Development of a well characterized PAPP-A2 ELISA to measure PAPP-A2 in maternal biological fluids

B. Kalra¹ A. Kumar¹, A.Tran¹, C. Oxvig², S.Shah¹ ¹AnshLabs, Webster, TX, ² University of Aarhus, Aarhus, Denmark

ABSTRACT

Relevance: Pregnancy-associated plasma protein-A2 (PAPP-A2) is a novel metalloproteinase identified as a homolog of PAPP-A in the metzincin superfamily of pappalysins. PAPP-A2 shares 46% sequence identity with PAPP-A. PAPP-A2 is a noncovalently linked dimer of two 220-kDa subunits. It exhibits robust proteolytic activity against IGFBP-5 and possibly also IGFBP-3. PAPP-A2 is expressed in a wide range of tissues and is abundant in placental syncytiotrophoblasts and the pregnant uterus. The physiological importance of PAPP-A2 is not fully known.

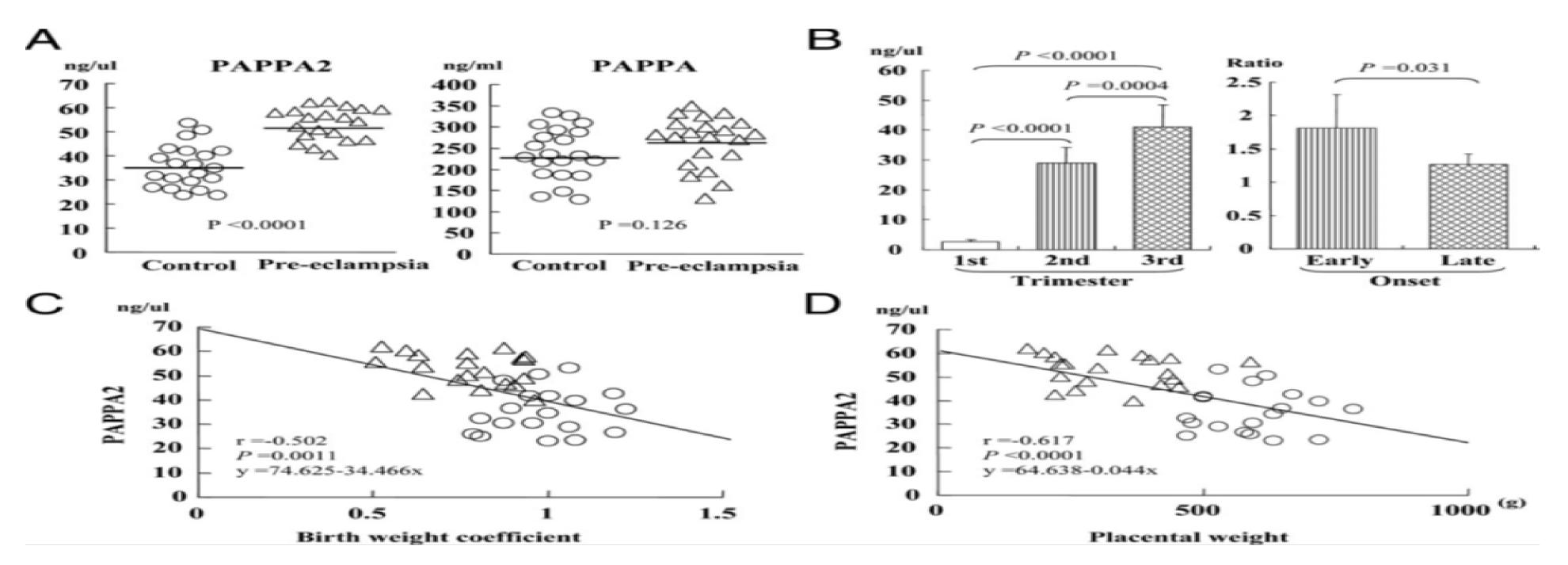
Methodology: We have developed a well characterized two-step sandwichtype enzymatic microplate ELISA to measure PAPP-A2 levels in the maternal serum and other biological fluids. The assay measures PAPP-A2 in 50 µL of sample (diluted 20-fold in sample diluent) against recombinant PAPP-A2 calibrators (0.1-10 ng/mL). The antibody pair used in the PAPP-A2 ELISA measures PAPP-A2 and does not detect proMBP, dimeric PAPP-A and PAPP-A-proMBP complex.

