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Immunoassay

REF **CMB0302**

100 tests

tPSA CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of tPSA (total prostatic specific antigen) in human serum.

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use

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IVD

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Introduction

tPSA (prostate specific antigen), a glycoprotein with a molecular weight of 34,000D, was first isolated by Wang *et.al.* in 1979[1]. tPSA is a kallikrein-like serine protease that is produced exclusively by the epithelial cells of the prostate. tPSA is immunologically specific for prostatic tissue, it is present in normal, benign hyperplastic, and malignant prostatic tissue, in metastatic prostatic carcinoma, and also in prostatic fluid and seminal plasma. It may serve as an accurate marker for assessing response to treatment in patients with prostatic cancer. Therefore, measurement of serum tPSA concentrations can be an important tool in monitoring patients with prostatic cancer and in determining the potential and actual effectiveness of surgery or other therapies. 30-50% of patients with benign prostatic hyperplasia have elevated serum tPSA concentrations, depending on the size of the prostate and the degree of obstruction, and the concentrations are increased in 25～92% of patients with prostate cancer, depending on tumor volume[2,3,4]. Elevated levels have not been reported for cancers of the lung, breast, colon, rectum, stomach, pancreas or thyroid.

Digital rectal examination, cystoscopic examination and prostate biopsy all can cause elevations of the serum tPSA concentration[2]. Conditions such as bacterial prostatitis and acute urinary retention also can increase the serum tPSA level[5,6,7].

Recent studies also indicate that tPSA measurements can enhance early prostate cancer detection when combined with DRE (digital rectal examination). When compared to PAP (prostatic acid phosphatase), tPSA is a more precise and useful marker in all clinical situations.

Measurement Principle

This assay is based upon the one-step sandwich method. The sample, Anti-PSA coated microparticles and enzyme labeled Anti- PSA are combined. During the incubation, tPSA present in the sample is allowed to react simultaneously with the two antibodies, resulting in the tPSA being sandwiched between the solid phase and enzyme-linked antibodies. After washing, a complex is generated between the solid phase, the tPSA within the sample and enzyme-linked antibodies by immunological reactions. Chemiluminescent Substrate is added and the complex catalyzes substrate, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is proportional to the amount of tPSA in the samples.

Materials provided

1. Calibrators

6 vials each containing 1.0 ml of Calibrator A through F with corresponding approximate tPSA concentrations showed in the following table. The matrix is PBS (phosphate buffered saline) containing BSA (bovine serum albumin). Contains a selection of preservatives.

Calibrators provided ready to use.

Calibrator	tPSA Concentration (ng/ml)
A	0
B	0.5
C	2
D	10
E	50
F	100

2. Reagent pack

Reagent pack provided ready to use.

- Enzyme Conjugate

1 vial containing 11.0 ml of horse radish peroxidase labeled mouse monoclonal Anti-PSA in Tris-HCl buffer containing BSA. Contains ProClin 300® preservative.

- Microparticles Solution

1 vial containing 2.3 ml of mouse monoclonal Anti-PSA coated microparticles in PBS buffer containing BSA. Contains a selection of preservatives.

Assay Analyzers on which the kit can be used

- AutoLumo A2000
- AutoLumo A2000 Plus

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzer which is AutoLumo A2000 or AutoLumo A2000 Plus.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample tube(s) or cup(s) for sample containing
4. Diluent Universal
5. Chemiluminescent Substrate
6. System Wash for washing the pipetting needle
7. Wash Buffer used in washing procedure
8. Distilled or deionized water

Metrological Traceability of Calibrators

The product calibrators are manufactured using PSA antigen and signal matched to our working calibrators, which are also signal matched to calibrators purchased from WHO (The World Health Organization) IS # 96/670, at each concentration level.

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. CAUTION: the calibrators contain human sourced components, which have been tested and found non-reactive for HBsAg, HIV-1 and HIV-2, HCV and Syphilis. It is recommended that all materials of human origin be considered potentially infectious. This assay contains materials of animal origin. Bovine components originate from countries where bovine spongiform encephalopathy (BSE) has not been reported.
6. Some reagents contain ProClin 300® may cause sensitization by skin contact, which must be avoided to contact with skin. This material and its container must be disposed in a safe way. If swallowed, seek medical advice immediately and show this container or label.
7. Do not smoke, drink, eat or use cosmetics in the working area.
8. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
9. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
10. Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
11. Do not use reagents beyond the labeled expiry date.
12. Do not mix or use components from kits with different batch codes.
13. When storing the calibrators, be certain the vials are securely sealed.
14. Ensure the microparticles are resuspended before loading it on the analyzer.
15. Avoid foam formation in all reagents and sample types (samples,

calibrators and controls).
16. Do not substitute any reagent in this kit from other manufacturers or other lots.
17. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.

Storage

1. Store the kit at 2-8℃. Do not freeze. Avoid strong light. When stored as directed, all reagents are stable until the expiration date.
2. Refrigerate the reagent pack at 2-10℃ for a minimum of 2 hours prior to use.
3. Store the reagents pack upright at 2-10℃ on the analyzer. They may be stored on the analyzer for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-8℃ in an upright position. For reagents stored off the analyzer, it is recommended that they be stored in their original trays and boxes to ensure they remain upright.
4. Once the reagent pack is open, it can be stored at 2-8℃ for 1 month.
5. Seal and return the remaining calibrators to 2-8℃, under that conditions the stability will be retained for 1 month. For longer use, store opened calibrators in aliquots and freeze at -20℃. Avoid multiple freeze-thaw cycles.

