/668 Reference Preparation will differ significantly from free 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 free PSA assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of free prostate specific antigen (free PSA) in human serum using the Access Immunoassay Systems. Access Hybritech free PSA is intended to be used with Hybritech (total) PSA to calculate the ratio of free PSA to total PSA expressed as a percentage (percent free PSA). Percent free PSA as measured by the Hybritech assays is indicated for use as an aid in distinguishing prostate cancer from benign prostatic conditions, when used in conjunction with Hybritech (total) PSA for prostate cancer detection in men aged 50 years and older with total PSA between 4[h] and 10[h] ng/mL with digital rectal examination findings that are not suspicious for cancer. Prostatic biopsy is required for diagnosis of cancer.

[h] Data are based on Hybritech Tandem calibration with a PSA cutoff of 4.0 ng/mL. The corresponding PSA cutoff based on WHO calibration is 3.1 ng/mL. A PSA range of 4-10 ng/mL with the Hybritech calibration corresponds to a PSA range of 3.1-7.8 ng/mL with the WHO calibration.

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]

Prostate-specific antigen 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.

PSA exists primarily as three forms in serum.[4] One form of PSA is believed to be enveloped by the protease inhibitor, alpha-2 macroglobulin[5] and has been shown to lack immunoreactivity. A second form is complexed to another protease inhibitor, alpha-1 antichymotrypsin (ACT).[5,6] The third form of PSA is not complexed to a protease inhibitor, and is termed free PSA.[5,6] The latter two forms are immunologically detectable in commercially available PSA assays and are referred to collectively as total PSA.

Previous reports have shown that measurement of PSA forms is useful in the differentiation of prostate cancer from benign prostatic conditions.[7,8] In patients with elevated PSA concentrations, men with prostate cancer tend to have lower percent free PSA (free PSA/total PSA) values than men with benign disease.[9,10,11,12,13] This difference in the distribution of percent free PSA values in men with and without cancer may be used to select cutoffs for biopsy decisions, maintaining 90% to 95% sensitivity, while sparing 20% to 30% of men with benign disease from biopsy.

Percent free PSA may also be used for risk assessment, to determine the probability of cancer for an individual patient. Lower percent free PSA values are associated with higher risk of cancer.[9,10,11,12,13]

A free PSA standard (WHO 96/668) containing seminal plasma-derived prostate-specific antigen (PSA)[14] was analyzed by an international collaborative study and established as the First International Standard for Prostate-Specific Antigen (Free) by the WHO. Calibration to the 1st (IS) (WHO 96/668), results in a ~ 20 % dose shift across the curve relative to the Hybritech calibration. The clinical free PSA cutoff (= 25 % free PSA) is the same with both the Hybritech and WHO calibrations. Calibration to the PSA WHO 96/670, using an adjusted cutoff of 3.1 ng/mL correlates results to the original Hybritech Tandem assay clinical performance.

Principles of the Procedure

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

Product Information

Access Hybritech free PSA Reagent Pack

Cat. No. 37210: 100 determinations, 2 packs, 50 tests/pack

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

R1a:	Paramagnetic particles coated with burro anti-goat, goat anti-biotin, and biotinylated mouse monoclonal anti-PSA antibodies in TRIS buffered saline, with surfactant, bovine serum albumin (BSA), < 0.1% sodium azide, and 0.1% ProClin** 300.
R1b:	Mouse monoclonal anti-free PSA alkaline phosphatase (bovine) conjugate diluted in phosphate buffered saline, with surfactant, BSA, proteins (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.[15]
- Xi. Irritant: 0.25% ProClin 300.

R 43: May cause sensitization by skin contact.

