

# Development of a well characterized PAPP-A2 chemiluminescence assay to measure PAPP-A2 in maternal biological fluids.

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## ABSTRACT

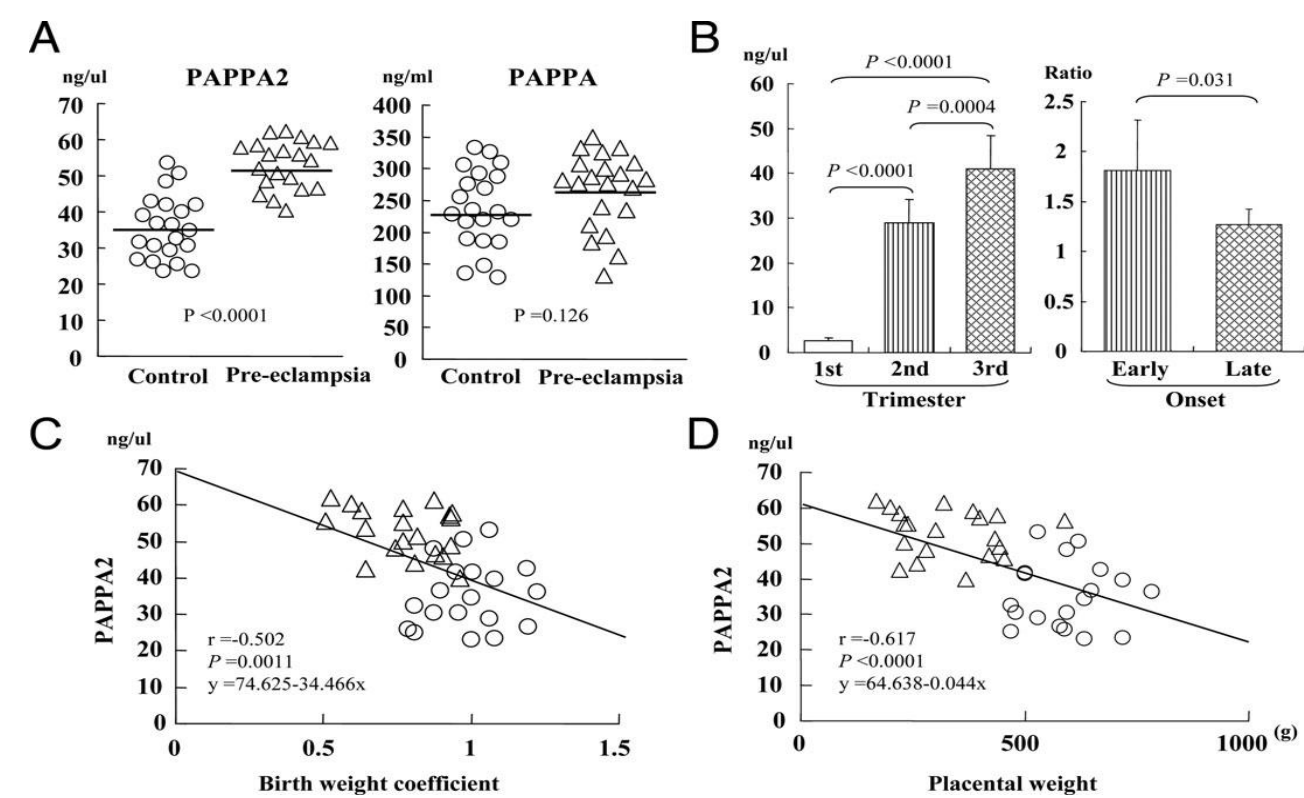
**Relevance:** Pregnancy-associated plasma protein-A2 (PAPP-A2) is a novel metalloproteinase identified as a homolog of PAPP-A in the metzincin superfamily of pappalysins. PAPP-A2 shares 46% sequence identity with PAPP-A. PAPP-A2 is a noncovalently linked dimer of two 220-kDa subunits. It exhibits proteolytic activity against IGFBP-5 and 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 known.

**Methodology:** We have developed a well characterized two-step sandwich-type enzymatic microplate CLIA to measure PAPP-A2 levels in the maternal serum and other biological fluids. The assay measures PAPP-A2 in 50  $\mu$ L of sample (diluted 20 folds in sample diluent) against recombinant PAPP-A2 calibrators (0.25-25 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 4.46% at 1.01ng/mL, 7.38% at 1.83ng/mL, 2.94% at 2.87ng/mL and 3.88% at 7.48ng/mL. The limit of detection calculated using six serum samples in the range of 0.125-1.9ng/mL over 12 runs is 0.1ng/mL. The functional sensitivity of the assay at 20% CV was 0.18ng/mL. Dilution studies showed an average recovery of 98-110%. The median PAPP-A2 value on second trimester samples (n=65) was 47.09ng/mL.

