Pregnancy Associated Plasma Protein-A (PAPP-A)
Test System
Product Code: 12625-300

1.0 INTRODUCTION

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

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 been of interest 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 used to detect problems that affect a fetus and, therefore, an infant. Screening for markers of PAPP-A and the occurrence of trisomy disorders, particularly Trisomy 21, also known as Down’s Syndrome. Along with a few other markers like APP, uE3, and HCG, preeclampsia in PAPP-A 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 increase as gestation time progresses. 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 like APP, uE3, and HCG, preeclampsia in PAPP-A have been found to elude to trisomy disorders.

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 different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immunobilization takes place during the assay at the surface of a microwell plate through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal 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 microwells resulting in the immobilization of the complex. The interaction is illustrated by the following equation:

$$\text{Abs} = \text{Biotinylated Monoclonal Antibody (Excess Quanity)}$$

$\text{Ag}_{\text{PAPP-A}} + \text{Native Antigen (Variable Quantity)}$

$$\text{Abs}_{\text{Biotinylated Monoclonal Antibody} - \text{Anti body-Antigen Complex (Variable Quantity)}}$$

$\text{ka} = \text{Rate Constant of Association}$

$\text{k}_d = \text{Rate Constant of Dissociation}$

4.0 REAGENTS

Materials Provided:

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

B. PAPP-A Enzyme Reagent – 12 ml/vial icon A

C. PAPP-A Biotin Reagent – 12 ml/vial icon B

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

E. PAPP-A Diluent – 5.0 ml – icon U

F. Streptavidin Coated Plate – 96 wells – icon W

G. Wash Solution Concentrate – 2ml/vial – icon C

H. Substrate Solution – 12ml/vial – icon S

I. Stop Solution – 8ml/vial – icon I

Note: 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. Good laboratory procedures for handling blood products can be found in the Center for Disease Control’s “Biosafety in Microbiological and Biomedical Laboratories,” 2nd Edition, 1985, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirements.

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 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 the individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with the PAPP-A standard. 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 solution to 1000ml with distilled or deionized water. Store at room temperature for up to 60 days.

2. Pipette 0.010ml (10µl) of the appropriate serum reference, control or specimen into a 96-well microplate. Add 0.100ml (100µl) of the PAPP-A Biotin Reagent to all wells.

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

4. Incubate at room temperature for 15-20 minutes.

5. Add 0.350ml (350µl) of wash buffer to (see Reagent Preparation Section), for each sample, standard and control. Decant the plate, blot the plate dry with absorbent paper.

6. Repeat four (4) additional times for a total of five (5) washes.

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

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

9. Incubate for 30 minutes at room temperature.

10. Dissolve and incubate for 30 minutes at room temperature.

11. Centrifuge the specimen to separate the serum from the cells.

12. The specimens shall be blood, serum in type, and the usual variations. For accurate comparison to established normal values, the sample(s) may be stored at temperatures of -20°C for up to 60 days.

13. Follow the manufacturer’s instruction for proper usage. If a squeeze bottle is employed, fill each well by directing the content of the container avoiding “air” bubbles. Do not dispense the wash. Decant the wash and repeat wash four additional times.

14. Add 0.100ml (100µl) of substrate solution to all wells. Allow the substrate to react for 15 minutes

15. Incubate at room temperature for 15-20 minutes.

16. Add 0.050ml (50µl) of wash solution to each well and gently mix. Discard the wash and repeat the addition of wash for at least 30 seconds. Add reagents in the same order to minimize reaction time differences between wells.

17. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm). The results shall be read within fifteen (15) minutes of adding the stop solution.

Note: Dilute the samples suspected of concentrations higher than 32 µg/ml with PAPP-A diluent and multiply result by dilution factor. For each sandwich set, add 40µl of diluent to 10µl of high concentration sample.

10.0 CALCULATION OF RESULTS

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

1. Record the absorbance obtained from the printout of the microplate reader as in Table 1.

2. Plot the absorbance for each duplicate serum reference versus the corresponding PAPP-A concentration in µg/ml on linear log scale.

3. Establish a linear curve for each reagent.

4. Calculate the concentration of PAPP-A in µg/ml for each serum test sample.

5. Analyze data by linear regression to calculate slopes and y-intercepts.

6. Calculate the concentration of PAPP-A in µg/ml by comparing the absorbance values to the linear curve.

7. Establish a linear curve for each reagent.

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14. Calculate the concentration of PAPP-A in µg/ml by comparing the absorbance values to the linear curve.
1. It is important that the time of reaction in each well is held constant to achieve reproducible results.

2. Four out of six quality control pools should be within the listed ranges and assay requirements. If the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

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

4. If test kits are altered, such as by mixing parts of different kits, the sensitivity was ascertained by determining the variability of the 0 µg/ml serum calibrator and using the 2x (95% certainty) statistic to calculate the minimum dose.

5. 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.

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. The within and between assay precisions of the PAPP-A AccuBind® ELISA test system were determined by analyses on four calibrators fall within 10% of the assigned concentrations. The least square regression equation and the correlation coefficient were computed for this PAPP-A ELISA in comparison with the reference method.

# 14.2 Sensitivity

The PAPP-A AccuBind® ELISA Test System has a sensitivity of 0.125 µg/ml. The sensitivity was ascertained by determining the variability of the 0 µg/ml serum calibrator and using the 2x (95% certainty) statistic to calculate the minimum dose.

# 14.3 Accuracy

The PAPP-A AccuBind® ELISA Test System was compared with a reference immunoassay method. Biological specimens from low, normal and relatively high PAPP-A level populations were used; the values ranged from 0.1 µg/ml – 36 µg/ml. The total number of such specimens was 50. The least square regression equation and the correlation coefficient were computed for this PAPP-A ELISA in comparison with the reference method.

# 13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" adult population, the expected ranges for the PAPP-A AccuBind® ELISA 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.

# 15.0 REFERENCES