S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

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

Specimen Collection and Preparation

1. No special preparation of the patient sample is necessary. Specimens for free PSA 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 both free and total PSA.[16] A repeat total PSA measurement in the case of borderline elevation is recommended.[17]
2. Transrectal needle biopsy has also been shown to cause transient increases in free PSA and persisting total PSA elevations,[16,17] thus a six-week waiting period between needle biopsy and PSA sampling has been recommended.
3. Serum is the recommended sample for the Access Hybritech free PSA assay. Plasma samples should **not** be used.
4. 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.
5. 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]
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.[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, total PSA, or percent free PSA.[18] 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 free PSA Reagent Packs

Materials Required But Not Provided

1. Access Hybritech free PSA Calibrators
   Cat. No. 37215
   Two options for calibration are provided with the Access Hybritech free PSA Calibrators, Hybritech calibration or WHO calibration.
   Hybritech calibration: concentrations are zero and approximately 0.5, 2.0, 5.0, 10 and 20 ng/mL.
   WHO calibration: concentrations are zero and approximately 0.4, 1.6, 4.1, 8 and 16 ng/mL.
2. Access Hybritech free PSA Quality Control (QC) or other commercially available control material.
   Cat. No. 37219
   Access Hybritech free 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 and 13 ng/mL.
   WHO calibration: concentrations are approximately 0.8 and 10 ng/mL.
3. Access Substrate
   Cat. No. 81906
4. **Access, Access 2, SYNCHRON LXi, UniCel DxC 600i:**
   Access Wash Buffer II, Cat. No. A16792
   UniCel DxI:
   UniCel DxI Wash Buffer II, Cat. No. A16793

Procedural Comments

1. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.
2. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.
3. Use 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 free 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.

Free PSA concentrations are dependent on the standard used to calibrate the assay. Free PSA concentrations based on calibration to the WHO 96/668 Reference Preparation will differ significantly from free 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.[21] Include Access Hybritech free PSA QC or other commercially available quality control materials that cover at least two levels of analyte. Access Hybritech free 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. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish mean values and acceptable ranges to assure proper performance. Quality control results that do not fall within acceptable ranges may indicate invalid test results. Examine all test results generated since obtaining the last acceptable quality control test point for this analyte. Refer to the appropriate system manuals and/or Help system for information about reviewing quality control results.

Results

Patient test results are determined automatically by the system software using a smoothing cubic spline 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 0.005–20 ng/mL Hybritech calibration or 0.005–16 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., < 0.005 ng/mL for both Hybritech and WHO calibration).
   - If a sample contains more than the stated value of the highest Access Hybritech free PSA Calibrator (S5), report the result as greater than that value (i.e., > 20 ng/mL Hybritech calibration or > 16 ng/mL WHO calibration). Alternatively, dilute one volume of sample with 4 or 9 volumes of Access Hybritech free PSA Calibrator S0 (zero). After assaying the diluted sample, multiply the obtained value by the appropriate dilution factor of 5 or 10, respectively. 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.[22,23]
   Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
3. The Access Hybritech free 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 (free, total, or percent free PSA) should not be interpreted as absolute evidence for the presence or absence of prostate cancer. Elevated total PSA concentrations or decreased percent free PSA may be observed in the serum of patients with non-malignant disorders, as well as those with prostate cancer. Furthermore, low total PSA concentrations or elevated percent free PSA are not necessarily indicative of the absence of cancer. Serum free and total 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.

4. The Access Hybritech free PSA assay does not demonstrate any "hook" effect up to 20,000 ng/mL with Hybritech calibration or 15,800 ng/mL with WHO calibration.
5. 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.
6. Free PSA concentrations are dependent on the standard used to calibrate the assay. Free PSA concentrations based on calibration to the WHO 96/668 Reference Preparation will differ significantly from free 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]

Calculation of percent free PSA

Free PSA values alone have not been shown to be effective in patient management and should not be used. Both total PSA and free PSA concentrations should be determined on the same serum specimen and used to calculate the percentage of free PSA. Percent free PSA values are then used for patient management.

