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CORE – PSA DEVICE

Rapid test for detection of Prostate Specific Antigen in serum / plasma / whole blood

INTRODUCTION

CORE -PSA is a rapid, semi-quantitative, two site sandwich immunoassay for the detection of Prostate Specific Antigen (PSA) levels in human serum / plasma / whole blood.

SUMMARY

PSA is a glycoprotein produced almost exclusively by the epithelial component of the prostate gland. It has a molecular mass of 33 kDa and is a single-chain glycoprotein with 237 amino acid residues. PSA is synthesized in the epithelial cells along the acini and in the ductal epithelium of the prostate gland. Its function is to bring about lysis of the semen coagulum thus accounting for the critical role it plays in male fertility.

The tissue specificity of PSA makes it the most useful tumour marker available for the diagnosis and treatment of prostate cancer. Complete removal of the prostate should result in an undetectable PSA level. Any measurable PSA after radical prostatectomy would indicate residual prostate tissues or metastasis. In such patients, increasing PSA concentrations after successful surgery strongly indicate recurrent disease. However, if the detectable serum PSA after radical prostatectomy is a result of incomplete resection of the gland and not persistent disease, the level should remain unchanged on extended follow-up.

CORE-PSA is a rapid test for the semi-quantitative determination of PSA in human serum / plasma / whole blood.

PRINCIPLE

CORE-PSA utilizes the principle of Immunochromatography, a unique two-site immunoassay on a nitrocellulose membrane. The conjugate pad contains two components - monoclonal anti-PSA antibody conjugated to colloidal gold and rabbit IgG conjugated to colloidal gold. As the test specimen flows through the membrane assembly of the device, the highly specific monoclonal anti-PSA antibody-colloidal gold conjugate complexes with the PSA in the specimen and travels on the membrane due to capillary action along with the rabbit IgG-colloidal gold conjugate. This complex moves further on the membrane to the test region (T) where it is immobilized by another specific monoclonal anti-PSA antibody coated on the membrane leading to formation of a pink to pink-purple coloured band. A detectable coloured band is formed if PSA level is equal to or higher than 4 ng/ml. The absence of this coloured band in the test region indicates PSA concentration < 4 ng/ml in the specimen.

The rabbit IgG-colloidal gold conjugate and unbound complex, if any, moves further to the reference region (R) that contains pre-calibrated anti-rabbit IgG antibodies, corresponding to 10 ng/ml PSA, immobilised on the membrane. The intensity of the coloured band formed at the reference region (R) corresponds to a PSA concentration of 10 ng/ml. This reference band would form even in a negative specimen. Semi-quantitative information about the concentration of PSA can be deduced by comparing the intensity of the test band against the reference band.

The unreacted conjugate and unbound complex, if any, move further on the membrane and are subsequently immobilized by the anti-rabbit antibodies coated on the membrane at the control region (C), forming a pink to pink-purple coloured band. This control band acts as a procedural control and serves to validate the test results.

NORMAL REFERENCE VALUES

0-4 ng/ml	: Normal
4-10 ng/ml	: Probable benign prostatic hypertrophy (BPH)
Above 10 ng/ml	: Probable adenocarcinoma of the prostate

REAGENTS AND MATERIALS SUPPLIED

Each kit contains:

- A. Individual pouches each containing a -
 1. Test device: Membrane assembly pre-dispensed with monoclonal anti-PSA antibody-colloidal gold conjugate, rabbit IgG-colloidal gold conjugate, monoclonal anti-PSA antibody and anti-rabbit antiserum coated at the respective regions.

- 2.Desiccant pouch
- 3.Sample dropper
- B. Sample Running Buffer: 0.1 M Tris buffer with 0.1% Sodium azide.
- C. Package Insert

Cat. No.	PSA-121210	PSA-121212
Tests	10	25

STORAGE AND STABILITY

The sealed pouches in the test kit may be stored between 4-30°C for the duration of shelf life as indicated on the pouch / carton. DO NOT FREEZE.

NOTE

1. For in vitro diagnostic use only. NOT FOR MEDICINAL USE. For professional use
2. Do not use beyond expiration date.
3. Read the instructions carefully before performing the test.
4. Handle all specimens as if potentially infectious.
5. Follow standard biosafety guidelines for handling and disposal of potentially infectious material.
6. Sample Running buffer contains sodium azide (0.1%), avoid skin contact with this reagent. Azide may react with lead and copper in the plumbing and form highly explosive metal oxides. Flush with large volumes of water to prevent azide build-up in the plumbing. If desiccant colour at the point of opening the pouch has turned from blue to pink or colourless, another test device must be run.

