Pregnancy Associated Plasma Protein-A (PAPP-A) Test System
Product Code: 12675-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of PAPP-A Concentration in Human Serum by a Microplate Enzyme Immunoassay, Chemiluminescence

2.0 SUMMARY AND EXPLANATION OF THE TEST

Trisomy 21, 18, and 13 are three conditions that can cause ill effects to a fetus and, therefore, an infant. Screening for markers and inherent trends has increased over the years. One of the proteins of interest for these trisomy disorders is PAPP-A, pregnancy associated plasma protein A. This protein can be found in the serum of every individual, but the concentration rises in maternal serum as gestation time increases. In studies, a correlation has been shown between the decreased level of PAPP-A and the occurrence of trisomy disorders, particularly Trisomy 21, also known as Down’s Syndrome. Along with a few other markers, levels of PAPP-A in pregnant women have been found to elude to trisomy disorders.

PAPP-A is produced primarily by the placenta during pregnancy. This glycoprotein has a molecular weight of 740,000 and tends to exist as a heterotetrameric form with PreMIA, a more basic protein in the breast. The concentration of PAPP-A in maternal blood increases over the time of the pregnancy as the placenta and fetus grow. Elevated levels of PAPP-A have been noted in cases of trisomy disorders. When tested during the first trimester of pregnancy, PAPP-A is the major marker for Down’s syndrome.

The Monobind PAPP-A test system is designed specifically for the testing of the heterotetrameric form of the protein. It is intended for use in the detection of maternal serum levels of this protein. Another form of PAPP-A also exists in serum, but it is a dimeric form associated with coronary and cardiac conditions. Tests developed for use with this dimeric protein are not designed to test for this dimeric form. When evaluation of this protein is done, it is often compared to the Multiple of Medians (MoM) and represented as a percentage of a predefined normal range. Without easy access to an IRP for PAPP-A, this method allows an easier way to compare results to levels established by the Test and finding methods, allowing for a type of reference value to be established.

3.0 PRINCIPLE

Immunoenzymometric sequential assay (Type 4): The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microwell well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-PAPP-A antibody. Upon mixing monoclonal biotinylated antibody, and a serum containing the native antigen, reaction results between the native antigen and the antibody, forming an Antibody-Antigen complex. The interaction is illustrated below following equation:

\[ \text{Ag}_{\text{PAPP-A}} + \text{BiAb} \xrightarrow{\text{Streptavidin C.W.}} \text{IC} \]

Simultaneously, the complex is deposited to the well through the affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below following equation:

\[ \text{Ag}_{\text{PAPP-A}} + \text{BiAb} \xrightarrow{\text{Streptavidin C.W.}} \text{IC} \]

After a suitable incubation period, the antibody-antigen bound fraction is separated from unbound antigen by decantation or aspiration. Another antibody (directed at a different epitope) labeled with an enzyme is added. Another interaction occurs to form an enzyme labeled antibody-antigen-biotinylated-antibody complex on the surface of the wells. Excess enzyme is washed off the wells after a wash step. The IC is added to the wells. The IC contains a high affinity and specificity antibodies (enzyme and antigenic antibody). The enzyme activity on the well is directly proportional to the native free antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

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 1&2 and HCV Antibodies by FDA required tests. 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. Proper 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)88-395. Safe Disposal of kit 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, care must be taken in sample collection. The blood should be collected in a plain red top tube without anticoagulants. A preservative and a “Tiger” bottle can be used. Allow the blood to clot. Centrifuge the specimen to separate the cells from the serum.

Specimens may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, it may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.020ml of the patient specimen to be assayed in duplicate.

In patients receiving therapy with high bioraphic doses (i.e. >5mg/diy), no sample should be taken until at least 8 hours after the last dose administration, preferably overnight to ensure fasting sample.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be prepared and tested according to the manufacturers’ recommendations. Each laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unmonitored change in experimental conditions. Controls or lots 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 solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be mixed to store at 2-30°C for up to 60 days.

