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

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

\[\text{(m)}\rightarrow\text{Bi}t\text{inol Monoclonal Antibody (Excess Quantity)}\]

\[\text{Ag (PAPP-A)} + \text{BtnAb (m)} = \text{Antigen-antibody complex (Variable Quantity)}\]

\[k_a = \text{Rate Constant of Association}\]

\[k_d = \text{Rate Constant of Dissociation}\]

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 a tracer is added. Another interaction occurs to form a tracer labeled antibody-antigen-biotinylated-antibody complex on the surface of the wells. Excess tracer is washed off via a wash step. A suitable signal reagent is added to produce light measurable with the use of a luminometer. The tracer activity on the tracer is directly proportional to the antigen concentration. By utilizing several different serum references of known antigen amount, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

\[\text{Enzyme labeled antibody (Excess Quantity)}\]

\[\text{Enzyme labeled antibody (Excess Quantity)} - \text{Antigen-Antibody Complex}\]

\[k_b = \text{Rate Constant of Association}\]

\[k_d = \text{Rate Constant of Dissociation}\]

1.0 INTRODUCTION

1.1 INTENDED USE: The Quantitative Determination of PAPP-A Concentration in Human Serum by a Microplate Enzyme Immunoassay, Chemicon Inc.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Trisomy 21, also known as Down’s Syndrome. Along with a few other markers like AFP, uE3, and hCG, trends in PAPP-A have been found to elude to trisomy disorders. 1,2,3,4,5,6

2.1 This glycoprotein has a molecular weight of 740,000 and tends to exist as a heterotetrameric dimer with ProMBP, proform major growth restriction, ectopic gravidity, preeclampsia or diabetes indicative of threatened abortion, preterm birth, intrauterine growth retardation, ectopic gravidity, atrioventricular septal defect, and exogenously added biotinylated monoclonal anti-PAPP-A antibody.

3.0 PRINCIPLE

Immunoenzymatic Sequential Assay (TYPE 4):

The essential reagents required for an immunoenzymatic assay include high affinity antibody (antibody and enzyme immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immunoassay takes place during the assay at the surface of a light reaction well plate through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-PAPP-A antibody.

4.0 REAGENTS

Materials Provided:

A. PAPP-A Calibrators – 0.5ml/vial - ICs A-F

Six (6) vials of serum reference for PAPP-A at concentrations of 0.00, 0.010 (100), 0.050 (500), and 1.000 (1000) mIU/mL. A preservative has been added. Store at 2-8°C.

B. PAPP-A Tracer Reagent – 12.0 ml/vial – Icon

One (1) vial of PAPP-A (Analog) horseradish peroxidase (HRP) conjugate in a protein based buffer with 1% I2. Store at 2-8°C.

C. PAPP-A Biotin Reagent – 12.0 ml/vial – Icon

One (1) vial of reagent contains anti-PAPP-A biotinylated rabbit IgG conjugate in buffer and preservative. Store at 2-8°C.

D. PAPP-A Control – 0.5ml/vial – Icon M

One (1) vial of serum reference for PAPP-A at concentration of 2.000 mIU/mL (exact value unknown). A preservative has been added. Store at 2-8°C.

E. PAPP-A Diluent – 5.0 ml/vial – Icon U

One (1) vial of human serum based buffer with salts, surfactants, and preservatives. Store at 2-8°C.

F. Light Reaction Wells 96 wells - Icon U

One 96-well microtiter plate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

G. Wash Solution Concentrate – 2ml/vial - Icon U

One (1) vial containing a surfactant in buffer saline. A preservative has been added. Store at 2-8°C

H. Signal Reagent A – 7ml/vial - Icon C

One (1) vial containing a luminiferous reagent. Store at 2-8°C. (See “Reagent Preparation”)

1. SIGNAL REAGENT B — 7ml/vial - Icon G

One (1) vial containing hydrogen peroxide (H2O2) in buffer. Store at 2-8°C. (See “Reagent Preparation”)

2. Working Signal Reagent Solution - Store at 2 - 8°C

Determine the amount of reagent needed and prepare by mixing equal parts of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 mL of A and 1 mL of B per two (2) eight well strips (a slight excess of solution is made). Discard the unused portion if not used within 36 hours after mixing. If complete utilization of the reagents is anticipated, within the above time frame, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

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

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 27°C).

**Test procedure should be performed by a skilled individual or trained personnel.**

1. Format the microplates’ 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. Pipette 0.010ml (10µL) of the appropriate serum reference, control or specimen into the assigned well.

