



DIAGNOSTIC AUTOMATION, INC.

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IVD



See external label



2°C-8°C



Σ=96 tests

REF

#4229-6

PAPP-A (Pregnancy Associated Plasma Protein A) ELISA

Cat. No. 4229-6

CLINICAL INDICATION

Risk evaluation of fetal aneuploidies in the first trimester of pregnancy.

CLINICAL RELEVANCE

PAPP-A (Pregnancy associated plasma protein A) is a protein produced by the developing placenta. PAPP-A may be measured in maternal serum; its concentration increases rapidly after the 7th week of pregnancy and its potential clinical usefulness is greatest in the first trimester. A low PAPP-A concentration has been reported as a potentially useful marker in antenatal screening for Down Syndrome in conjunction with age and free βHCG measurement.

TEST PRINCIPLE

The DIAGNOSTIC AUTOMATION, INC. PAPP-A (Sandwich) EIA is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a polyclonal anti PAPP-A antibody. An aliquot of patient sample containing endogenous PAPP-A is incubated in the coated well with a sample buffer. After incubation the unbound material is washed off. In the second incubation step a sandwich complex is formed with anti PAPP-A antibody peroxidase conjugate. Having added the substrate solution, the intensity of color developed is proportional to the concentration of PAPP-A in the patient sample.

REAGENTS

Materials provided

Test kit for 96 determinations, containing

1. Microtiter Wells: 12 Strips à 8 wells (wells breakable) coated with polyclonal anti PAPP-A antibody
2. Reference Standards (lyoph.), 6 vials, concentration after reconstitution with 150 µl sample buffer: 0, 1, 2.5, 5.0, 15 and 30 µg/ml
3. Control sera, 2 vials (lyoph.), level 1 (0.90 - 2.10 µg/ml) and level 2 (5.90 - 13.9 µg/ml) (exact control ranges see vial label)
4. Sample Buffer: 25 ml
5. HRP Enzyme Conjugate (10X conc.), 1.5 ml, complex containing Horseradish Peroxidase
6. Conjugate Diluent:, 14 ml
7. Substrate Solution - TMB - 14 ml
8. Stop Solution: 0,5 M H₂SO₄, 14 ml
9. Washing solution (concentrated): 40X concentrated, 30 ml

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microtiterplate reader (450 ± 10 nm), e.g.

2. Precision micropipettes with disposable tips for 10, 200 and 1000 μ l
3. Standard refrigerator
4. Absorbent paper
5. Deionized water

STORAGE CONDITIONS

When stored at 2° to 8° C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date. PAPP-A Enzyme Complex, Substrate Solution, Wash Solution and Standards must be stored at 2° to 8° C. Microtiter wells must be stored at 2° to 8° C. Once the foil pouch has been opened care should be taken to seal it tightly again. The immuno-reactivity of the coated microtiter wells is stable for 3 months in the resealed tightly closed pouch containing the desiccant.

WARNINGS AND PRECAUTIONS FOR USERS

1. Test methods are not available which can offer complete assurance that Hepatitis B virus, Human Immunodeficiency Virus (HIV/HTLV-III/LAV), or other infectious agents are absent from the reagents in this kit. Therefore, all human blood products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation, where it exists (e.g. USA Center for Disease Control/National Institute of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories", 1984).
2. Avoid contact with Stop Solution - 0.5 M H₂SO₄. It may cause skin irritation and burns.
3. Replace caps on reagents immediately. Do not switch caps.
4. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
5. Do not pipette reagents by mouth.
6. For in-vitro diagnostic use only!
7. Do not mix or use components from kits with different lot numbers.

SPECIMEN COLLECTION AND PREPARATION

For Serum the usual precautions for venipuncture should be observed. No special pretreatment of the sample is necessary. The specimen may be stored at 2-8° C for up to 24 hours, and should be frozen at -10° C or lower for longer periods.

Do not use grossly hemolyzed or grossly lipemic specimens.

PERFORMANCE OF THE ASSAY

GENERAL REMARKS:

1. All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
2. Once the test has been started, all steps should be completed without interruption.
3. Use new disposable plastic pipette tips for each reagent, standard or specimen in order to avoid cross contamination. For the dispensing of the Substrate Solution and the Stop Solution avoid pipettes with metal parts.
4. Before starting the assay, it is recommended that all reagents be ready, caps removed, all needed wells secured in holder etc. This will ensure equal elapsed time for each pipetting step without interruption.
5. As a general rule the enzymatic reaction is linearly proportional to time and temperature. This makes interpolation possible for fixed physico-chemical conditions. If in a test run the absorbance of Zero Standard is lower than 1,0 or above the upper performance limit of your microtiterplate spectrophotometer you can extend or reduce the incubation time of the final enzymatic formation of color to 45 or 15 minutes accordingly.
6. The Substrate Solution should be colorless or slightly blue or green. If the solution is dark blue the reagent is unusable and must be discarded.

