BECKMAN COULTER

CE 2797

ACCESS
Immunoassay Systems

Instructions For Use

Access Hybritech PSA
Prostate-Specific Antigen

REF 37200

FOR PROFESSIONAL USE ONLY

Rx Only

PRINCIPLE

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

Except for skin cancer, 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.[2,3] It is also the second leading cause of cancer deaths among American men.[3] 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.[4] Historically, a majority of prostate cancers had advanced beyond the gland at the time of diagnosis.[5] 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.[5,6,7]

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.[8] 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.[9] Moreover, PSA may serve as an accurate marker for monitoring advancing clinical stage in untreated patients,[10] as well as assessing response to therapy.[11,12,13,14] 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.[15]

Prostate specific antigen (PSA) was identified and purified by Wang and co-workers in 1979.[16] PSA is a single chain glycoprotein with a molecular weight of approximately 34,000 daltons, containing 7% carbohydrate by weight.[16] PSA exists primarily as three forms in serum.[17] One form of PSA is believed to be enveloped by the protease inhibitor, alpha-2 macroglobulin[17] and has been shown to lack immunoreactivity. A second form is complexed to another protease inhibitor, alpha-1 antichymotrypsin (ACT).[17,18,19] The third form of PSA is not complexed to a protease inhibitor, and is termed free PSA.[17,18,19] 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.[20] However, it has been reported that the concentration of free PSA usually ranges from 5 to 50% of the total PSA in serum.[21]

Additional studies have also shown that various immunoassays react differently to these two forms in serum.[20,21] 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.[22] PSA is present in normal, benign hyperplastic, and malignant prostatic tissue, and also in prostatic fluid and seminal plasma.[23] PSA has not been detected in cancers of the lung, colon, rectum, stomach, pancreas or thyroid.[24] Purified PSA lacks any acid phosphatase activity and does not react with antibodies against PAP and vice versa.[25] 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.[26] 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.

Table 1.0 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[27,28]
0.75	0.64	PSA velocity suspicious for prostate cancer if PSA 4.0-10.0 ng/mL[27,28]
2.0	1.6	PSA velocity for aggressive prostate cancer[27,29]

Table 1.0 Hybritech Calibration and WHO Calibration PSA Values, Continued

Hybritech Calibration PSA Value (ng/mL)	WHO Calibration PSA Value (ng/mL)	Description
2.5	2.0	Total PSA value to trigger biopsy[29,30,31]
4.0	3.1	Total PSA value to trigger biopsy[8]
10.0	7.8	Upper end of threshold for biopsy[8]
20.0	15.6	Prostate cancer risk stratification[32,33]

METHODOLOGY

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 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.

SPECIMEN

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.

REAGENTS

PRODUCT INFORMATION

Access Hybritech PSA Reagent Pack

Ref. 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 2 hours before use on the instrument.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Stable at 2 to 10°C for 28 days after initial use.
- Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.
- If the reagent pack is damaged (i.e., broken elastomer), discard the pack.

Well	Contents	Ingredients
R1a:	3.37 mL	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:	3.1 mL	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.

*ProClin is a trademark of LANXESS Corp.

WARNING AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- For hazards presented by the product refer to the following sections: REACTIVE INGREDIENTS and GHS HAZARD CLASSIFICATION.

REACTIVE INGREDIENTS

⚠ CAUTION

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

GHS HAZARD CLASSIFICATION

PMP (Compartment R1a)	DANGER	
		
		
	H316	Causes mild skin irritation.
	H317	May cause an allergic skin reaction.
	H318	Causes serious eye damage.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P310	Immediately call a POISON CENTER or doctor/physician.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		Ethoxylated lauryl alcohol 1 - 5%
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
MxPSA - ALP (Compartment R1b)	WARNING	
		
	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		Sodium Azide < 0.15%
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

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

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

1. Access Hybritech PSA Calibrators
   Ref. 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.
   Ref. 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
   Ref. No. 37206
4. Access Substrate
   Ref. No. 81906
5. Access Wash Buffer II, Ref. No. A16792
   UniCel DxI Wash Buffer II, Ref. No. A16793
   UniCel DxI Access Immunoassay Systems Wash Buffer II, Ref. No A79784
   (Diluent pack for use with the UniCel DxI system onboard dilution feature.)

