CE 2265

CANCHECK® - PSA

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

DEVICE

INTENDED USE

CANCHECK® - 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.

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

PRINCIPLE

CANCHECK® - PSA utilizes the principle of agglutination of antibodies/ antisera with respective antigen in immuno-chromatography format along with use of nano gold particles as agglutination revealing agent. The conjugate pad contains two components - Agglutinating sera for PSA conjugated to colloidal gold and rabbit globulin conjugated to colloidal gold. As the test specimen flows through the membrane assembly of the device, the highly specific Agglutinating sera for PSA- colloidal gold conjugate complexes with the PSA in the specimen and travels on the membrane due to capillary action along with the rabbit globulin-colloidal gold conjugate. This complex moves further on the membrane to the test region (T) where it is immobilized by another specific Agglutinating sera for PSA 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 globulin-colloidal gold conjugate and unbound complex, if any, moves further to the reference region(R) that contains pre-calibrated Agglutinating sera for rabbit globulin 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 10ng/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 Agglutinating sera for rabbit globulin coated on the membrane at the control region (C), forming a pink to pink-purple coloured band. The control band formation is based on the 'Rabbit / Agglutinating sera for Rabbit globulin' system. Since it is completely independent of the analyte detection system, it facilitates formation of consistent control band signal independent of the analyte concentration. 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 adenocarcenoma of the prostate.

REAGENTS AND MATERIALS SUPPLIED

CANCHECK® - PSA kit contains:

A. Individual pouches, each containing :
   1. DEVICE Membrane assembly pre-dispensed with Agglutinating sera for PSA - colloidal gold conjugate, rabbit globulin-colloidal gold conjugate, Agglutinating sera for PSA and Agglutinating sera for rabbit globulin coated at the respective regions.
   2. Desiccant pouch.
   3. PIPETTE Disposable Plastic Sample Applicator.

B. BUF Sample Running buffer in a dropper bottle.

C. Package insert.

STORAGE AND STABILITY

The sealed pouches in the test kit & the kit components may be stored between 4°C to 30°C for the duration of shelf life as indicated on the pouch/ carton. DO NOT FREEZE. After first opening of the sample running buffer bottle, it can be stored between 4°C to 30ºC for remaining duration of its shelf life.

REF	50600010	50600025
Σ	10	25

NOTES

Read the instructions carefully before performing the test. For in vitro diagnostic use only. NOT FOR MEDICINAL USE. For professional use only. Do not use beyond expiry date. Do not intermix reagents from different lots. Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS# 7646-79-9) should be kept to a minimum. Inhalation / swallowing may cause harm. Handle all specimens as if potentially infectious. Follow standard biosafety guidelines for handling and disposal of potentially infectious material. 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. **CANCHECK® - 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°C to 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 should be used for testing.
11. Refrigerated specimens must be brought to room temperature prior to testing.

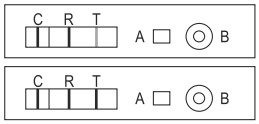
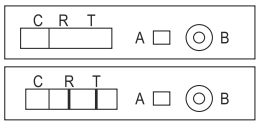
PRE-ANALYTICAL FACTORS AFFECTING PSA VALUES

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 (DRE)	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 **CANCHECK® - 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 applicator. ***Once opened, the device must be used immediately.***
4. Label the test device with patient's identity.
5. Tighten the cap of the sample running buffer bottle provided with the kit in clockwise direction to pierce the buffer bottle nozzle.
6. Place the testing device on a flat horizontal surface.
7. Holding the sample applicator vertically, carefully dispense exactly **one drop** (25µl) of serum/plasma/whole blood into the specimen port 'A'.
8. Add **five drops** of sample running buffer into the buffer port 'B'.
9. 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** ● If intensity of the Test band (T) is less than the Reference band - PSA concentration is **between 4-10ng/ml.** ● 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 no band is visible at fifteen minutes. The test should also be considered invalid if reference band and/or test band appears and no control band appears. Verify the test procedure and repeat the test with a new device.