SPECIMEN COLLECTION AND PREPARATION

1. **CORE-PSA** uses human serum / plasma / whole blood as specimen.
2. No special preparation of the patient is necessary prior to specimen collection by approved techniques. However, please refer chart below of "PRE-ANALYTICAL FACTORS" for appropriate time of collection of sample.
3. For whole blood, collect blood with a suitable anticoagulant such as EDTA or Heparin or Oxalate and use the freshly collected blood.
4. Whole blood should be used immediately and should not be frozen.
5. Though fresh specimen is preferable, in case of delay in testing, it may be stored **at 2-8 °C for maximum up to 24 hrs.**
6. If serum is to be used as specimen, allow blood to clot completely. Centrifuge to obtain clear serum.
7. Repeated freezing and thawing of the specimen should be avoided.
8. Do not use turbid, lipaemic and hemolysed serum/plasma.
9. Do not use hemolysed, clotted or contaminated blood specimens.
10. Specimen containing precipitates or particulate matter must be centrifuged and the clear supernatant only used for testing.
11. Refrigerated specimens must be brought to room temperature prior to testing.

PRE-ANALYTICAL FACTORS AFFECTING PSA VALUES

Several factors affect PSA results in immunoassays. The time of sample collection is crucial to obtain a correct picture. The best time to collect sample is before any procedure / event like DRE, ejaculation etc. (depicted below in the chart). However, if the procedure has already taken place, then the following chart would indicate the time of sample collection.

Pre-analytical factors	Effect on Total-PSA	When to collect sample
Digital Rectal Examination	Elevated	After 1 week
Ejaculation	Elevated	After 48 hours
Needle biopsy	2-50 fold increase	After 6 weeks

Transurethral resection of prostate	6-50 fold increase	After 6 weeks
Prostate massage	Minor increase	After 1 week
Transrectal Ultrasound	Elevated	After 1 week
Cystoscopy	Elevated	After 1 week
Finasteride therapy	Lowered by 50%	Before therapy

TESTING PROCEDURE AND INTERPRETATION OF RESULTS

1. Bring the kit components of **CORE-PSA** device to room temperature before testing.
2. Open a foil pouch by tearing along the “notch”.
3. Remove the testing device and the sample dropper. Once opened, the device must be used immediately.
4. Label the device with specimen identity.
5. Place the testing device on a flat horizontal surface.
6. Holding the dropper vertically, carefully dispense exactly **one drop** (25µl) of serum / plasma / whole blood into the specimen port “A”.
7. Add **five drops** of sample running buffer into the reagent port “B”.
8. At the end of **15 minutes** read results as follows:

Negative Result

Presence of two coloured bands at Reference (R) and Control (C) regions indicate absence of PSA or the concentration of PSA in the specimen is below 4 ng/ml.



Positive Result

a) If intensity of the Test band (T) is less than the Reference band - PSA concentration is **between 4-10ng/ml**.



b) If intensity of the Test band is equal to or greater than the Reference band - PSA concentration is **≥ 10ng/ml**.



Invalid Result

The test is invalid if the Control band and/or Reference band is not visible at fifteen minutes. Verify the test procedure and repeat the test with a new device.

PERFORMANCE CHARACTERISTICS

Internal Evaluation

In an in-house study, the performance of **CORE-PSA** device was evaluated using a panel of 155 specimens of positive (of varying reactivity) and negative sera in comparison with commercially available ELISA kit. 100% correlation with ELISA was observed. The results of the evaluation are as follows:

SPECIMEN DATA	Total	CORE-PSA	Commercially available ELISA
Number of specimen tested	155	155	155
Number of Positive serum/plasma specimens	10	10	10
Number of Negative serum/plasma specimens	100	100	100
Number of Negative whole blood specimens	45	45	45

Based on this evaluation:

Sensitivity of CORE-PSA : 100%

Specificity of CORE-PSA : 100%

External Evaluation N°1

30 samples were evaluated in parallel with **CORE-PSA** & CHEMILUMINISCENCE method by a NABL-accredited reputed reference laboratory in India. 100% correlation with CHEMILUMINISCENCE was observed. The results of the evaluation are as follows:

SPECIMEN DATA	Total	CORE-PSA	CHEMILUMINISCENCE assay
Number of specimen tested	30	30	30
Number of Positive specimen	10	10	10
Number of Negative specimen	20	20	20

