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# Immunoassay

REF **CMB0502**

***tests 100***

# fPSA CLIA Microparticles

*This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of fPSA (free 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 |
| EC REP | authorized representative in the European Community | | date of manufacture |

EC REP

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## Introduction

PSA (prostate specific antigen), a glycoprotein with a molecular weight of 34,000D, was first isolated by Wang *et. al.* in 1979[1]. It is mainly found in the cytoplasm of prostate acinar cells and ductal epithelium.[2] In addition to being present in normal tissue, PSA is also present in prostatic cancerous tissue, benign hyperplastic tissue, in prostatic fluid and seminal plasma, and is therefore a useful clinical marker for prostate cancer[3, 4, 5]. Two immunoreactive forms of PSA are found in serum: free and complexed PSA. The complexed form is given by the binding of ACT (alpha-1-antichymotrypsin) to the active site of PSA. The PSA molecule which is not bound to the serine protease inhibitor ACT, named Free PSA, is found in lower concentrations than the complexed form[6].

Current methods of screening men for prostate cancer utilize the detection of the major PSA-ACT form. Levels of 4.0 ng/ml or higher are strong indicators of the possibility of prostatic cancer. However, elevated serum PSA levels have also been attributed to benign prostatic hyperplasia and prostatitis, leading to a large percentage of false positive screening results. A potential solution to this problem involves the determination of free PSA levels. Preliminary studies have suggested that the percentage of free PSA is lower in patients with prostate cancer than those with benign prostatic hyperplasia. Thus, the measurement of free serum PSA in conjunction with total PSA, can improve specificity of prostate cancer screening in selected men with elevated total serum PSA levels, which would subsequently reduce unnecessary prostate biopsies with minimal effects on cancer detection rates.

The proportion, or percent, of free PSA determined by comparing the concentration of free PSA to the concentration of total PSA has been proposed as a way to improve the discrimination between BPH and prostate cancer, especially in those men with intermediate levels of total serum PSA[7, 8, 9].

## Measurement Principle

This assay is based upon the two-step sandwich method. In the first step, sample and anti-fPSA coated microparticles and sample diluent are combined. During the incubation, fPSA antigen present in the sample binds to the antibody coated on the paramagnetic microparticles. After the washing, in the second step, enzyme conjugate is added to the reaction mixture. During the incubation, the enzyme labeled anti-fPSA reacts with the antigens attached to the solid phase in the first step. After a second washing, a complex is generated among the solid phase, the fPSA antigen within the sample and the antibodies in the enzyme conjugate 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 fPSA in the samples.

## Materials provided

1. Calibrators

6 vials each containing 1.0 mL of Calibrator A through F. The matrix is PBS (phosphate buffered saline) buffer containing BSA (bovine serum albumin). Contains 2‰ ProClin 300® and 1‰ Bronidox preservatives.
Calibrators provided ready to use.

2. Reagent pack

Reagent pack provided ready to use.

| Σ | 50*1 | 100*1 | 100*2 | 100*5 | 50*2 |
|---|---|---|---|---|---|
| Microparticles Solution | 1.2mL*1 | 2.3mL*1 | 2.3mL*2 | 2.3mL*5 | 1.2mL*2 |
| Enzyme Conjugate | 5.5mL*1 | 11.0mL*1 | 11.0mL*2 | 11.0mL*5 | 5.5mL*2 |
| Sample Diluent | 3.0mL*1 | 5.5mL*1 | 5.5mL*2 | 5.5mL*5 | 3.0mL*2 |

- Microparticles Solution

Mouse monoclonal Anti-fPSA coated microparticles in PBS buffer containing BSA. Contains 1‰ ProClin 300® and 1‰ Bronidox preservatives.

- Enzyme Conjugate

Horseradish-peroxidase labeled mouse monoclonal anti-fPSA in Tris-HCl buffer containing BSA. Contains 2‰ ProClin 300® and 0.2‰ Bronidox preservatives.

- Sample Diluent

Saline. Contains 2‰ ProClin 300® and 0.2‰ Bronidox preservatives.

## Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzer which is AutoLumo A2000 Plus, AutoLumo A2000 Plus B or AutoLumo A1000.