EQUIPMENT AND MATERIALS

R1	Access Hybritech PSA Reagent Packs

CALIBRATION

CALIBRATION INFORMATION

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.[39]

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.[40] 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. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish mean values and acceptable ranges to assure proper performance. Quality control results that do not fall within acceptable ranges may indicate invalid test results. Examine all test results generated since obtaining the last acceptable quality control test point for this analyte. Refer to the appropriate system manuals and/or Help system for information about reviewing quality control results.

TESTING PROCEDURE(S)

PROCEDURAL COMMENTS

1. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.
2. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.
3. Use twenty five (25) µL of sample for each determination in addition to the sample container and system dead volumes. Use fifty (50) µL of sample in addition to the sample container and system dead volumes for each determination run with the DxI system onboard dilution feature. 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.

RESULTS INTERPRETATION

Patient test results are determined automatically by the system software. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient 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.

REPORTING RESULTS

EXPECTED RESULTS

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.[8] 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 2.0.

[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.

Table 2.0 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 (26.9-34.3)	104	102	69 (68%)	33 (32%)
DRE +	946 (15%)	626	136	22 (18.5-25.0)	83	78	53 (68%)	25 (32%)
PSA ≤ 4.0 DRE-	4,750 (75%)	0	N/A	N/A	N/A	N/A	N/A	N/A
PSA > 4.0 DRE-	678 (11%)	414	94	23 (18.7-26.8)	52	52	40 (77%)	12 (23%)
PSA ≤ 4.0 DRE+	701 (11%)	446	48	11 (7.9-13.6)	31	28	24 (86%)	4 (14%)
PSA > 4.0 DRE+	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

† 95% Confidence Interval (Lower limit - Upper Limit)

DRE: Digital Rectal Examination

N/A: Not Available - Not Part of Study Protocol

[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 3.0 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 3.0 % 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 4.0.

Table 4.0 Percent of Biopsied Subjects with Cancer Corresponding to Test Results[h]

Results Category		Percent of Biopsied Subjects with Cancer		Number of Biopsied Subjects with Cancer
		%	(95% CI)[††]	
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 5.0.

Table 5.0 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 6.0. 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 6.0 % 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	1,469	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

Table 6.0 % Distribution of PSA (ng/mL)[h], Continued

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)
Other	394	98	2	0	0	0
TOTAL	1,154	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.

PROCEDURAL NOTES

LIMITATIONS

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). When the DxI system onboard dilution feature is used, the system will report Hybritech-calibrated results less than 127.5 ng/mL as < 127.5 ng/mL and WHO calibrated results less than 110.5 ng/mL as < 110.5 ng/mL.
   - 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 or Wash Buffer II. 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. The DxI system onboard dilution feature automates the dilution process, using one volume of sample with 9 volumes of UniCel DxI Access Immunoassay Systems Wash Buffer II, allowing samples to be quantitated up to approximately 1,500 ng/mL for Hybritech calibration and 1,210 ng/mL for WHO calibration. 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.[41,42]

   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.[39]

PERFORMANCE CHARACTERISTICS

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 7.0 Imprecision with the Hybritech Calibration[h]

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 8.0 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, 1,500 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 9.0 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
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.[43] 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
UniCel DxI 600 v. UniCel DxI 800	218	0.10-145.15	0.39 (-0.18 to 0.96)	1.005 (0.992 to 1.019)	0.990

[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.

ADDITIONAL INFORMATION

For a patient/user/third party in the European Union and in countries with identical regulatory regime (Regulation 2017/746/EU on In vitro Diagnostic Medical Devices); if, during the use of this device or as a result of its use, a serious incident has occurred, please report it to the manufacturer and/or its authorized representative and to your national authority.

The Summary of Safety and Performance is available from the EUDAMED database: ec.europa.eu/tools/eudamed

REVISION HISTORY

Revision A

New release of IVDR compliant IFU.

Revision B

Updated Translations.

Revision C

Added Translations.

Revision D

Updated ProClin trademark statement.

Revision E

Updated Translations.

SYMBOLS KEY

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

REFERENCES

1. Sturgeon CM, et al., National Academy of Clinical Biochemistry laboratory medicine practice guidelines for use of tumor markers in testicular, prostate, colorectal, breast, and ovarian cancers. In Chapter 3: Tumor Markers in Prostate Cancer by Lilja H., et al. Clin Chem. 2008 Dec;54(12):e11-79.