Based on this evaluation:

Sensitivity of CORE-PSA : 100%

Specificity of CORE-PSA : 100%

External Evaluation n°2

196 samples were evaluated in parallel with **CORE-PSA** & chemiluminescence method by a hospital laboratory in France. The results of the evaluation are as follows:

Commercial kit on analyzer	Core PSA			Total
	<4 ng/mL	4-10 ng/mL	≥10 ng/mL	
<4 ng/mL	120	5	0	125
4-10 ng/mL	1	28	1	30
≥10 ng/mL	0	3	38	41
Total	121	36	39	196

Agreement with chemiluminescence method on analyzer is 95%, with a kappa= 0,905. 9 of 10 discrepant results are either comprised between 4 and 10 ng/mL or > 10 ng/mL. Such a result will be followed by a quantification of both free PSA and Total PSA. 1 sample was found <4 ng/mL with **CORE-PSA** and measured 4,42 ng/mL by the chemiluminescence method.

REMARKS

1. The sensitivity and specificity of the PSA test and the threshold at which a result should prompt a biopsy are unclear. The results of prostatic biopsies are often considered as gold standard, but biopsies are generally performed only when the results of a PSA test or digital rectal examination arouse concern, which leads to a workup bias with respect to defining the sensitivity and specificity of the PSA test, and to an overestimation of the sensitivity of the test in particular. Moreover, the majority of small prostate cancers present in many older men is not clinically important and should not be included in the spectrum of disease used to determine the sensitivity of the PSA test. To overcome these problems, Gann *et al* assessed the relation between PSA

levels in base-band serum specimens and the subsequent clinical diagnosis of prostate cancer among the male subjects in the Physicians' Health Study. Based on this study, a PSA value of 4.0 ng/ml has been accepted as the upper limit of the normal level.

2. Based on many independent investigations it is now clear that the PSA level increases with advancing age. As men age, the prostate gland enlarges and contains more PSA-producing tissue. Bigger prostates are associated with higher PSA values. In fact, the PSA concentration correlates directly with prostate size.

To improve clinical staging, prostate-specific antigen (PSA) levels (> 10 ng/ml), sonographic tumour volume (> 3 cc), maximum tumour diameter, length of capsular tumour abutment, and overall impression of capsular irregularity suggesting periprostatic tumour spread may also be assessed.

4. A recent trial evaluating the effect of finasteride on PSA serum concentrations determined that a patient who has taken finasteride for at least 12 months would be able to multiply his PSA concentration by a factor of 2 to establish what that value would have been had he not been taking the drug. According to the same study, the ability of finasteride to lower the PSA levels begins to decline at age 80, and the PSA concentration subsequently begins to increase.

Interferences due to heterophile antibodies, Rheumatoid Factors and other nonanalyte substances in patient's serum, capable of binding antibodies multivalently and providing erroneous analyte detection in immunoassays, has been reported in various studies. Though **CORE-PSA** uses sufficient amounts of blocking reagents to inhibit the majority of this interference; nevertheless, some samples with high titers may still express clinically important assay interference. Both laboratory professionals and clinicians must be vigilant to this possibility of antibody interference. Results that appear to be internally inconsistent or incompatible with the clinical presentation should invoke suspicion of the presence of an endogenous artifact and lead to appropriate in vitro investigative action.

6. The membrane is laminated with an adhesive tape to prevent surface evaporation. Air pockets or patches may appear, which do not interfere with the test results. Presence of a band at the test region even if low in intensity or formation is a positive result.

7. The deliberate slow reaction kinetics of **CORE-PSA** is designed to maximize and enhance reaction time between sample capture and tracer elements to improve test sensitivity.

8. Most positive results develop within 15 minutes. However, certain sera sample may take a longer time to flow. Therefore, negatives should be confirmed only at 30 minutes. Do not read results after 30 minutes.

9. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

10. **CORE-PSA** should be used as a screening test in clinically suspected cases only, and its results should be confirmed by a quantitative method before taking clinical decisions.

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Symbols used on CORE- PSA Labels

	Consult instructions for use
	Storage temperature
	Use by
LOT	Batch code
REF	Catalogue number
IVD	In vitro diagnostic medical device
	Device
	Disposable Plastic Dropper
	Sample Running Buffer
	Date of Manufacture
	Manufactured By
	Contains sufficient for <n> tests

Manufactured by:



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