## 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 ([REF] CMO0101/CMO0102/CMO0103)
6. System Wash for washing the pipetting needle ([REF] CMO0401/CMO0403)
7. Wash Buffer used in washing procedure ([REF] CMO0301/CMO0302 CMO0303/CMO0304/CMO0305/CMO0306)
8. Distilled or deionized water

## Metrological Traceability of Calibrators

The product calibrators are manufactured using fPSA antigen and signal matched to our working calibrators, which are also signal matched to calibrators purchased from WHO (The World Health Organization) IS # 96/668, 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: This assay contains materials of human origin and animal origin, which all considered potentially infectious.
6. Do not smoke, drink, eat or use cosmetics in the working area.
7. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
8. 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.
9. Do not use reagents beyond the labeled expiry date.
10. Do not mix or use components from kits with different batch codes.
11. When storing the calibrators, be certain the vials are securely sealed.
12. Ensure the microparticles are resuspended before loading it on the

analyzer.
13. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
14. Do not substitute any reagent in this kit from other manufacturers or other lots.
15. 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 unsealed reagents pack upright on the analyzer or 2-10℃ 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 2-10℃ in an upright position.
4. Seal and return the remaining calibrators at 2-8 ℃ immediately after the experiment, under which conditions the stability will be retained for 28 days, for longer use, store opened calibrators in aliquots and freeze at -20 ℃, under which conditions the stability will be retained for 2 months. 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. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
7. Insufficient processing of sample, or disruption of the sample during transportation may cause depressed results.
8. Avoid grossly hemolytic, lipemic or turbid samples.
9. 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.
10. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
11. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
12. 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.
13. 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 contaminationnalysis. 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 input manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests
- Place the sample tube(s) or cup(s) on the sample rack, 25 $\mu$L of samples for each test. But consider the sample container and 150 $\mu$L of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample rack 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 Sample Diluent to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of fPSA 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 input manually.
- Transfer the calibrators into the sample tube (s) or cup(s) and place them on the sample rack. Conduct duplicate detection on the system.
- Load the sample rack 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.

## Measurement Results

The sample test results are determined automatically by the system software. The amount of fPSA in the samples is determined from the measured light production by means of the stored calibration data. 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 acceptable ranges. 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. fPSA is elevated in BPH (benign prostatic hyperplasia). Clinically an elevated fPSA value alone is not of diagnostic value as a specific test for differential diagnosis of BPH. The ratio of fPSA/tPSA is a better marker and should be used in conjunction with other clinical observations DRE (Digital Rectal Examination) and diagnostic procedures (prostate biopsy).
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. In vitro tests were performed on 27 commonly used pharmaceuticals. No interference with the assay was found on 27 pharmaceuticals.
8. This test measures concentrations within the range of 0.05- 50 ng/mL.

## Biological Reference Interval

A normal value of 0.944 ng/mL (95% confidence interval) was obtained by testing 687 healthy individuals. When the ratio of fPSA/tPSA is $<10\%$, the prostate cancer should be suspected and further tissue biopsy is needed. When the ratio of fPSA/tPSA is $>25\%$, there is little possibility of prostate cancer. 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

3 samples were assayed in duplicate, twice per day across 20 testing days. Data from this study are summarized in the following table.

| Sample | n | Mean | Between-batch | Total |
|---|---|---|---|---|
| | | | %CV | %CV |
| 1 | 80 | 1.27 | 2.16 | 3.44 |
| 2 | 80 | 3.5 | 2.17 | 4.27 |
| 3 | 80 | 14.5 | 1.87 | 3.39 |

*Representative data; results in individual laboratories may vary from these data.

2. Analytical Sensitivity

Limit of Blank≤0.05ng/mL
Limit of Detection=0.1ng/mL
Limit of Quantitation: 0.1ng/mL with a coefficient of variation of ≤ 20 %.

3. Interference

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

| Interferent | Concentration |
|---|---|
| Bilirubin | 65 mg/dL |
| Hemoglobin | 500 mg/dL |
| Triglyceride | 1500 mg/dL |

4. Measurement Accuracy by Correlation

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

| Correlation Method | Number of Samples | Intercept | Slope | Correlation Coefficient |
|---|---|---|---|---|
| Linear Regression | 1337 | -0.1718 | 1.0134 | 0.97 |

5. High Dose Hook Effect

A sample spiked with fPSA up to 15000 ng/mL gives a result more than the last calibrator point (e.g.50 ng/mL).

## Literature References


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