Important: Percent free PSA (% fPSA) can only be calculated if the results were derived from the same type of calibration (Hybritech or WHO). Therefore, never mix Hybritech and WHO calibrations when calculating % fPSA.

$$\frac{\text{Hybritech free PSA (ng/mL)}}{\text{Hybritech total PSA (ng/mL)}} \times 100 = \frac{\text{WHO free PSA (ng/mL)}}{\text{WHO total PSA (ng/mL)}} \times 100 = \text{Percent free PSA}$$

Expected Values

A multicenter, prospective clinical trial was conducted to test the effectiveness of percent free PSA as an aid in distinguishing prostate cancer from benign prostatic conditions, when used in conjunction with Hybritech (total) PSA for prostate cancer detection.[13] Although the free PSA results in this trial were generated with the Hybritech Tandem free PSA assay, the Access Hybritech free PSA assay has been developed using the same monoclonal antibodies employed in the Tandem free PSA assay and has been standardized to provide the same clinical performance. The WHO calibration was established based on the First International Standard for Free PSA (WHO 96/668), 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 free PSA assay.

All subjects were between 50 and 75 years of age, with serum PSA values between 4[h] and 10[h] ng/mL and digital rectal examination (DRE) findings that were not suspicious for cancer. These men represent the "diagnostic gray zone," in which total PSA has identified the men as high risk (25% cancer rate compared to a 4% cancer rate for the general population of men over 50 years of age), but where specificity could be improved. All men had undergone ultrasound-guided six-sector needle biopsies of the prostate, and thus had a histologically confirmed diagnosis prior to determination of free PSA concentrations. The study was blinded; pathologists did not have access to percent free PSA values, and laboratory technicians did not have access to diagnoses. Exclusion criteria included acute prostatitis, urinary tract infection, prior transurethral resection of the prostate (TURP), or recent prostatic manipulation or medications that might alter serum PSA concentrations.

A total of 773 men participated in the study. Median age for both cancer and benign disease subjects was 64 years. The study population of 86% Caucasian, 9% African-American, 3% Hispanic, and 2% Asian. Table 1 shows the expected values for free PSA (ng/mL), total PSA (ng/mL), and percent free PSA [(free PSA/total PSA) x 100%] for this population of men.

[h] Data are based on Hybritech Tandem calibration with a PSA cutoff of 4.0 ng/mL. The corresponding PSA cutoff based on WHO calibration is 3.1 ng/mL. A PSA range of 4-10 ng/mL with the Hybritech calibration corresponds to a PSA range of 3.1-7.8 ng/mL with the WHO calibration.

Table 1: Hybritech free PSA (ng/mL), Total PSA (ng/mL) and Percent free PSA (%): Expected Values, by Diagnosis[h]

		Benign n = 394	Cancer n = 379	Total n = 773
Free PSA	**Median**	1.0	0.7	0.9
	Mean ± SD	1.1 ± 0.6	0.8 ± 0.5	1.0 ± 0.6
	Range	0.2–4.9	0.2–3.6	0.2–4.9
Total PSA	**Median**	5.6	5.9	5.8
	Mean ± SD	6.0 ± 1.6	6.2 ± 1.7	6.1 ± 1.6
	Range	4.0–10.0	4.0–10.0	4.0–10.0
% free PSA	**Median**	17.9	12.2	15.3
	Mean ± SD	19.0 ± 7.8	13.4 ± 6.8	16.3 ± 7.9
	Range	4.3–52.2	2.3–42.1	2.3–52.2

[h] Data are based on Hybritech Tandem calibration with a PSA cutoff of 4.0 ng/mL. The corresponding PSA cutoff based on WHO calibration is 3.1 ng/mL. A PSA range of 4-10 ng/mL with the Hybritech calibration corresponds to a PSA range of 3.1-7.8 ng/mL with the WHO calibration.

In a prostate cancer detection program, DRE and PSA testing would identify men with non-suspicious DRE results and PSA between 4[h] ng/mL and 10[h] ng/mL. Free PSA and percent free PSA would then be determined on these patients, and results would be used as an aid in patient management.

The multicenter clinical trial results demonstrated that percent free PSA may be used in two ways:

1. individual patient risk assessment to aid in management decisions, or
2. a single cutoff (men with values less than or equal to a certain cutoff would be candidates for additional follow-up procedures such as biopsy).