3. Add 0.350ml (350µL) of the PAPP-A Tracer Reagent to all wells.

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

5. Cover and incubate for 45 minutes at room temperature.

Discard the 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) or aspirate. Repeat four (4) additional times for a total of five (5) washes.

8. Automatic or manual plate washer can be used. If a manual plate washer is employed, fill each well with 0.350mL of wash. If an automatic plate washer is employed, fill each well with 0.350mL of wash. Decant the wash and repeat four (4) additional times.

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

10. Rinse the microplate gently with 0.350ml (350µL) of wash buffer (see Reagent Preparation Section). Add 0.010ml (10µL) of luminol in buffer to each well. Store at 2-8°C.

11. Add 0.350ml (350µL) of wash buffer (see Reagent Preparation Section). Add 0.010ml (10µL) of luminol in buffer to each well. Store at 2-8°C.

12. Add 0.100ml (100µL) of working signal reagent to all wells (see Reagent Preparation Section.). Always add reagents in the same well in order to minimize reaction time differences between wells.

DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION

Incubate the plate at room temperature. Read the relative light units (RLUs) obtained from the luminometer as outlined in Example 1.

14. Read the relative light units (RLUs) in each well for 0.2 – 1.0 seconds. The results should be read within thirty (30) minutes of adding the working signal reagent.

Note: Dilute the samples suspected of concentrations higher than 10,000 mIU/mL in PAPP-A diluent and re-assay. The result by dilution factor: For a 1:5 dilution add 40µl of ‘0’ mIU/mL calibrator to 150µl of sample high concentration sample.

10.0 CALCULATION OF RESULTS

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

1. Record the RLUs (Relative Light Units) obtained from the luminometer as outlined in Example 1.

2. Plot the RLUs for each duplicate serum reference versus the corresponding PAPP-A concentration in mIU/L on linear graph paper.

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

4. To determine the concentration of PAPP-A for an unknown, locate the average RLUs for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read
the concentration (in mL/L) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average RLU (19420) of the unknown intersects the calibration curve at (1607 mL/L) PAPP-A concentration (See Figure 1)*.  

Note 1: Computer data reduction software designed for chemiluminescence assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

**EXAMPLE 1**

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>N</th>
<th>X</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Level 1</td>
<td>10</td>
<td>370.5</td>
<td>8.54</td>
</tr>
<tr>
<td>Control Level 2</td>
<td>10</td>
<td>1747.7</td>
<td>133.32</td>
</tr>
<tr>
<td>Control Level 3</td>
<td>10</td>
<td>4575.3</td>
<td>453.38</td>
</tr>
</tbody>
</table>

**TABLE 2:** Within Assay Precision (values in mL/L)

**TABLE 3:** Between Assay Precision (values in mL/L)

**1.2 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 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 maximal 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 Immunocassays (Boscato LM, Stuart MC). "Heterophilic antibodies: a problem for all immunocassays" 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 would produce false test results, or if results are incorrect 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 (MoM) established for the laboratory when assessing patient samples. By dividing the value of the patient sample by the MoM will give a percent value that is used frequently for evaluation.

**TABLE 1**

<table>
<thead>
<tr>
<th>Term of Gestation (full weeks)</th>
<th>PAPP-A Concentration (mIU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>650</td>
</tr>
<tr>
<td>10</td>
<td>1200</td>
</tr>
<tr>
<td>11</td>
<td>1700</td>
</tr>
<tr>
<td>12</td>
<td>2600</td>
</tr>
<tr>
<td>13</td>
<td>3900</td>
</tr>
</tbody>
</table>

**14.0 PERFORMANCE CHARACTERISTICS**

14.1 Precision

The within and between assay precision of the PAPP-A AccuLite® CLIA test system were determined by analyses on three different levels of control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

**14.2 Sensitivity**

The PAPP-A AccuLite® CLIA Test System has a sensitivity less than 2.36 mL/L. The sensitivity was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

**14.3 Accuracy**

The PAPP-A AccuLite® CLIA Test System was compared with a reference method. Biological specimens from low, normal and relatively high PAPP-A level populations were used; the values ranged from 15 mIU/L – 331099 mIU/L. The total number of such specimens was 50. The least square regression equation and the correlation coefficient were computed for this PAPP-A CLIA in comparison with the reference method.

**15.0 REFERENCES**


Revision: 1 Date: 2013-AUG-06 DCO: 0893

**TABLE 4**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free hCG</td>
<td>ND</td>
</tr>
<tr>
<td>aFPI</td>
<td>ND</td>
</tr>
<tr>
<td>hPL</td>
<td>ND</td>
</tr>
<tr>
<td>hSTh</td>
<td>ND</td>
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