Validation Total imprecision calculated on 4 samples over 12 runs, 4 replicates per run, using CLSI EP5-A guidelines was 4.7% at 1.03ng/mL, 4.% at 1.8ng/mL, 4.6% at 2.6ng/mL and 4.4% at 3.13ng/mL. The limit of detection calculated using six serum samples in the range of 0.091-3.085ng/mL over 12 runs is 0.071ng/mL.

The functional sensitivity of the assay at 20% CV was 0.08ng/mL. Dilution studies showed an average recovery of 100-110%. The median PAPP-A2 value on a first trimester samples (n=65) and second trimester samples (n=65) were 30.76ng/mL and 42.14ng/mL, respectively. When potential interferents (hemoglobin, triglycerides and bilirubin) were added at two times their physiological concentration, PAPP-A2 concentrations were within ±10% of the control.

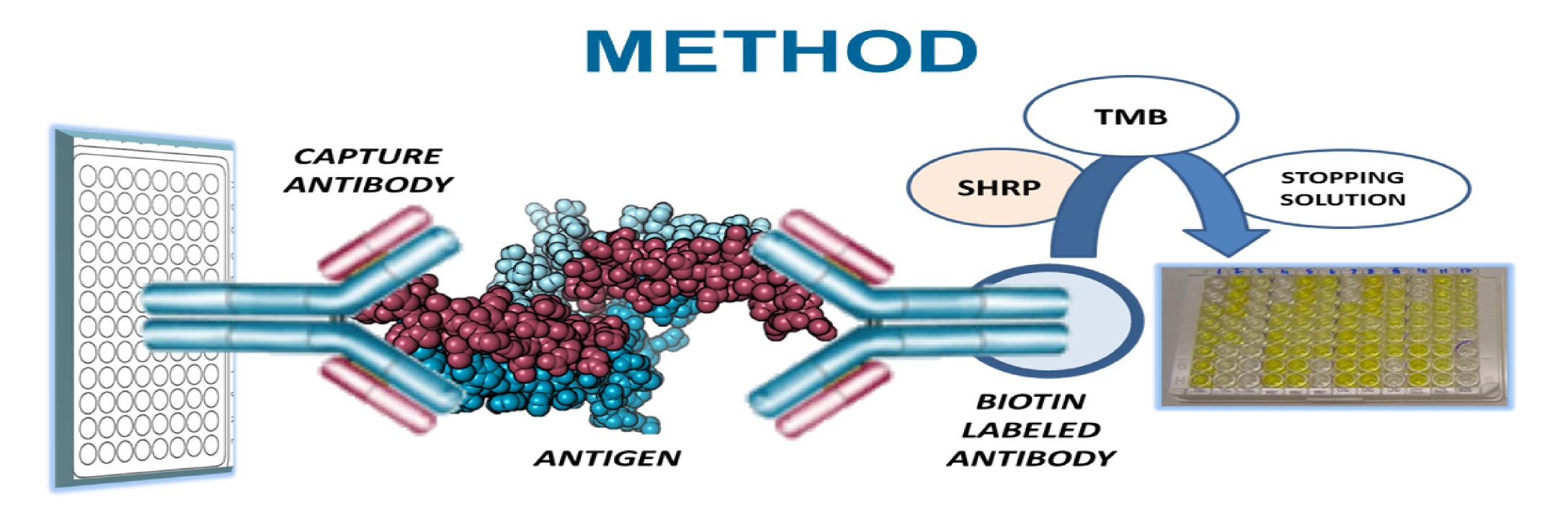
Conclusions: A quantitative, robust and fully characterized microplate PAPP-A2 ELISA has been developed to measure PAPP-A2 in maternal serum. The approximate median PAPP-A2 levels found in a first and second trimester maternal serum can be measured with < 5 % CV using this assay. The performance of the assay is acceptable for investigation of clinical utility in a variety of pregnancy-related disorder.