7. During incubation with Substrate Solution avoid direct sunlight on the microtiter plate.

TEST PROCEDURE 1 (manual procedure)

REAGENT PREPARATION

- Reference Standards: Reconstitute the lyophilized contents of the standard vial with 150 µl Sample buffer. **Note:** The reconstituted standards are stable for 7 days at 2-8°C. For longer storage freeze at -20°C.
- PAPP-A Control Sera: Reconstitute the lyophilized controls with 150 µl Aqua dest. each. Aliquot and store at -20° C after reconstitution.
- Wash Solution: Dilute 30 ml of concentrated Wash Solution with 1170 ml deionized water to a final volume of 1,2 Liters. The diluted Wash Solution is stable for 2 weeks at room temperature.
- Enzyme Conjugate: 30 min. before use dilute 1.0 ml of concentrated Enzyme Conjugate with 10 ml Conjugate Diluent. **Please note:** The Enzyme Conjugate has to be prepared fresh 30 min. before use and cannot be stored longer than 24 hours. If more than one test run is performed, dilute only the quantity required for each test run.

ASSAY PROCEDURE

1. Secure the desired number of coated microtiter wells in the holder
2. Dispense 10 µl Standard/Control/Sample into the respective wells. It is recommended to run standards and samples in dual determination.
3. Add 100 µl Sample Buffer into each well. Incubate the plate for 30 min. at room temperature.
4. Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted Wash Solution, 300 µl per well. Strike the wells sharply on absorbent paper to remove residual droplets.
5. Dispense 100 µl of diluted Enzyme Conjugate (see Reagent Preparation) into all wells.
6. Incubate the plate 30 min. at room temperature.
7. Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted wash solution (300 µl per well). Strike the wells sharply on absorbent paper to remove residual droplets.
8. Add 100 µl of Substrate Solution (TMB) to each well at timed intervals.
9. Incubate for 15 min. at room temperature.
10. Stop the enzymatic reaction by adding 50 µl of Stop Solution to each well and determine the absorbance of each well at 450 ± 10 nm.
11. Please note: Dispense the Stop Solution into the centre of the well so that a complete mixing with the Substrate Solution is achieved.

TEST PROCEDURE 2 (procedure for automated instrumentation or optional version for manual use)

REAGENT PREPARATION

- Reference Standards: Reconstitute the lyophilized contents of the standard vial with 1.5 ml Sample Buffer. **Note:** The reconstituted standards are stable for 7 days at 2-8°C. For longer storage freeze at -20°C.
- PAPP-A Control Sera: Reconstitute the lyophilized controls with 1.5 ml Sample Buffer. Aliquot and store at -20° C after reconstitution.
- Wash Solution: Dilute 30 ml of concentrated Wash Solution with 1170 ml deionized water to a final volume of 1,2 Liters. The diluted Wash Solution is stable for 2 weeks at room temperature.
- Enzyme Conjugate: 30 min. before use dilute 1.4 ml of concentrated Enzyme Conjugate with 14 ml Conjugate Diluent. **Please note:** The Enzyme Conjugate has to be prepared fresh 30 min. before use and cannot be stored longer than 24 hours. If more than one test run is performed, dilute only the quantity required for each test run.
- Sample preparation: Dilute 50-µl samples with 450 µl Sample Buffer.

ASSAY PROCEDURE

1. Secure the desired number of coated microtiter wells in the holder

2. Dispense 100 µl Standard/Control/Sample into the respective wells. It is recommended to run standards and samples in dual determination.
3. Incubate the plate for 30 min. at room temperature.
4. Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted Wash Solution, 300 µl per well. Strike the wells sharply on absorbent paper to remove residual droplets.
5. Dispense 100 µl of diluted Enzyme Conjugate (see Reagent Preparation) into all wells.
6. Incubate the plate 30 min. at room temperature.
7. Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted wash solution (300 µl per well). Strike the wells sharply on absorbent paper to remove residual droplets.
8. Add 100 µl of Substrate Solution (TMB) to each well at timed intervals.
9. Incubate for 15 min. at room temperature.
10. Stop the enzymatic reaction by adding 50 µl of Stop Solution to each well and determine the absorbance of each well at 450 ± 10 nm.
11. **Please note:** Dispense the Stop Solution into the centre of the well so that a complete mixing with the Substrate Solution is achieved.