Individual Patient Risk Assessment

Percent free PSA may be used to determine the relative risk of prostate cancer in individual men. Family and patient history can be used in combination with percent free PSA results to determine the best individualized patient management decisions.

Table 2 shows the probability of detecting prostate cancer with needle biopsy, based on total PSA and percent free PSA results. PSA results in this table were obtained from a prior multi-center study evaluating the efficacy of total PSA for prostate cancer detection,[24,25] and percent free PSA results were obtained from the current study.

It can be seen that rising PSA levels increase the risk of detectable cancer. Percent free PSA can further stratify risk for men with PSA values between 4[h] ng/mL and 10[h] ng/mL and non-suspicious digital rectal examination results. Lower percent free PSA values indicate higher risk. The risk of cancer ranged from 8% to 56% for this population. For purposes of comparison, the risk of prostate cancer is 4% for the general population of men over 50 years of age.[24]

[h] Data are based on Hybritech Tandem calibration with a PSA cutoff of 4.0 ng/mL. The corresponding PSA cutoff based on WHO calibration is 3.1 ng/mL. A PSA range of 4-10 ng/mL with the Hybritech calibration corresponds to a PSA range of 3.1-7.8 ng/mL with the WHO calibration.

Table 2: Probability of Prostate Cancer, Based on PSA and Percent free PSA Results[h]
(for Men with Non-Suspicious DRE Results, Regardless of Patient Age)

PSA (Hybritech Calibration)	PSA (WHO Calibration)	Probability of Cancer
0–2 ng/mL	0–1.6 ng/mL	1%
2–4 ng/mL	1.6–3.1 ng/mL	15%
4–10 ng/mL	3.1–7.8 ng/mL	25%
> 10 ng/mL	> 7.8 ng/mL	> 50%

Percent free PSA	Probability of Cancer
0–10%	56%
10–15%	28%
15–20%	20%
20–25%	16%
> 25%	8%

[h] Data are based on Hybritech Tandem calibration with a PSA cutoff of 4.0 ng/mL. The corresponding PSA cutoff based on WHO calibration is 3.1 ng/mL. A PSA range of 4-10 ng/mL with the Hybritech calibration corresponds to a PSA range of 3.1-7.8 ng/mL with the WHO calibration.

Important: Percent free PSA (% fPSA) can only be calculated if the results were derived from the same type of calibration (Hybritech or WHO). Therefore, never mix Hybritech and WHO calibrations when calculating % fPSA.

$$\frac{\text{Hybritech free PSA (ng/mL)}}{\text{Hybritech total PSA (ng/mL)}} \times 100 = \frac{\text{WHO free PSA (ng/mL)}}{\text{WHO total PSA (ng/mL)}} \times 100 = \text{Percent free PSA}$$

Percent free PSA values should not be interpreted as definitive evidence for the presence or absence of prostate cancer. Prostatic biopsy is required for diagnosis of cancer.

The clinical trial results also demonstrated that older men were at higher risk than younger men. The probability of cancer by percent free PSA (%fPSA) values and age is shown in Table 3 and Figure 1.