INTRODUCTION



Correlation of circulating PAPP-A and -A2 concentrations with various clinical parameters. (A) Serum concentration of PAPP-A (right) and -A2 (left) measured by ELISA. Maternal blood samples were obtained either at the time of diagnosis or during surgery. The concentrations were compared between from uncomplicated pregnancy (circles) and from preeclampsia (triangles). The horizontal bars indicate mean values. (B) Correlation between PAPP-A2 and gestational week or disease severity. (C) Correlation between and normalized birth weight. Open circles indicate control uncomplicated pregnancy, whereas open triangles indicate preeclampsia. A regression line is shown with correlation coefficients and P-values. (D) Correlation between PAPP-A2 concentration and placental weight.

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RESULTS

Antibody pairing

Antibody pairing	ıg							
Capture Antibody	4C	1N	2C	4C	2C	1N	2C	4C
Detection Antibody	2C	5N	5N	5N	8N	9N	9N	9N
CALIBRATORS	Optical Density							
Cal A (0.0 ng/mL)	0.04	0.201	0.087	0.061	0.085	0.108	0.067	0.054
Cal B (0.1 ng/mL)	0.09	0.19	0.14	0.11	0.14	0.13	0.11	0.11
Cal F (10.0 ng/mL)	3.21	1.82	3.67	3.58	3.78	1.43	3.51	3.89
Sample ID	PAPP-A2 Concentration in (ng/mL)							
C-Terminal truncated PAPP-A2 (20 ng/mL)	0.06	14.20	0.06	0.06	0.06	15	0.06	0.06
Amniotic Fluid 1:19dilution	7.14	2.74	4.43	4.08	5.79	2.63	4.90	4.29
1 st Trimester Serum 1:9 dilution	14.97	0.06	0.06	0.06	0.06	0.06	0.06	0.06
1 st Trimester Serum 1:9 dilution	9.20	2.09	2.70	2.482	3.61	2.31	2.76	2.51
1 st Trimester Serum 1:9 dilution	6.22	1.96	2.65	2.41	3.35	2.12	2.70	2.07
2 nd Trimester Serum 1:9 dilution	5.58	0.06	0.79	0.75	1.35	0.06	0.76	0.72
2 nd Trimester Serum 1:9 dilution	15.85	0.06	1.55	1.40	2.46	0.06	1.44	1.19
Non-Pregnant Female	0.21	0.06	0.13	0.22	0.16	0.06	0.15	0.14

Limit of Detection: The lowest amount of PAPP-A2 in a sample that can be detected with a 95% probability (n=24) is 0.071 ng/mL. The value was determined by processing six serum samples in the range of 0.091 to 3.085 ng/mL. Three assay runs per day were performed over four days with all samples run in duplicate per run.

Quantitation: at 20% total imprecision is 0.08 value was determined by 🔂 8.0 processing eight samples in the range of 5 4.0 0.091-3.08 ng/mL over twelve runs and four days in duplicates (n=24) following CLSI EP17 guidelines.

PAPP-A2 (ng/mL)

Imprecision: Reproducibility of the PAPP-A2 ELISA was determined on four serum pools. Serum pools were run in replicates of four/assays and twelve runs

Sample	Mean Conc.	Within Run		Between Run		Total	
	(ng/mL)	SD	% CV	SD	% CV	SD	% CV
Pool-1	1.029	0.044	4.25%	0.020	1.94%	0.048	4.67%
Pool-2	1.813	0.059	3.28%	0.046	2.53%	0.075	4.14%
Pool-3	2.651	0.111	4.18%	0.050	1.89%	0.122	4.59%
Pool-4	3.128	0.115	3.68%	0.076	2.42%	0.138	4.41%

Linearity of Dilution: First and Second Trimester Pregnancy sera diluted in calibrator A/Sample diluent.