2. Working Signal Reagent

Dilute reagent amount to reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B (10μl for each 100μl of sample). Add 1ml of A and 1ml of B per two (2) eight wells strips (A slight excess of solution is made). Discard the unused portion if not used within 36 hours after mixing. Reagents are stable up to 24 hours after the above anticipated, within the time above time frame, the contents of the above set label and record.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum controls and references to room temperature (20 - 27°C). If the procedure should be performed by a skilled individual or trained professional**

1. Format the microtubes’ wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.

2. Prepare 100μl (10μl) of the appropriate serum reference, control or specimen into the assigned well.

3. Add 0.100ml (100μl) of the PAPP-A Biotin Reagent to all wells.

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

5. Cover and incubate for 30 minutes at room temperature.

6. Add patient contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

7. Add 0.350ml (350μl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) and aspirate. Repeat step (6) washes for a total of five (5) washes.

8. Add 0.100ml (100μl) of PAPP-A Tracer Reagent to all wells.

9. Cover and incubate for 30 minutes at room temperature.

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

11. Add 0.350ml (350μl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) and aspirate. Repeat step (6) washes for a total of four (4) times.

12. Repeat four (4) additional times for a total of five (5) washes. Avoid automatic or manual plate washer can be used. Follow the manufacturer’s instruction for proper usage.

13. Add 0.100ml (100μl) of working Signal Reagent to all wells. Incubate for 3 minutes.

14. Discute for 5 minutes at room temperature in the dark.

15. Add 0.100ml (100μl) of Signal Reagent B into Signal Reagent A and label accordingly.

Note 1: Do not use the signal reagent solution if older than 36 hours.

Note 2: Do not use reagents that are contaminated or have bacteria growth.

Note 3: Discard (tap and stop) mixing (4 cycles) for 5-8 seconds/cycle is more efficient than one continuous (20-30 seconds) cycle to achieve homogeneity. A plate mixer can be used to perform the mixing cycles.

Note 4: It is extremely important to accurately dispense the correct volume with a calibrated pipette and by
In order for the assay results to be considered valid the following criteria should be met:
1. The dose response curve should be within the established parameters.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant for reproducible results.
2. Pipetting of samples should not extend beyond ten (10) mm from the edge of the well to avoid air bubbles.
3. Highly hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of signal reagent solution initiates a kinetic reaction; therefore, the solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Failure to remove adhering solution adequately in the aspiration or decantation step(s) may result in poor reproducibility and spurious results.
7. Use components from the same lot. No intermixing of reagents is performed by a skilled individual or trained professional.
8. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential.
9. Any deviation from Monobind's IFU may yield inaccurate results.

12.2 Interpretation

1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.
2. Laboratory results alone are only one aspect for determining the cause of the observed changes.
3. The reagents for AccuLite® CLIA procedures have been formulated to optimize maximal interference; however, potential interaction between rare serum specimens and test chemicals is possible. 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)). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history, and all other clinical findings.
4. For valid test results, adequate 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 incompletely 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.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" adult population, the expected ranges for the PAPP-A AccuLite® CLIA Test System are detailed in Table 2.

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 expected values established by the manufacturer only until an in-house validation can be determined by the analyst using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The total precision of the PAPP-A AccuLite® CLIA Test System was determined by analyses on six different levels of pool control and patient sera. The mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2.

14.3.3 Method Comparison

The PAPP-A AccuLite® CLIA Test System was compared with a reference immunometric method. Biological specimens from low, normal and relatively high PAPP-A levels were used; the values ranged from 0.1 µg/ml – 36 µg/ml. The total number of samples was 50. The sources of systematic error and the correlation coefficient were computed for this PAPP-A ELISA in comparison with the reference method.

14.4 Specificity

The % cross-reactivity of the PAPP-A antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between doses of interfering substance to dose of PAPP-A needed to displace the same amount of labeled analog.