PERFORMANCE CHARACTERISTICS

Internal Evaluation

In an in-house study, the performance of **CANCHECK® - 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	CANCHECK® - 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 **CANCHECK® - PSA** - 100%.
Specificity of **CANCHECK® - PSA** - 100%.

External Evaluation 1

A panel of 100 samples comprising of PSA negative and PSA positive (varying concentrations), were evaluated with **CANCHECK® - PSA** and the results obtained were compared with results of a commercial ELISA kit as under:

SPECIMEN DATA	Total	CANCHECK® - PSA	Commercial ELISA
Number of specimen tested	100	100	100
Number of Positive serum specimens (> 10 ng/ml)	20	20	20
Number of Positive serum specimens (4-10 ng/ml)	30	30	30
Number of Negative serum samples	50	50	50

Based on this evaluation:
Sensitivity of **CANCHECK® - PSA** - 100%.
Specificity of **CANCHECK® - PSA** - 100%.

External Evaluation 2

A panel of 196 samples comprising of varying levels of PSA , were evaluated with **CANCHECK® - PSA** and simultaneously tested in parallel with Commercial ELISA. The results obtained are as shown below:

SPECIMEN DATA	Commercial ELISA	CANCHECK® - PSA
PSA < 4 ng/ml	125	121
PSA 4-10 ng/ml	30	36
PSA > 10 ng/ml	41	39
Total	196	196

External Evaluation 3

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

SPECIMEN DATA	Total	CANCHECK® - PSA	Chemiluminiscence Assay
Number of specimen tested	30	30	30
Number of Positive specimens	10	10	10
Number of Negative specimens	20	20	20

Intra-assay Precision study

Replicates of 10 tests of **CANCHECK® - PSA** of two different lots were tested at a time with a known PSA negative specimen, a PSA positive specimen of conc. 4-10ng/ml and >10ng/ml each.
Result: No variation in results was observed in each of the lot, indicating 100% co-relation.

Inter-assay Precision study

A known PSA negative specimen, a PSA positive specimen of conc. 4-10ng/ml and >10ng/ml each, were tested three times on three different days, using two different lots of **CANCHECK® - PSA**.
Result: No variation in results was observed , indicating 100% co-relation.

Interference Study

The following substances, upto the given indicated concentrations were found to be non- interfering with **CANCHECK® - PSA** i.e showed negative test results :

Name of compound	Concentration
Bilirubin	20mg/dL
Hemoglobin	1000mg/dL
Triglyceride	30g/L
Ascorbic acid	10mg/dL
Acetylsalicylic acid	50mg/dL
Ibuprofen	70mg/dL
CEA	10µg/ml
AFP	10µg/ml
LH	10 IU/ml
hCG	1000 IU/ml

LIMITATIONS OF THE TEST

(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. (3) 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. (5) 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 **CANCHECK® - 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) **CANCHECK- PSA** has no high dose hook effect upto 1,00,000ng/ml PSA. (7) 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. (8) The deliberate slow reaction kinetics of **CANCHECK® - PSA** is designed to maximize and enhance reaction time between sample capture and tracer elements to improve test sensitivity. (9) 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. (10) 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. (11) **CANCHECK® - 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.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

(1) W. Jeffrey Allard, Zeqi Zhou & Kwok K. Yeung., 1998, Clin. Chem., 44:6, 1216-1223. (2) Lothar Thomas. Clinical Laboratory Diagnostics - Use and assessment of Clinical Laboratory Results. TH-Books. 1998. 963-965 (3) F.T. Kreutz & M. R. Suresh, 1997, Clin. Chem., 43:4, 649-656. (4) Teitz Textbook of Clinical Chemistry. Second Edition, WB Saunders Publishing. 1994: 907-910. (15) Data on file: Zephyr Biomedicals.

SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Date of Manufacture	Do not reuse
Manufacturer	IVD — *In vitro* Diagnostic Medical Device	This side up	BUF — Sample Running Buffer
Use by	REF — Catalogue Number	DEVICE — Device	EC REP — Authorised Representative in the European Community
Contains sufficient for <n> tests	LOT — Batch Number / Lot Number	PIPETTE — Disposable Plastic Sample Applicator	

Manufactured by:

Zephyr Biomedicals

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EC REP

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