**Conclusions:** A quantitative, robust and fully characterized microplate PAPP-A2 CLIA has been developed to measure PAPP-A2 in maternal serum. The approximate median PAPP-A2 levels found in 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 disorders.

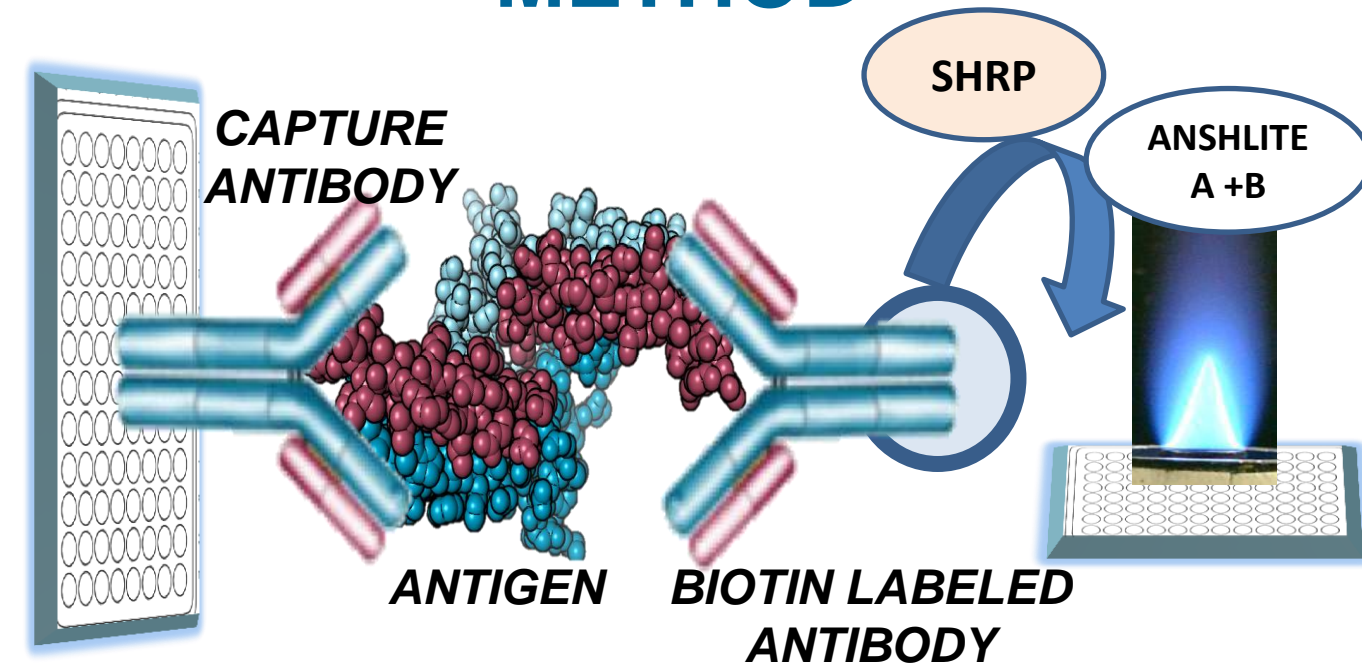
## INTRODUCTION



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 sera 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 PAPP-A2 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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## METHOD



## RESULTS

**Analytical Specificity:** The antibody pair used in the PAPP-A2 CLIA measures bioactive PAPP-A2 (full length) and does not detect other variants of PAPP-A2 (Truncated C-Terminal).

**Limit of Detection:** The lowest amount of PAPP-A2 in a sample that can be detected with a 95% probability (n=24) is 0.171 ng/mL. The value was determined by processing six serum samples in the range of 0.12 to 1.9 ng/mL. Two assay runs per day were performed over six days with all samples run in duplicate per run.

**Limit of Quantitation:** The estimated minimum PAPP-A2 dose achieved at 20% total imprecision is 0.184 ng/mL. The value was determined by processing seven samples in the range of 0.19-1.9 ng/mL over twelve runs and six days in duplicates (n=24) following CLSI EP17 guidelines.

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

Sample	Mean conc. (ng/mL)	Within run		Between run		Total	
		SD	%CV	SD	%CV	SD	%CV
Pool-1	1.008	0.026	2.63%	0.036	3.60%	0.045	4.46%
Pool-2	1.836	0.055	2.98%	0.123	6.69%	0.134	7.32%
Pool-3	2.875	0.057	1.99%	0.062	2.16%	0.085	2.94%
Pool-4	7.479	0.277	3.71%	0.087	1.16%	0.290	3.88%

### Cross Reactivity and Interference:

S. No	Analyte	Conc. (ug/mL)	% Difference to Control
1	PAPP-A	10	ND
2	ProMBP	0.05	ND
3	Hemoglobin	1350	4.98
4	Triglyceride	5000	-3.71
5	Bilirubin	600	0.246