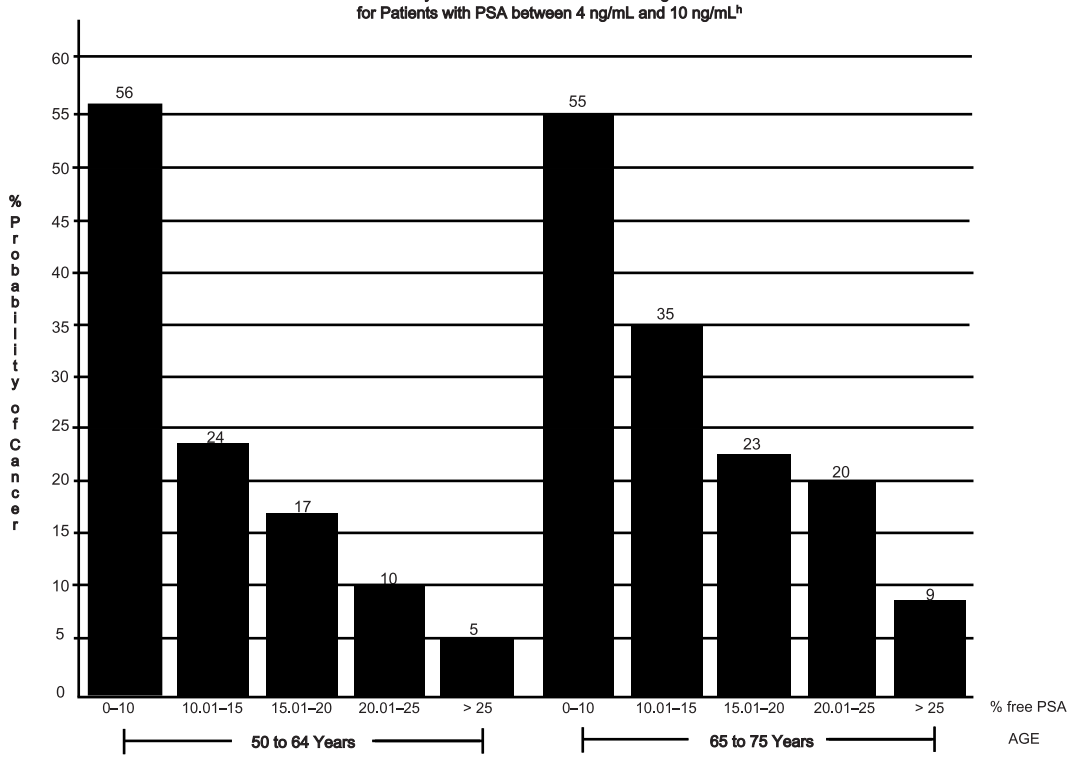
Table 3: Probability of Prostate Cancer[h]
(For Men with Non-Suspicious DRE Results and PSA Between 4 ng/mL and 10 ng/mL, by Patient Age)

% free PSA	Patient Age	
	50 to 64 Years	65 to 75 Years
0.00 to 10.00%	56%	55%
10.01 to 15.00%	24%	35%
15.01 to 20.00%	17%	23%
20.01 to 25.00%	10%	20%
≥ 25.01%	5%	9%

[h] Data are based on Hybritech Tandem calibration with a PSA cutoff of 4.0 ng/mL. The corresponding PSA cutoff based on WHO calibration is 3.1 ng/mL. A PSA range of 4-10 ng/mL with the Hybritech calibration corresponds to a PSA range of 3.1-7.8 ng/mL with the WHO calibration.

Figure 1: Probability of Cancer

by Percent free PSA and Patient Age
for Patients with PSA between 4 ng/mL and 10 ng/mL[h]



[h] Data are based on Hybritech Tandem calibration with a PSA cutoff of 4.0 ng/mL. The corresponding PSA cutoff based on WHO calibration is 3.1 ng/mL. A PSA range of 4-10 ng/mL with the Hybritech calibration corresponds to a PSA range of 3.1-7.8 ng/mL with the WHO calibration.

Single Cutoff

Rather than using risk assessment, a cutoff approach to patient management can also be used. Table 4 shows sensitivity (percentage of cancers detected) and specificity (percentage of biopsies avoided in men without cancer) for various percent free PSA cutoffs. A cutoff of ≤ 25% free PSA was selected based on data from the clinical trial. When men with values of 25% free PSA **or less** were biopsied, 95% of cancers were detected. The majority of men with PSA values between 4[h] ng/mL and 10[h] ng/mL have benign disease. In this clinical trial, 20% of biopsied men with benign disease and a percent free PSA value greater than the 25% free PSA cut-off could have been spared from biopsy.

The cutoff of ≤ 25% free PSA is based on results from this clinical trial. Additional follow-up may be recommended for men with percent free PSA values above 25%, if the physician believes it is necessary based upon other factors in the patient's medical or family history.

