Pregnancy Associated Plasma Protein-A (PAPP-A) Test System

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of PAPP-A Concentration in Human Serum by a Microparticle 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 traits can start as early as the 10th week of gestation. 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 during pregnancy associated plasma protein A. This protein can be taken during the assay at the surface of a microplate well as a percent of an in-house establish median. Without easy allowance for a type of reference value to be established.2,5,8,9

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 dimer with ProMBP, proform major basic protein. The concentration of PAPP-A in maternal blood increases over the time of the pregnancy as the placenta and fetus grow because the placenta is the organ that produces the trophoblast. In general, the concentration level of this protein in maternal serum is indicative of threatened abortion, preterm birth, intrauterine growth restriction, ectopic pregnancy, preclampsia or diabetes mellitus. When tested during the first trimester of pregnancy, PAPP-A is the 2,5,8,9

The Monobind PAPP-A test system is designed specifically for the testing of the heterotetrameric form important during pregnancy. Another form of PAPP-A also exists in serum, but it is a dimeric form associated with corin and cardiac conditions. Tests developed for use on pregnancy patients 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 percent of an in-house median. Without easy access to an IRP for PAPP-A, this method allows an easier way to compare results from different laboratories and results in cases of identical tests, allowing for a type of reference value to be established. 5,8,9

3.0 PRINCIPLE

Immunoenzymometric Sequential Assay (TYPE 4): The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with a 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 microplate well through the interaction of streptavidin coated on the well and excessively added biotinylated monospecific anti-PAPP-A antibody.

When monoclonal biotinylated antibody is mixed with a serum containing the PAPP-A antigen, a reaction results between the PAPP-A antigen and the antibody, to form an antibody-antigen complex. Simultaneously the biotin attached to the antibody binds to the streptavidin coated on the microplates resulting in immobilization of the complex. The interaction is illustrated by the following equation:

\[
\text{Ag(PAPP-A)} + \text{BtnAb} \rightarrow \text{Ag(PAPP-A) - BtnAb}
\]

\[
\text{Ag(PAPP-A)} - \text{BtnAb} \rightarrow \text{Ag(PAPP-A) - BtnAb} + \text{Ab} + \text{Ab} + \text{Ab}
\]

\[
\text{Ag(PAPP-A)} - \text{BtnAb} + \text{Ab} + \text{Ab} + \text{Ab}
\]

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 via a wash step. A suitable substrate is added to produce color measurable with the use of a microplate spectrophotometer. 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.

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 1&2 and HCV Antibody. For in-raw data the concentration of the reagents is anticipated, without the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label according to the following equation:

\[
\text{Ag(PAPP-A)} - \text{BtnAb} \rightarrow \text{Ag(PAPP-A) - BtnAb} + \text{Ab} + \text{Ab} + \text{Ab}
\]

Required But Not Provided:

1. PAPPA Calibrators – 3 x 0.010ml (100 µl), 0.050ml (50 µl) and 0.100ml (100 µl) with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100 µl) and 0.350ml (350 µl) with a precision of better than 1.5%.
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Luminometer
5. Absorbent Paper for cleaning the microplate wells.
6. Plastic wrap or microplate covers for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.

4.0 REAGENTS

A. PAPP-A Calibrators – 0.5 ml/vial – Icons A-F
B. PAPP-A Tracer Reagent – 12 ml/vial – Icon
C. PAPP-A Biotin Reagent – 12 ml/vial – Icon
D. PAPP-A Control – 0.5 ml/vial – Icon M
E. PAPP-A Calibrators – 0.5 ml/vial – Icons A-F
F. Streptavidin Coated Plate – 96 wells – Icon

1. Test System

2. Pimatex AB

3. Hemin

4. Microplate Luminometer

5. Absorbent Paper for cleaning the microplate wells.
6. Plastic wrap or microplate covers for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timers.

9. *Precautions*

In patients receiving therapy with high blood glucose (i.e. >250mg/dl), no test should be taken until at least 8 hours after the last blood test, preferably overnight to ensure fasting sample.