2. SEER Cancer Stat Facts: Prostate Cancer. National Cancer Institute. Bethesda, MD, https://seer.cancer.gov/statfacts/html/prost.html. Accessed March 2021.

3. American Cancer Society, Key Statistics for Prostate Cancer. https://www.cancer.org/cancer/prostate-cancer/about/key-statistics.html. Accessed March 2021.

4. Walsh PC. Why make an early diagnosis of prostate cancer. J Urol 1992;147: 853-854.

5. Catalona WJ, Smith DS, Ratliff TL, Dodds KM, Coplen DE, Yuan JJ, Petros JA, Andriole GL. Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. New Engl J Med 1991;324: 1156-1161.

6. Mettlin C, Lee F, Drago J, Murphy GP. The American Cancer Society National Prostate Cancer Detection Project-Findings on the detection of early prostate cancer in 2425 men. Cancer 1991;67: 2949-2958.

7. Lee F, McHugh TA, Solomon MH, Dorr RP, Siders DB, Kirscht JL, Christensen LL, Mitchell A. Transrectal ultrasound, digital rectal examination, and prostate-specific antigen: Preliminary results of an early detection program for prostate cancer. Scand J Urol Nephrol 1991;137 (Suppl): 101-105.

8. Catalona WJ, Richie JP, Ahmann FR, Hudson MA, Scardino PT, Flanigan RC, deKernion JB, Ratliff TL, Kavoussi LR, Dalkin BL, Waters WB, MacFarlane MT, Southwick PC. Comparison of digital rectal examination and serum prostate specific antigen in the early detection of prostate cancer: Results of a multicenter clinical trial of 6,630 men. J Urol 1994; 151: 1283-1290.

9. Stamey TA, Yang N, Hay AR, McNeal JE, Freiha FS, Redwine E. Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. New Engl J Med 1987;317: 909-916.

10. Stamey TA, Kabalin JN. Prostate specific antigen in the diagnosis and treatment of adenocarcinoma of the prostate. I. Untreated patients. J Urol 1989;141: 1070-1075.

11. Killian CS, Yang N, Emrich LJ, Vargas FP, Kuriyama M, Wang MC, Slack NH, Papsidero LD, Murphy GP, Chu TM. Prognostic importance of prostate-specific antigen for monitoring patients with stages B2 to D1 prostate cancer. Cancer Res 1985;45: 886-891.

12. Stamey TA, Kabalin JN, McNeal JE, Johnstone IM, Freiha FS, Redwine E, Yang N. Prostate specific antigen in the diagnosis and treatment of adenocarcinoma of the prostate. II. Radical prostatectomy treated patients. J Urol 1989;141: 1076-1083.

13. Stamey TA, Kabalin JN, Ferrari M. Prostate specific antigen in the diagnosis and treatment of adenocarcinoma of the prostate. III. Radiation treated patients. J Urol 1989;141: 1084-1087.

14. Stamey TA, Kabalin JN, Ferrari M, Yang N. Prostate specific antigen in the diagnosis and treatment of adenocarcinoma of the prostate. IV. Anti-androgen treated patients. J Urol 1989;141: 1088-1090.

15. Schacht MJ, Garnett JE, Grayhack JT. Biochemical markers in prostatic cancer. Urol Clin No Amer 1984;11: 253-267.

16. Wang MC, Valenzuela LA, Murphy GP, Chu TM. Purification of a human prostate specific antigen. Invest Urol 1979; 17: 159-163.

17. Lilja H. Structure, function and regulation of the enzyme activity of prostate specific antigen. World J Urol 1993; 11: 188-191.

18. Lilja H, Christensson A, Dahlen V, et al. Prostate-specific antigen in human serum occurs predominately in complex with alpha 1 antichymotrypsin. Clin Chem 1991;37:1618-1625.

19. Graves HC. Standardization of immunoassays for prostate-specific antigen. Cancer 1993; 72: 3141-3144.

20. Graves HC. Issues on standardization of immunoassays for prostate-specific antigen. Clin Invest Med 1993; 16: 415-424.

21. McCormack RT, Rittenhouse HG, Finlay JA, Sokoloff RL, Wang TJ, Wolfert RL, Lilja H, Oesterling JE. Molecular forms of prostate-specific antigen and the human kallikrein gene family: A new era. Urology 1995;45: 729-744.

22. Nadji M, Tabei SZ, Castro A, Chu TA, Murphy GP, Wang MC, Morales AR. Prostate specific antigen: An immunological marker for prostate neoplasms. Cancer 1981; 48: 1229-1232.

