Cat. No. RF450

DADE BEHRING

Dimension® clinical chemistry system
Heterogeneous Immunoassay Module

PSA

Prostate Specific Antigen Flex® reagent cartridge

*CAUTION: United States 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. Prior to changing assays, the laboratory MUST confirm baseline values for patients being serially monitored.

*Intended Use: The PSA method for the Dimension® clinical chemistry system with the heterogeneous immunoassay module is an *in vitro* diagnostic test intended to quantitatively measure prostate specific antigen (PSA) in human serum:

1. as an aid in the detection of prostate cancer when used in conjunction with digital rectal exam (DRE) in men 50 years or older. Prostate biopsy is required for diagnosis of cancer.
2. as an aid in the management (monitoring) of prostate cancer patients.

*Summary: Prostate specific antigen (PSA) is a serine protease of approximately 30,000 Daltons produced by the epithelial cells of the prostate gland.[1,2] The level of PSA in serum and other tissues is normally very low. In malignant prostate disease (prostatic adenocarcinoma) and in non-malignant disorders such as benign prostate hypertrophy (BPH) and prostatitis, the serum level of PSA may become elevated.[3]

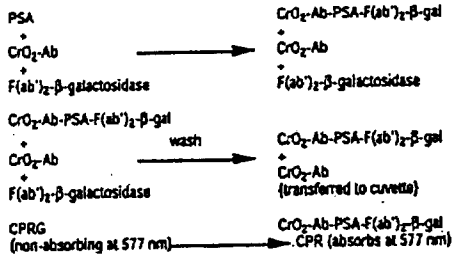
Most of the PSA protein in serum is complexed with either α1-antichymotrypsin (ACT) or α2-macroglobulin.[4,5] The PSA protein associated with α 2-macroglobulin is encapsulated and unavailable for measurement by current immunoassay systems. About 90% of the PSA in serum exists as PSA-ACT complex and 10% is free. The Dimension® PSA assay measures both the free and the ACT bound components of serum PSA.

The specificity of PSA to prostate tissue makes it a significant marker in the early detection and management of prostate diseases.

Prostate cancer is the most common type of cancer found in men in the United States and the second leading cause of male cancer mortality, accounting for more than 30,000 deaths in 1999.[6] Prior to the use of PSA for early detection of prostate cancer, the traditional method of digital rectal examination (DRE) detected considerably fewer tumors.[3,7] The most sensitive method for early detection of prostate cancer uses both DRE and PSA and in January 1992, the American Urological Association (AUA) endorsed annual examination for early detection of prostate cancer with DRE and PSA beginning at age 50.[8] An abnormal DRE or an elevated PSA may suggest the presence of prostate cancer, however a prostate biopsy is required for final diagnosis.

PSA testing is also accepted as a test in the monitoring of previously diagnosed prostate cancer patients.[9,10] Serum levels of PSA are most useful when sequential values are obtained and monitored over time. After complete removal of the prostate gland (radical prostatectomy), PSA levels should decline to a very low or nondetectable level. A rise of the serum PSA level in prostatectomy patients indicates residual prostate tissue, recurrence or metastasis of the disease.[11] Serum PSA levels during radiation treatment should decline and remain at baseline while the patient is in remission.[12]

Principles of Procedure: The PSA method is a one step enzyme immunoassay based on the "sandwich" principle. Sample is incubated with chromium dioxide particles coated with monoclonal antibodies specific for a binding site on PSA, and conjugate reagent (β-galactosidase labelled monoclonal antibodies specific for a second binding site on the PSA molecule) to form a particle/PSA/conjugate sandwich. Unbound conjugate and analyte are removed by magnetic separation and washing. The sandwich bound β-galactosidase is combined with the chromogenic substrate chlorophenol red-β-d galactopyranoside (CPRG). Hydrolysis of CPRG releases a chromophore (CPR). The color change measured at 577 nm due to formation of CPR is directly proportional to the concentration of PSA present in the patient sample.



Reagents

Wells[a]	Form	Ingredient	Concentration[b]	Source
1	Liquid	PSA Ab-β-galactosidase[c]	0.09 mg/mL	Mouse, monoclonal
3	Tablet[d]	Antibody-CrO_2[c]	1.8 mg/mL	Mouse, monoclonal
4,5,6	Tablet[d]	CPRG	10.3 mg/mL	
7	Liquid	Substrate diluent	42 mg/mL	

a. Wells are numbered consecutively from the wide end of the cartridge.
b. Nominal value in hydrated cartridge.
c. Antibody titer and conjugate activity may vary from lot to lot.
d. Tablets contain excipients, buffers, and stabilizers.