	Dilution	Expected	Observed	
SAMPLE	Dilution	Value	Value	% Recovery
	Factor (1:X)	(ng/mL)	(ng/mL)	
	1:10	8.134	N/A	N/A
_	1:20	4.067	4.239	104
1	1:40	2.034	2.126	105
	1:80	1.017	1.070	105
	1:10	7.541	N/A	N/A
	1:20	3.771	3.808	101
2	1:40	1.885	1.996	106
	1:80	0.943	1.025	109
3	1:10	6.636	N/A	N/A
	1:20	3.318	3.599	108
	1:40	1.659	1.751	106
	1:80	0.830	0.882	106
	1:10	5.245	N/A	N/A
	1:20	2.623	2.693	103
4	1:40	1.311	1.372	105
	1:80	0.656	0.824	126

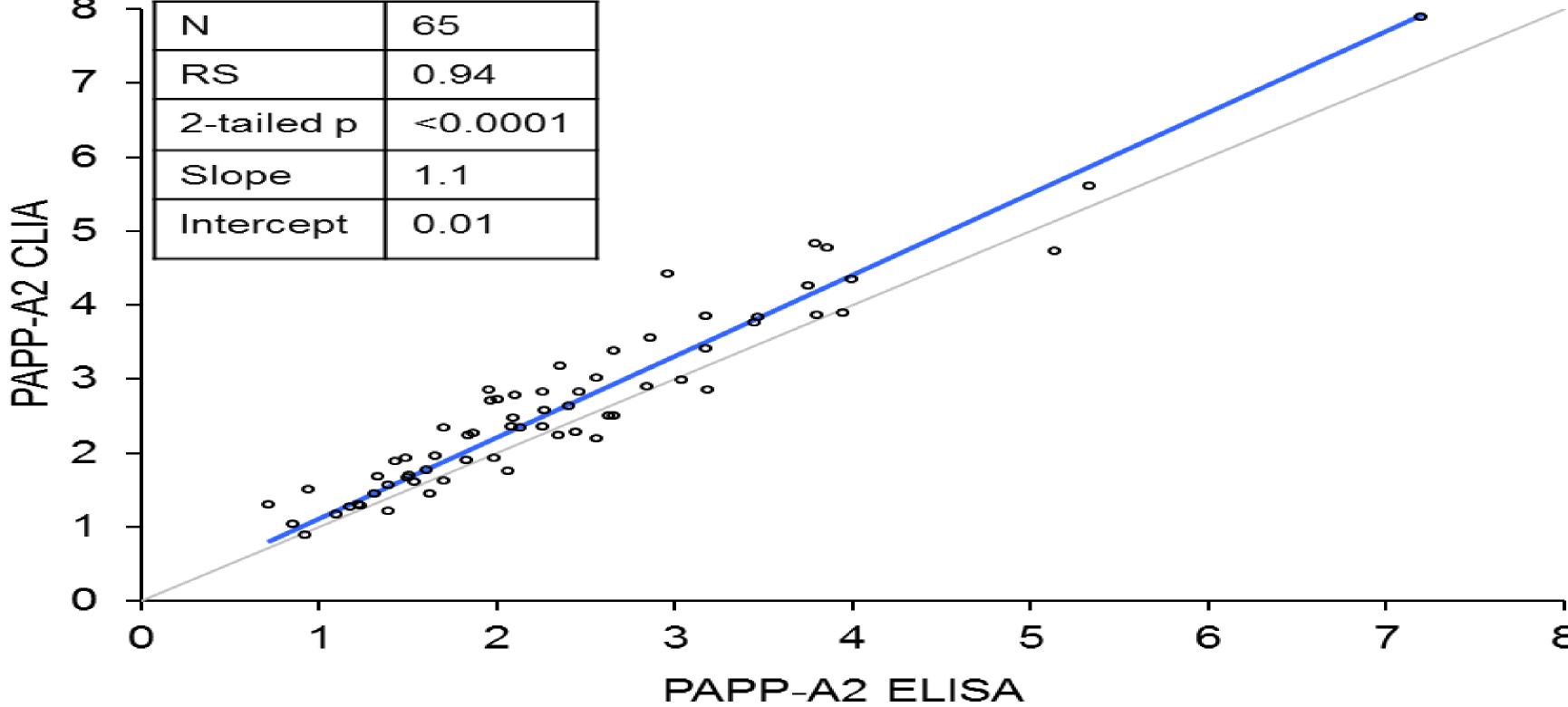
Spike Recovery: Sample spiked with rPAPP-A2