23. Wang MC, Papsidero LD, Kuriyama M, Valenzuela LA, Murphy GP, Chu TM. Prostatic antigen: A new potential marker for prostatic cancer. Prostate 1981; 2: 89-96.

24. Frankel AE, Rouse RV, Wang MC, Chu TM, Herzenberg LA. Monoclonal antibodies to a human prostate antigen. Cancer Res 1982; 42: 3714-3717.

25. Papsidero LD, Wang MC, Valenzuela LA, Murphy GP, Chu TM. A prostate antigen in sera of prostatic cancer patients Cancer Res 1980; 40: 2428-2432.

26. Stamey TA, Teplow DB, Graves HC. Identity of PSA purified from seminal fluid by different methods: comparison by amino acid analysis and assigned extinction coefficients. Prostate 1995;27:198-203.

27. Carter HB, Pearson JD, Metter EJ, et al. Longitudinal evaluation of prostate-specific antigen levels in men with and without prostate disease. JAMA. 1992; 267:2215-20.

28. Carter HB, Ferrucci L, Kettermann A, et al. Detection of life-threating prostate cancer with prostate-specific antigen velocity during a window of curabliity. J Natl Cancer Inst. 2006;98:1521-7.

29. Punglia RS, D' Amico AV, Catalona WJ, et al. Effect of verification bias on screening for prostate cancer by measurement of prostate-specific antigen. N Eng J Med. 2003;349:335-342

30. Catalona WJ, Smith BS, Ornstein DK. Prostate cancer detection in men with serum PSA concentrations of 2.6 to 4.0 ng/mL and benign prostatic examination: Enhancement of specificity with free PSA measurements. JAMA 1997;277:1452-1455.

31. Babaian RJ, Johnston DA, Naccarato W, et al. The incidence of prostate cancer in a screening population with a serum prostate specific antigen between 2.5 and 4.0 ng/mL: Relationship to biopsy strategy. J Urol 2001;165:757-760.

32. Armitage TG, Cooper EH, Newling DW, Robinson MR, Appleyard, I. The value of the measurement of serum prostate specific antigen in patients with benign prostatic hyperplasia and untreated prostate cancer. Br J Urol 1988 Dec;62(6):584-9.

33. D'Amico AV, Whittington R, Malkowicz SB, Schultz D, Blank K, Broderick GA, et al. Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer. JAMA, 280:969,1998.

34. Ornstein DK, Rao GS, Smith DS, Ratliff TL, Basler JW, Catalona WJ. Effect of digital rectal examination and needle biopsy on serum total and percentage of free prostate specific antigen levels. J Urol 1997; 157: 195-198.

35. Yuan JJ, Coplen DE, Petros JA, Figenshau RS, Ratliff TL, Smith DS, Catalona WJ. Effects of rectal examination, prostatic massage, ultrasonography and needle biopsy on serum prostate specific antigen levels. J Urol 1992; 147: 810-814.

36. Woodrum DL, French C, Shamel LB. Stability of free PSA in serum samples under a variety of sample collection and sample storage conditions. Urology 1996; 48 (Suppl): 33-39.

37. Woodrum DL, French CF, Hill TM, Roman SJ, Slatore HL, Shaffer JL, York LG, Eure KL, Loveland KG, Gasior GH, Southwick PC, Shamel LB. Analytical performance of the Tandem®-R free PSA immuno-assay measuring free prostate specific antigen. Clin Chem 1997; 43:1203-1208.

38. Woodrum DL, York L. Two year stability of free and total PSA in frozen serum samples. Urology 1998; 52: 247-251.

39. Lilja H, et al. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines (LMPG): Practice Guidelines and Recommendations for Use of Tumor Markers in the Clinic, Prostate Cancer (Section B), Draft 2006. National Academy of Clinical Biochemistry.

40. Cembrowski GS, Carey RN. Laboratory quality management: QC ⇌ QA. ASCP Press, Chicago, IL, 1989.

41. Kricka L. Interferences in immunoassays - still a threat. Clin Chem 2000; 46: 1037-1038.

42. Bjerner J, et al. Immunometric assay interference: incidence and prevention. Clin Chem 2002; 48: 613 - 621.

43. Approved Guidelines - Protocols for determination of limits of detection and limits of quantitation, EP17-A. October 2004. National Committee for Clinical Laboratory Standards.

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