FINAL REACTION STABILITY

It is recommended that the wells be read within 10 minutes following step 11.

CALCULATION OF RESULTS

Any microwell reader capable of determining the absorbance at 450 ± 10 nm may be used, PAPP-A value of each serum sample obtained as follows:

1. Using linear-linear or semi log graph paper, construct a standard curve by plotting the average absorbance (Y) of each Reference Standard against its corresponding PAPP-A value by simple interpolation from this standard curve.
2. Use the average absorbance of each serum sample to determine the corresponding PAPP-A value by simple interpolation from this standard curve.
Regression Program allow the reading and computer assisted interpretation using a four parameter logistic function.

NORMAL VALUES

An extensive clinical evaluation of the MOM-values is now in its final stages. It is recommended that each laboratory determine its own reference ranges. The following data are for guidance only.

SUMMARY DIAGNOSTIC AUTOMATION, INC. PAPP-A ELISA

Week of pregnancy	Valid N	Median mg/L	Minimum mg/L	Maximum mg/L	Mean mg/L	Std. Dev.
8	9	2.60	1.20	6.30	2.91	1.49
9	21	4.20	1.60	18.72	5.09	3.70
10	40	7.56	0.26	24.00	9.17	5.45
11	130	11.0	1.72	100.0	12.20	9.91
12	112	15.06	2.31	45.00	16.12	7.87
13	44	17.00	4.04	46.00	19.28	9.68
14	10	21.51	11.0	47.44	23.93	10.06

Conversion Factor:

1 mIU/ml = 4.5 mg/L

1 ng/ml = 222 mIU/L

ASSAY CHARACTERISTICS

SENSITIVITY

The analytical sensitivity of the PAPP-A ELISA was calculated from the mean minus 2SD of 14 replicate analyses of zero standard. Analytical Sensitivity is $\leq 0.19 \mu\text{g/ml}$.

PRECISION WITHIN-RUN

The intra-assay (within-run) variation was determined by repeated measurements of control samples.

Sample 1		Sample 2	
Mean	1,09	Mean	9,562
SD	0,04	SD	0,27
CV (%)	4,08	CV (%)	2,85
N	21	N	21

PRECISION BETWEEN-RUN

The inter-assay (between-run) variation was determined by repeated measurements of control samples in the three different kit lots.

Sample 1		Sample 2	
Mean	1,13	Mean	10,09
SD	0,07	SD	0,48
CV(%)	6,39	CV(%)	4,74
N=	24	N=	24

RECOVERY TEST

We estimated the analytical recovery of PAPP-A in the ELISA at 3 different concentrations in serum samples. Increasing amounts of unlabelled PAPP-A were added to the samples with various initial PAPP-A concentrations. Each sample (non spiked and spiked) was assayed. PAPP-A concentration was measured and the percentage recovery rates were calculated.

Sample	Endogenous PAPP-A ($\mu\text{g/ml}$)	Added PAPP-A ($\mu\text{g/ml}$)	Measured Conc. PAPP-A ($\mu\text{g/ml}$)	Expected Conc. PAPP-A ($\mu\text{g/ml}$)	Recovery (%)
1 Serum	1,00	0,00	1,09	3,00	100,3
		2,00	3,01		
		4,00	5,18		
		8,00	9,39		
2 Serum	10,00	0,00	10,15	12,00	105,7
		2,00	12,69		
		4,00	14,19		
		8,00	18,44		
			18,00	102,5	

DILUTION TEST

Two samples (serum) having different PAPP-A levels were serially diluted with zero standard and the PAPP-A content in the diluted samples were assayed by the ELISA. Four dilutions were performed for each sample and the percentage recovery rates were calculated.

Sample	Endogenous PAPP-A ($\mu\text{g/ml}$)	Measured Conc. PAPP-A ($\mu\text{g/ml}$)	Expected Conc. PAPP-A ($\mu\text{g/ml}$)	Recovery (%)
1 Serum	Undil.	18,11	18,11	100,0
	1:2	9,06	9,06	
	1:4	4,88	4,53	

	1:8	2,38	2,26	105,3
	1:16	1,05	1,13	92,6
2 Serum	Undil.	10,14	10,14	
	0,902	5,16	5,07	101,8
	0,608	2,51	2,53	99,1
	0,411	1,17	1,27	92,6
	0,310	0,60	0,63	94,7


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