Precautions: Used cuvettes contain human body fluids; handle with appropriate care to avoid skin contact or ingestion.

For *in vitro* diagnostic use

Reagent Preparation: Mixing and diluting are automatically performed by the Dimension® system.

Storage Instructions: Store at 2-8°C.

Expiration: Refer to carton for expiration date of individual unopened reagent cartridges. Sealed or unhydrated cartridge wells on the instrument are stable for 30 days. Once wells 1,3 and 7 have been entered by the instrument, they are stable for 15 days. Once wells 4,5 and 6 have been entered by the instrument, they are stable for 5 days.

Specimen collection: Serum can be collected by normal procedures.[13] Serum specimens may be refrigerated at 2-8°C for up to 14 days. For longer storage, samples may be frozen at -20°C.[14] Mix thoroughly after thawing. Avoid repeated freezing and thawing.

Specimens should be free of particulate matter. To prevent the appearance of fibrin in serum samples, complete clot formation should take place before centrifugation. Clotting time may be increased due to thrombolytic or anticoagulant therapy.

An erroneously elevated PSA level can be observed if the serum specimen from a patient is collected following digital rectal examination (DRE), needle biopsy or transurethal resection.[14]

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.

Known Interfering Substances

The following substances do not interfere with the PSA method when present in serum at the concentrations indicated. Systemic inaccuracies (bias) due to these substances are less than 10% at a PSA level of 3.0 to 5.0 ng/mL:

Compound	Concentration
Acetaminophen	20 mg/dL [1322 µmol/L]*
Albumin	6 g/dL [60 g/L]
Amikacin	15 mg/dL [256 µmol/L]
Aminoglutethimide	6 ng/mL [26 nmol/L]
Ampicillin	5 mg/dL [143 µmol/L]
Ascorbic acid	3 mg/dL [170.3 µmol/L]
Bilirubin	60 mg/dL [1026 mmol/L]
Caffeine	10 mg/dL [515 µmol/L]
Carbamazepine	12 mg/dL [508 µmol/L]
Chloramphenicol	25 mg/dL [774 µmol/L]
Chlordiazepoxide	2 mg/dL [67 µmol/L]
Chlorpromazine	5 mg/dL [157 nmol/L]
Cimetidine	10 mg/dL [397 µmol/L]
Cholesterol	500 mg/dL [12.9 mmol/L]
Creatinine	30 mg/dL [2652 µmol/L]
Cyclophosphamide	25 mg/dL [896 µmol/L]

Dextran 75	1250 mg/dL [12.5 g/L]
Diazepam	2 mg/dL [70 µmol/L]
Diethylstilbestrol	0.02 mg/dL [700 nmol/L]
Digoxin	5 ng/mL [6.4 nmol/L]
Doxorubicin-HCl	7 mg/dL [121 µmol/L]
Erythromycin	20 mg/dL [272 µmol/L]
Estramustine phosphate	20 mg/dL [343 µmol/L]
Ethanol	350 mg/dL [76 mmol/L]
Ethosuximide	30 mg/dL [2125 µmol/L]
Finasteride	0.2 mg/dL [5.4 µmol/L]
Flutamide	1 mg/dL [36 µmol/L]
Furosemide	2 mg/dL [60 µmol/L]
Gentamicin	12 mg/dL [251 µmol/L]
Goserelin acetate	0.01 mg/dL [79 nmol/L]
Hemoglobin	1000 mg/dL [0.62 mmol/L] (monomer)
Heparin	8000 U/L [8000 U/L]
Human plasma kallikrein	100 µg/mL [1 µmol/L]
Ibuprofen	40 mg/dL [1939 µmol/L]
Immunoglobulin G	8 g/mL [60 g/L]
Ketoconazole	7 mg/dL [132 µmol/L]
Leuprolide acetate	10 mg/dL [86 µmol/L]
Lidocaine	6 mg/dL [256 µmol/L]
Lipemia	3000 mg/dL [33.9 mmol/L] (triglyceride)
Lithium	3.5 mg/dL [5.07 mmol/L]
Megestrol acetate	2 mg/dL [52 µmol/L]
Methotrexate	300 mg/dL [7 mmol/L]
Nicotine	2 mg/dL [123 µmol/L]
Penicillin G	25 U/mL [25,000 U/L]
Pentobarbital	10 mg/dL [442 µmol/L]
Phenobarbital	15 mg/dL [646 µmol/L]
Phenytoin	10 mg/dL [396 µmol/L]
Primidone	10 mg/dL [458 µmol/L]
Propoxyphene	0.4 mg/dL [12 µmol/L]
Prostatic Acid Phosphatase	1000 ng/mL [1000 µg/L]
Protein (low)	4 g/dL [40 g/L]
Protein (high)	12 g/dL [120 g/L]
Rheumatoid factor	750 IU/mL [750 IU/mL]
Salicylic acid	50 mg/dL [3.62 mmol/L]
Theophylline	25 mg/dL [1388 µmol/L]
Urea	500 mg/dL [179 µmol/L]
Uric acid	20 mg/dL [1.2 mmol/L]
Valproic acid	50 mg/dL [3467 µmol/L]

a. Système International d'Unités [SI units] are in brackets.

