1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Total Prostate Specific Antigen (PSA) Concentration in Human Serum by a Microenzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Prostate Specific Antigen (PSA) is a serine protease with chymotrypsin-like activity. The prostate is a gland in the form of a normal antigen of the prostate, but is not found in any other normal or malignant tissue.

PSA is found in benign, malignant and metastatic prostate cancer. Since prostate cancer is the second most prevalent form of male malignancy, the detection of elevated PSA levels plays an important role in the early diagnosis. Serum PSA levels have been found to be more useful than prostatic acid phosphatase (PAP) in the diagnosis and management of patients due to increased sensitivity.

In this method, IFPSA calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of PSA) are added and the reactants mixed. Reaction between the PSA antibodies and native PSA forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-PSA antibody bound conjugate is separated from the unbound enzyme-PSA conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known total prostate specific antigen (PSA) levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen’s activity can be correlated with tPSA concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3): The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immunization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-PSA antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antigen, and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

\[
\text{EnzAb(p)} + \text{AgtPSA} + \text{BtnAb(m)} \rightarrow \text{(Enzyme Antibody-Antigen-Biotinylated Antibody Complex)}
\]

3.1 Required But Not Provided:

1. Pipettor(s) capable of delivering 0.025, 0.050 & 0.100 ml (25, 50, & 100 µl) volumes with a precision of better than 1.5%.

2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350 ml (100 & 350µl) volumes with a precision of better than 1.5%.

3. Microplate washers or a squeeze bottle (optional).

4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.

5. Absorbent Paper for blotting the microplate wells.

6. Plastic wrap or microplate covers for incubation steps.

7. Pipettor aspirator (optional) for wash steps.

8. Timer.

9. Quality control materials

5.0 PRECAUTIONS

For In Vitro Diagnostic Use

Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV & HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, “Biosafety in Microbiological and Biomedical Laboratories,” 2nd Edition, 1989. HHS Publication No. (CDC) 88-8395.

Safe disposal of components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain red top venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen is frozen, it cannot be thawed within this time, the sample(s) may be stored at temperatures -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.001 ml (50 µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every assay period. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. Wash Buffer

Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-8°C for up to 60 days.

2. Working Substrate Solution – Stable for one year

Pour contents of the amber vial labeled Solution ‘A’ into the clear vial labeled Solution ‘B’. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly.

2.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20 - 23°C).

**Test Procedure should be performed by a skilled individual or trained professional**

1. Format the microplates’ wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. Place one replication of each into the assigned well.

2. Pipette 0.050ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.

3. Add 0.100ml (100µl) of the iPSA Enzyme Reagent to each well. It is very important to dispense all reagents close to the bottom of the coated well.

4. Swirl the microplate gently for 20-30 seconds to mix and cover.

5. Incubate for 30 minutes at room temperature.

6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.

7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decad (t and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer’s instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.

8. Add 0.100ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences between wells.

**DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION**

9. Incubate at room temperature for fifteen (15) minutes.

10. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.

11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of iPSA in unknown specimens.

1. Record the absorbance obtained from the printout of the instrument for each sample.

2. Plot the absorbance for each duplicate serum reference versus the corresponding iPSA concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).

3. Draw the best-fit curve through the plotted points.

4. To determine the concentration of iPSA for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be used as outlined in Example 1). In the following example, the average absorbance (1.142) intersects the dose response curve at (23.6 ng/ml) iPSA concentration (See Figure 1).

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.
8. Use components from the same lot. No intermixing of reagents from different batches.

9. Patient specimens with PSA concentrations above 100 ng/ml may be diluted (for example 1/10 or higher) with normal female serum (PSA = 0 ng/ml) and re-assayed. The sample’s concentration is obtained by multiplying the result by the dilution factor (10).

10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind’s IFU may yield inaccurate results.

11. All applicable national standards, regulations and laws, including but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.

12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

13. Cross contamination - as required by CE Mark Standards Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. Interpretation and results of interpretation must be performed by a skilled individual or trained professional.

2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other current conditions.

3. The reagents for AccuBind® ELISA procedure have been formulated to eliminate maximal interference; however, potential interactions between different serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato, LM, Stuart, MC. “Heterophilic antibodies: a problem for all immunoassays” Clin Chem. 1988; 3427-33). The results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.

4. For valid test results, accurate controls and other parameters must be within the listed ranges and assay requirements.

5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.

6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

7. PSA is elevated in benign prostate hypertrophy (BPH) and prostate cancer conditions.5 Clinically, an elevated PSA value alone is not of diagnostic value. PSA is often used in conjunction with other clinical manifestations (observations) and diagnostic procedures (prostate biopsy). Free PSA determinations may be helpful in the diagnosis of BPH and prostate cancer conditions.2

8. Due to the variation in the calibration used in IPSA/PSA test kits and differences in epitopic recognition of different antibodies, it is always suggested that the patient sample should be tested with IPSA/PSA test kits made by the same manufacturer. (Monobind inc. offers a IPSA ELISA test that should be used for consistency reasons, when needed.)

13.0 PERFORMANCE CHARACTERISTICS

Healthy males are expected to have values below 4 ng/ml.4

<table>
<thead>
<tr>
<th>Expected Values for IPSA AccuBind® ELISA Test System</th>
<th>Healthy Males</th>
<th>≤4 ng/ml</th>
</tr>
</thead>
</table>

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of “normal”-persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

15.0 REFERENCES