[h] Data are based on Hybritech Tandem calibration with a PSA cutoff of 4.0 ng/mL. The corresponding PSA cutoff based on WHO calibration is 3.1 ng/mL A PSA range of 4-10 ng/mL with the Hybritech calibration corresponds to a PSA range of 3.1-7.8 ng/mL with the WHO calibration.

Table 4: Sensitivity and Specificity for Various Percent free PSA Cutoffs
Recommended Cutoff: ≤ 25% free PSA
(Biopsy men with values less than or equal to this cutoff)

% free PSA Cutoffs	Sensitivity (# of cancers detected/# of total cancers)			Specificity (# of non-cancers detected/# of total non-cancers)		
	%	(n/N)	95% CI*	%	(n/N)	95% CI*
≤ 25%	**95%**	**(358/379)**	**92–97%**	**20%**	**(80/394)**	**16–24%**
≤ 32%	98%	(373/379)	96–99%	6%	(25/394)	4–9%
≤ 55%	100%	(379/379)		0%	(0/394)	

* 95% CI = 95% Confidence Interval

Table 5 shows that the cancers occurring in men with a percent free PSA value above the 25% cutoff (i.e., those cancers which would be missed if men above the cutoff were not biopsied) are found primarily in older men with larger glands. Older men (those with less than a 10 year life expectancy) are often not affected by nor treated for prostate cancer. Thus, use of percent free PSA would result in a recommendation for biopsy in younger men, those most likely to gain from early detection.

The volume finding is clinically advantageous. Men with percent free PSA values near and above the cutoff tend to have large glands (benign prostatic hyperplasia), whereas men with cancer have lower percent free PSA values which tend to cluster progressively further away from the cutoff. Thus, when the recommendation is made not to biopsy men above the cutoff, this is the group with the lowest risk of cancer and the highest probability of benign disease (see Table 2 and discussion in previous section, "Individual Patient Risk Assessment").

Table 5: Characteristics of Cancer Subjects Above and Below Cutoff:
Patient Age and Prostate Volume

% free PSA Cutoff	Median Patient Age	Median Prostate Volume
> 25% free PSA	68 years	48 cc
≤ 25% free PSA	63 years	34 cc

Specific Performance Characteristics

Dilution Recovery (Linearity)

Ten serum samples containing elevated free PSA concentrations were diluted with Access Hybritech free PSA Calibrator S0 (zero) and assayed in quadruplicate at multiple dilutions. Observed free PSA concentrations versus expected concentrations were analyzed by linear regression. The correlation coefficients (r) varied between 0.9986 and 1.000.

Imprecision

This assay exhibits total imprecision of less than 7% across the assay range for Hybritech and WHO calibration. Reproducibility of the Access Hybritech free PSA assay was determined in one study by assaying five free PSA controls (three of which were serum based) in duplicate across 20 runs on the Access Immunoassay System. The data presented were calculated based on CLSI EP5-A guidelines.

Table 6: Imprecision[h] with the Hybritech Calibration

Sample	Mean (n=40) (ng/mL)	Within-Run (%CV)	Between-Run (%CV)	Total (%CV)
1	0.29	1.68	1.36	2.16
2	1.04	1.79	3.41	3.85
3	1.73	1.75	1.98	2.65
4	7.72	2.02	1.77	2.68
5	12.87	2.89	1.71	3.36

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

Table 7: Imprecision with the WHO Calibration

Sample	Mean (n=40) (ng/mL)	Within-Run (%CV)	Between-Run (%CV)	Total (%CV)
1	0.23	1.69	1.51	2.26
2	0.84	1.95	3.41	3.93
3	1.40	1.76	2.33	2.92
4	6.26	2.01	1.78	2.68
5	10.34	2.80	1.78	3.32

Analytical Specificity/Interferences

1. Cross-reactivity with **PSA-ACT** was determined to be less than 1%.
2. Serum 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 3.8–14.1 g/dL (38–141 g/L) do not interfere with the Access Hybritech free PSA assay.
3. Various concentrations of drugs were added to serum samples containing free 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 free PSA from the serum samples.