Procedure

Materials Needed

PSA Flex® reagent cartridge, Cat. No. RF450
Reaction Vessels, Cat. No. RXV1
Chemistry Wash, Cat. No. RD701
Sample Probe Cleaner, Cat. No. RD703
Sample Diluent, Cat. No. 791092901

Test Steps

Sampling, reagent delivery, mixing, separation, processing and printing of results are automatically performed by the Dimension® system with the heterogeneous immunoassay module. For details of this processing, refer to your Dimension® system manual.

Test Conditions

Reaction Vessel

• Sample size	40 µL
• Antibody- CrO_2	30 µL
• Antibody-ß-galactosidase	50 µL
• Temperature	37.0° C
• Incubation period	10.6 minutes

Reaction Cuvette

• Transfer Volume	50 µL
• CPRG Reagent Volume	150 µL
• Diluent Volume	200 µL
• Temperature	37.0 ± 0.1° C
• Reaction period	3.5 minutes
• Wavelength	577 and 700 nm
• Type of measurement	Bichromatic Rate
• Units	ng/mL [µg/L]

*Calibration

The general calibration procedure is described in your Dimension® system manual.

The following information should be considered when calibrating the PSA method:

• Assay Range	0.1 - 100.0 ng/mL [µg/L]
• Reference Material	PSA Calibrator Cat. No. RC450
• Suggested Calibration Levels	0.0, 4.0, 10.0, 45.0, 105.0 ng/mL [µg/L]
• Calibration Scheme, Replicates	5 @ Level 1 2 @ Levels 2 & 3 3 @ Levels 4 & 5
• Calibration Frequency:	Each new reagent cartridge lot. Every 90 days for any lot.
• Assigned Coefficients:	C_0 -165.0 C_1 1261.0 C_2 -2.7 C_3 185.0 C_4 0.5

Quality Control

At least once each day of use, analyze two levels of Dade Behring PSA Control (Cat. # RC451). The results obtained should fall within acceptable limits defined by the laboratory. For further details refer to your Dimension® system manual.

A system malfunction may exist if the following five test precision is observed:

PSA Concentration	S.D.
4.0 ng/mL [µg/L]	0.20 ng/mL [µg/L]
45.0 ng/mL [µg/L]	1.99 ng/mL [µg/L]

Results: The instrument automatically calculates and prints the concentration of PSA in ng/mL [µg/L].

Limitations of Procedure

Results:	> 100 ng/mL [µg/L]
Manual dilution:	Make appropriate dilution with Sample Diluent to obtain results within assay range. Enter dilution factor. Reassay. Resulting readout is corrected by dilution.
Autodilution:	Refer to your Dimension® system manual.
Results:	< 0.1 ng/mL [µg/L] should be reported as less than 0.1 ng/mL [µg/L].

PSA levels may be lower in patients who receive hormonal therapy and may not adequately reflect the presence of residual or recurrent disease.[15]

One step sandwich immunometric assays are susceptible to a high-dose "hook effect", where an excess of antigen prevents simultaneous binding of the capture and detection antibodies to a single analyte molecule.[16] The PSA method shows no hook effect up to 51,000 ng/mL [µg/L].

Patient samples may contain heterophilic antibodies that could react in immunoassays to give falsely elevated or depressed results. This assay has been designed to minimize interference from heterophilic antibodies.[17]

The instrument reporting system contains error messages to warn the operator of specific malfunctions. Any report slip containing such error messages should be held for follow-up. Refer to your Dimension® system manual.

*Expected values in detection of prostate cancer: The following table summarizes the results of a retrospective study using samples from 699 men aged 50 years or older who were referred to a urologist for determination of the presence of prostate cancer. These samples were collected from 38 clinical sites across the United States. All of these men had a biopsy performed. Samples were tested for PSA using the Dimension® PSA method. The PSA results, corresponding DRE and biopsy results are shown below. The study demonstrated that PSA testing, when used in conjunction with DRE, was more effective in detecting prostate cancer than DRE alone.