Sample

1. Collect serum samples in accordance with correct medical practices.
2. Do not use heat-inactivated samples. Do not use sodium azide preservative in samples.
3. Do not use samples with obvious microbial contamination.
4. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
5. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
6. Insufficient processing of sample, or disruption of the sample during transportation may cause depressed results.
7. Avoid grossly hemolytic, lipemic or turbid samples.
8. Cap and store the samples at 18-25℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8℃ up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20℃. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortex or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
9. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy etc. in appearance prior to use to ensure consistency in the results.
10. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
11. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
12. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

Measurement Procedure

1. Check the consumable materials
- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit
- Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests
- Place the sample tube(s) or cup(s) on the sample holder, 25 μl of sample and calibrators for each test. But consider the sample container and 150 μl of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample holder and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the sample to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers sample into the reaction vessel
  - Adds Microparticles Solution and Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of tPSA in the sample
  - Discards the used reaction vessel
  - Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibrate the curve
- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample tube (s) or cup(s) and place them on the sample holder. Conduct duplicate detection on the system.
- Load the sample holder and input calibrators' information on the system software interface.
- Select "run" to start the test and generate the calibration curve, calibration is required every 28 days.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements
  - A reagent kit and Chemiluminescent Substrate with new batch code is used
  - Beyond the expiration date of a calibration curve
  - Important parts of the analyzer are replaced or repaired
- Refer to the Assay Analyzer's operation manual.

5. Dilute the sample

Samples with a tPSA value exceeding 100ng/ml may be diluted with the automated dilution method. Diluent Universal is used to dilute the samples. After dilution by the analyzer, the software automatically takes the

dilution into account when calculating the sample concentration.

Measurement Results

The sample test results are determined automatically by the system software utilizing a 4 parameter logistic curve fit data reduction method. The amount of tPSA in the samples is determined from the measured light production by means of the stored calibration data. Sample test results can be reviewed using the appropriate screen or printed out. Refer to the Assay Analyzer's operation manual on reviewing sample results.

Control Procedure

The recommended control requirement for this assay is to purchase control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels. When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and may require retesting. Assay recalibration may be necessary. It is recommended that each laboratory establish its accepted range to ensure proper test performance.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. tPSA is elevated in BPH (benign prostatic hyperplasia). Clinically an elevated tPSA value alone is not of diagnostic value as a specific test for cancer and should only be used in conjunction with other clinical manifestations (observations) and diagnostic procedures such as prostate biopsy and DRE (Digital Rectal Examination) report. Free PSA determinations may be helpful in regard to the differential diagnosis of BPH and prostate cancer conditions.
4. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
5. Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop HAMA (human Anti-mouse antibodies). HAMA can produce either falsely high or falsely low values in immunoassays which use mouse monoclonal antibodies. Additional information may be required for diagnosis.
6. This assay was designed and validated for use with human serum from individual patient and donor samples. Pooled samples must not be used since the accuracy of their test results has not been validated.
7. This test measures concentrations within the range of 0.1- 100ng/ml. If tPSA concentrations above the measuring range to be expected, it is recommended to dilute samples with Diluent Universal, the maximum dilution is 1:10 of this test, allowing samples to be quantitated up to approximately 1000 ng /ml.

Biological Reference Interval

A normal range of 4ng/ml (95% confidence interval) was obtained by testing male serum samples from 493 individuals defined as normal by clinician. It is recommended that each laboratory establish its own normal range which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

Performance Characteristics

1. Measurement Precision

This assay is designed to have a within-run precision of ≤8%. 2 pooled human serum based panel members (1 and 2) were assayed, using 1 batch of reagents, in replicates of 10. Data from this study are summarized in the following table.

Panel Member	Batch	n	Mean	Within-run Precision SD	%CV
1	1	10	3.21	0.19	6.12
2	1	10	27.83	1.63	5.86

This assay is designed to have a between-run precision of ≤15%. 2 pooled human serum based panel members (1 and 2) were assayed, using 1 batch of reagents, in replicates of 2, once per day across 10 testing days. Data from this study are summarized in the following table.

Panel Member	Batch	n	Mean	Between-run Precision SD	%CV
1	1	20	4.11	0.31	7.63
2	1	20	33.02	2.70	8.19

2. Analytical Sensitivity

Analytical sensitivity, defined as the concentration corresponding to the mean RLUs of 20 replicates of calibrator A plus 2 standard deviations, is ≤0.1 ng/ml.

3. Analytical Specificity

Cross reaction: the following substances and concentrations were tested and found no cross reaction with the test.

Substances	Concentration
AFP	500 ng/ml
CEA	500 ng/ml
Ferr	400 ng/ml

Interference: the following substances and concentrations were tested and found not to interfere with the test.

Interferent	Concentration
Bilirubin	65 mg/dl
Hemoglobin	400 mg/dl
Triglyceride	1500 mg/dl

4. Measurement Accuracy by Correlation

A comparison study was performed where samples were tested using this assay and a tPSA reference assay. Data were analyzed and are summarized in the following table.

Correlation Method	Number of Samples	Intercept	Slope	Correlation Coefficient
Linear Regression	160	0.1906	09563	0.99

5. High Dose Hook Effect

A sample spiked with tPSA up to 10000 ng/ml gives a result more than the last calibrator point (100 ng/ml).

Literature References

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