Table 8: Drug Interference Testing (Commonly Used Drugs)

Drug	Concentration
acetaminophen	0.2 mg/mL
aspirin	0.5 mg/mL
biotin	50 ng/mL
captopril	4 µg/mL
cimetidine	0.1 mg/mL
ciprofloxacin	46 µg/mL
clemastine	2.7 µg/mL
clomipramine HCl	2.7 µg/mL
doxycycline hyclate	2.6 µg/mL
finasteride	370 ng/mL
fluoxetine HCl	0.55 µg/mL
furosemide	20 µg/mL
hydrocodone bitartrate	240 ng/mL
ibuprofen	0.4 mg/mL
lovastatin	270 ng/mL
metoprolol tartrate	2.7 µg/mL
naproxen sodium	1 mg/mL
nifedipine	270 ng/mL
prednisone	1.65 µg/mL
sildenafil	0.2 mg/mL
sulfamethoxazole	117 µg/mL
(in combination with) trimethoprim	23.4 µg/mL
terazosin HCl	1.45 mg/mL

Analytical Sensitivity

The analytical sensitivity of the Access Hybritech free PSA assay is less than 0.005 ng/mL for both Hybritech and WHO calibration. Analytical sensitivity is defined as the concentration of free PSA corresponding to the response in Relative Light Units (RLU) that is two standard deviations greater than 20 replicate determinations of the zero calibrator (S0).

Comparison of Access Immunoassay Systems[h]

The following table provides the Deming regression statistics for the Access Hybritech free 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	116	0.02–18.7	0.00 (-0.03 to 0.03)	0.998 (0.992 to 1.004)	0.998
Synchron LXi 725 v. Access 2	60	0.01–15.7	0.05 (-0.03 to 0.12)	0.959 (0.945 to 0.972)	0.994
UniCel DxI 800 v. Access 2	107	0.06–18.1	0.01 (-0.04 to 0.05)	0.971 (0.946 to 0.978)	0.997
UniCel DxC 600i v. Access 2	107	0.03–19.99	0.020 (-0.015 to 0.054)	0.954 (0.946 to 0.964)	0.998
UniCel DxI 600 v. UniCel DxI 800	200	0.12–17.09	-0.07 (-0.14 to 0.01)	1.051 (1.038 to 1.065)	0.992

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

Access
Immunoassay Systems

Hybritech free PSA CALIBRATORS

REF 37215

Intended Use

The Access Hybritech free PSA Calibrators are intended to calibrate the Access Hybritech free PSA assay for the quantitative determination of free 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 free PSA Calibrators, Hybritech calibration or WHO calibration.

Hybritech Calibration: The measurand (analyte) in the Access Hybritech free 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 free PSA Calibrators is traceable by comparison with a set of primary reference calibrators standardized to the WHO First International Standard (1st IS) for free PSA (WHO 96/668).

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 free PSA Calibrators

Cat. No. 37215: S0, 5.0 mL/vial; S1–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 calibration card has a unique lot number specific to each calibration.

S0:	Buffered BSA, < 0.1% sodium azide and 0.25% ProClin** 300.
S1, S2, S3, S4, S5:	Human free PSA at levels of approximately 0.5, 2.0, 5.0, 10 and 20 ng/mL for Hybritech calibration (or 0.4, 1.6, 4.1, 8 and 16 ng/mL for WHO calibration) in buffered BSA, < 0.1% sodium azide and 0.25% 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.[26]
- 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.[15]
- 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: 0.25% 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.

Procedure

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.

Calibration Details

The Access Hybritech free PSA Calibrators are provided at six levels:

- For Hybritech calibration: zero and approximately 0.5, 2.0, 5.0, 10 and 20 ng/mL.
- For WHO calibration: zero and approximately 0.4, 1.6, 4.1, 8 and 16 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.

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