*Distribution of results:

	Biopsy Result		
	Benign	Malignant	Total
DRE +	114	90	204
DRE -	348	147	495
Mean PSA (ng/mL)	7.48	14.20	9.78
Total	462	237	699
PSA > 4.0, DRE -	290	135	425
PSA > 4.0, DRE +	84	80	164
Total	374	215	589
PSA ≤ 4.0, DRE -	58	12	70
PSA ≤ 4.0, DRE +	30	10	40
Total	88	22	110

The Predictive Power (PPV) was estimated as the probability of having a positive biopsy given a positive result for DRE, PSA and PSA with DRE. The results are as follows:

Method	PPV %	Interval
DRE + only	44.1	37.8 - 50.9
PSA > 4.0 only	36.5	32.6 - 40.4
PSA > 4.0 or DRE +	35.8	32.0 - 39.5
PSA > 4.0 and DRE +	48.8	41.1 - 56.5
PSA > 4.0 and DRE -	31.8	27.3 - 36.2

Serum PSA concentrations, regardless of the value, should not be interpreted as definitive evidence for the presence or absence of prostate cancer. Prostate biopsy is required for the diagnosis of cancer.

*Expected values in management of prostate cancer patients: In a separate population of 617 subjects at a major Medical Center, the distribution of PSA values is shown in the table below:

Distribution of PSA Values, Percent (%)

	Number of Subjects	0-4 (ng/mL)	4.1-10 (ng/mL)	10.1-30 (ng/mL)	30.1-60 (ng/mL)	>60 (ng/mL)
Healthy Subjects						
Males > 40 yrs	168	92.3	7.7	0.0	0.0	0.0
Males < 40 yrs	122	99.2	0.8	0.0	0.0	0.0
Females	31	100.0	0.0	0.0	0.0	0.0
Malignant Diseases						
Prostate	160	42.5	11.9	22.5	11.2	11.9
Gastrointestinal	25	92.0	8.0	0.0	0.0	0.0
Genitourinary	30	93.4	3.3	3.3	0.0	0.0
Pulmonary	25	100.0	0.0	0.0	0.0	0.0
Non-malignant Diseases						
BPH	56	89.3	7.1	3.6	0.0	0.0

Each laboratory should establish its own expected values for PSA as performed on the Dimension® system.

When changing PSA assays in the course of monitoring a patient, additional sequential testing should be carried out to confirm baseline values.

*Specific Performance Characteristics[f]

Reproducibility[g,h]

ng/mL	Mean SD (%CV)	Within-Run SD (%CV)	Total Material
Serum pool 1	4.21	0.10 (2.3)	0.15 (3.5)
Serum pool 2	50.11	1.00 (2.0)	1.57 (3.1)
Dade PSA Control 1	4.08	0.10 (2.4)	0.16 (3.9)
Dade PSA Control 2	48.67	0.92 (1.9)	1.36 (2.8)

f. All specific performance characteristics tests were run after normal recommended equipment quality control checks were performed (refer to your Dimension® system manual).

g. Reproducibility testing was done in accordance with the NCCLS Approved Guideline for Evaluation of Precision Performance of Clinical Chemistry Devices (EP5-A, Feb.1999).

h. Specimens at each level were analyzed in duplicate, once a day, for 20 days. The within-run and total standard deviations were calculated by analysis of variance method.

Correlation
Regression Statistics

Comparative Method	Slope	Intercept ng/mL [µg/L]	Correlation Coefficient	n[i]
acs® *plus*	1.06	-0.07	0.994	596

i. The range of PSA values in the correlation study was 0.00 to 99.90 ng/mL[µg/L].

Analytical Sensitivity

The sensitivity of the PSA method is 0.1 ng/mL [µg/L], and represents the lowest concentration of PSA that can be distinguished from zero. This sensitivity is defined as the concentration at two standard deviations above the level 1 (0 ng/mL [µg/L]) PSA Calibrator (n= 20).

Analytical Specificity

The following substance does not interfere with the PSA assay at the concentration indicated:

Substance	Concentration	Apparent PSA Concentration
Prostatic acid phosphatase	1000 ng/mL [µg/L]	0.01 ng/mL [µg/L]

Recovery

Known amounts of PSA were added to human serum samples with baseline PSA values of 0.10 to 1.32 ng/mL [µg/L]. Sample PSA concentrations were measured and the percent recovery calculated. Recovery ranged from 97.0 to 104.9% with an average recovery of 101.5%.

Bibliography:

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* An asterisk in the margin denotes a revised section.