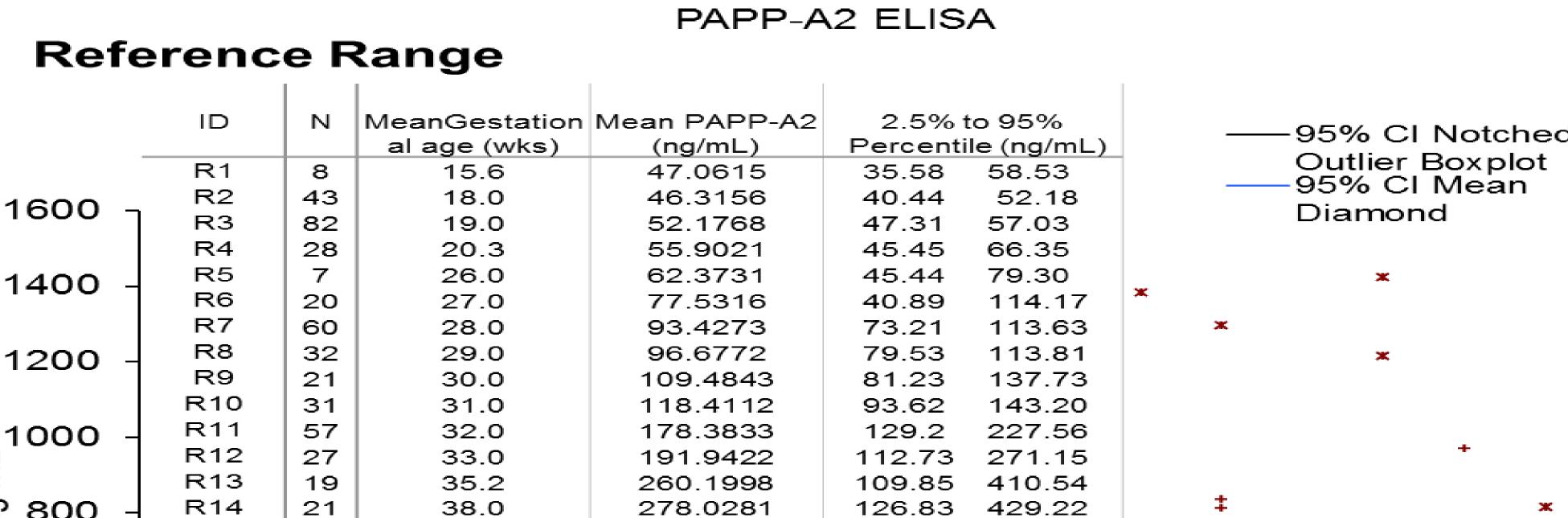
s	ample	Endogenous conc. (ng/mL)	Expected conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
	1	0.297	0.788 1.179	0.675 0.978	86 83
	2	0.302	0.793	0.699	88
		0.002	1.184	1.058	89
	3	0.329	0.819 1.208	0.698 1.009	85 84
	4 0.53	1.009	0.934	93	
	-	0.55	1.391	1.167	84

Cross Reactivity and Interference:

A 10	NID
I	ND
P 0.05	ND
bin 1350	3.62
ide 11000	-3.62
n 4200	5.14
r	obin 1350 ride 11000

Method Comparison





148.5405

247.8729

R1 R2 R3 R4 R5 R6 R7 R8 R9 R10R11R12R13R14R15R16R17R18

117.79 179.29

160.52 335.21

CONCLUSIONS

- ☐ A sensitive, reliable and easy-to-run microplate PAPP-A2 assay has been developed .The approximate median PAPP-A2 levels found in first and second trimester pregnancy can be measured within <5% CV using this assay.
- ☐ The assay has shown excellent analytical performance and is suitable for studies in the area of pregnancy related complications.

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