Sample may be refrigerated at 2-8°C for a maximum period of five days. If the sample has not been refrigerated or stored at room temperature as recommended within this time, the sample(s) 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 specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should ensure acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate un向きed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the

8.0 REAGENT PREPARATION

1. Wash Buffer

Dilute contents of wash solution to 1000ml with distilled or deionized water. Buffer can be stored at 2-30°C for up to 60 days.

2. Working Signal Reagent Solution – Store at 2-8°C. Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1ml of A and 1ml of B per two or eight well strips to be tested (A slight excess of solution is made). Discard the unused portion if not used within 36 hours. The mixing of the reagents is anticipated, without the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

9.0 TEST PROCEDURE

F. Streptavidin CW = Streptavidin immobilized on well
G. Ag(PAPP-A) - BtnAb = Antigen-antibody complex (Variable Quantity)

H. Signal Reagent A – 7 ml/vial – Icon CA
I. Signal Reagent B – 7 ml/vial – Icon CB
J. Product Instructions.
10.0 CALCULATION OF RESULTS

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

1. Record the absorbance obtained from the printout of the microtiter plate reader as outlined in Example 1.

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

3. Connect the points with a best-fit curve.

4. To determine the concentration of PAPP-A 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 µg/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance in the patient/sample (526341) intersects the dose response curve at (10.5 µg/ml) PAPP-A concentration (See Figure 1).

Note: Computer data reduction software designed for CLIA assay may also be used for the data reduction. If such software is utilized, the variation of the software should be ascertained.

EXAMPLE 1

<table>
<thead>
<tr>
<th>Sample L.D.</th>
<th>Well Number</th>
<th>RLUs</th>
<th>Mean RLU (B)</th>
<th>Value (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cal A</td>
<td>A1</td>
<td>60</td>
<td>62</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>A2</td>
<td>63</td>
<td>62</td>
<td>0</td>
</tr>
<tr>
<td>Cal B</td>
<td>B1</td>
<td>2684</td>
<td>2545</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>B2</td>
<td>2687</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal C</td>
<td>C A6</td>
<td>8696</td>
<td>8283</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>B6</td>
<td>7668</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal D</td>
<td>D C1</td>
<td>18743</td>
<td>18132</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>D1</td>
<td>17250</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal E</td>
<td>E1</td>
<td>50045</td>
<td>49194</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td>E2</td>
<td>48533</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>F1</td>
<td>100316</td>
<td>100000</td>
<td>32.0</td>
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<td></td>
<td>F2</td>
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<td>23391</td>
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<td>Ctrl #2</td>
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<td>57906</td>
<td>57682</td>
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<td></td>
<td>E4</td>
<td>57937</td>
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<td>Patient</td>
<td>G1</td>
<td>52087</td>
<td>52634</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>H1</td>
<td>52180</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FIGURE 1

1. The absorbance (OD) of calibrator 0 µg/ml should be ≥ 1.3.
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.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.

2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.

3. Highly hemolyzed or grossly contaminated specimens should not be tested.

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 wash step(s) may result in poor replication and spurious results.

7. Use components from the same lot. No intermingling of reagents from different batches.

8. 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.

9. 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 evaluation.

10. 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.

11. Risk Analysis - as required by CE Mark IVD Directive 98/79/EC (1988:3427-33). For diagnostic purposes, the patient population, the expected ranges for the PAPP-A AccuLite® CLIA test, and assay requirements must be within the listed ranges and assay requirements.

11.1 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 patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.

3. The reagents for AccuLite® CLIA procedures have been formulated to eliminate cross interference; however, potential interaction between rare 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 (Boscolo LM, Stuart MC “Heterophilic antibodies: a problem for all immunoassays” Clin. Chem. 1996;32:277-33). 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 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.

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 1.

It is recommended to compare values based on the Multiple of Median established for the laboratory where assessing patient samples. By dividing the value of the patient sample by the MoM will give a percent value that is used for frequency evaluation.

11.0 QC. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a dose response curve prepared with each assay. In addition, the RLUs of the calibrators have been normalized to 100,000 RLUs for the F calibrator (greatest light output). This conversion minimizes differences caused by efficiency of the various instruments that can be used to measure light output.

15.0 REFERENCES