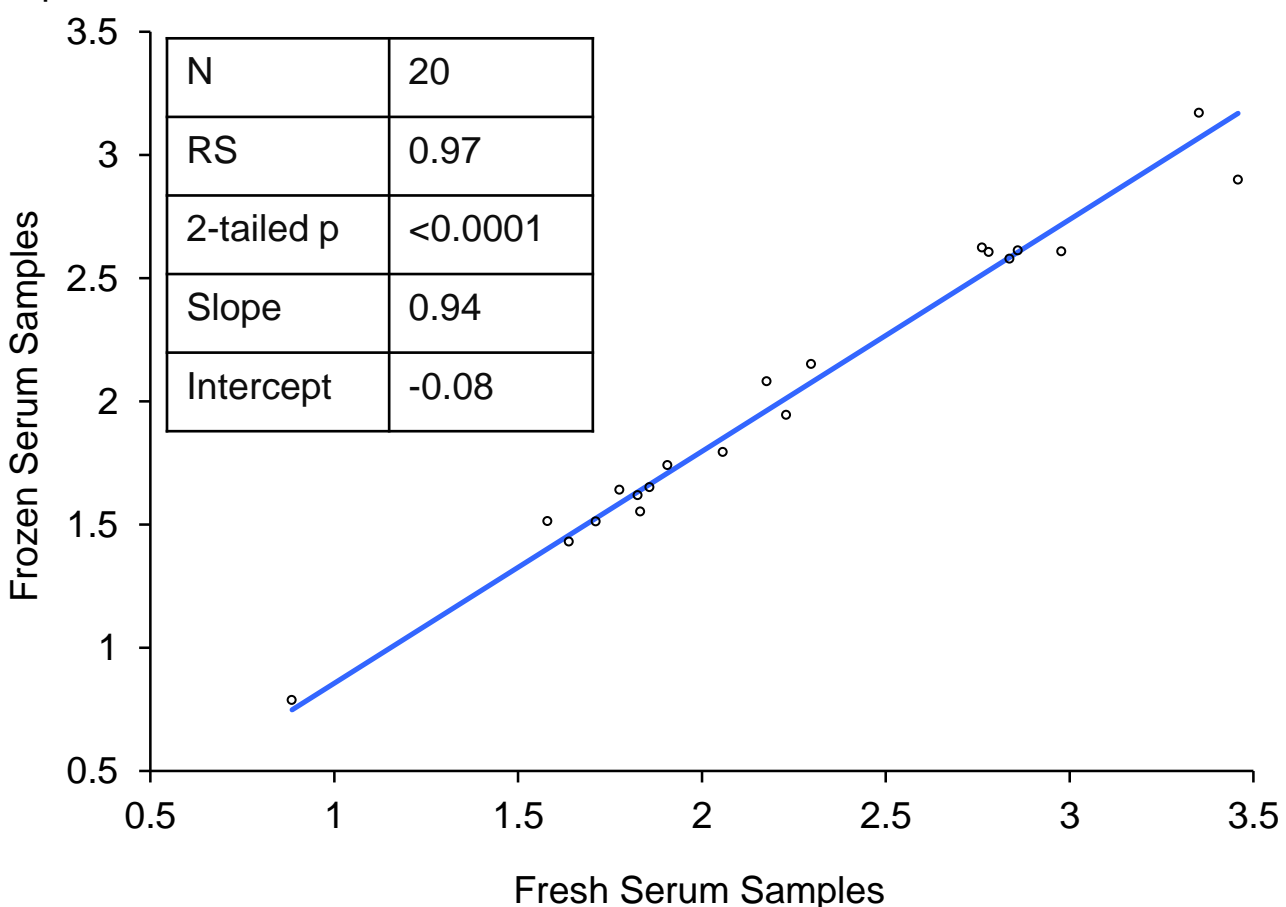
**Linearity of Dilution:** First and Second Trimester Pregnancy sera diluted in calibrator A/Sample diluent.

Sample ID	Dilution factor (1 in X)	Expected Value (ng/mL)	Observed Value (ng/mL)	% Recovery
S1	1:10	12.918	NA	NA
	1:20	6.459	6.558	102%
	1:40	3.230	3.389	105%
	1:80	1.615	1.789	111%
	1:160	0.807	0.914	113%
S2	1:10	18.118	NA	NA
	1:20	9.059	9.039	100%
	1:40	4.530	4.697	104%
	1:80	2.265	2.389	105%
	1:160	1.132	1.241	110%
S3	1:10	1.173	NA	NA
	1:20	0.587	0.606	103%
	1:40	0.293	0.317	108%
	1:80	0.147	0.172	117%
	1:160	0.073	0.081	110%
S4	1:10	6.607	NA	NA
	1:20	3.303	3.018	91%
	1:40	1.652	1.649	100%
	1:80	0.826	0.824	100%
	1:160	0.413	0.417	101%

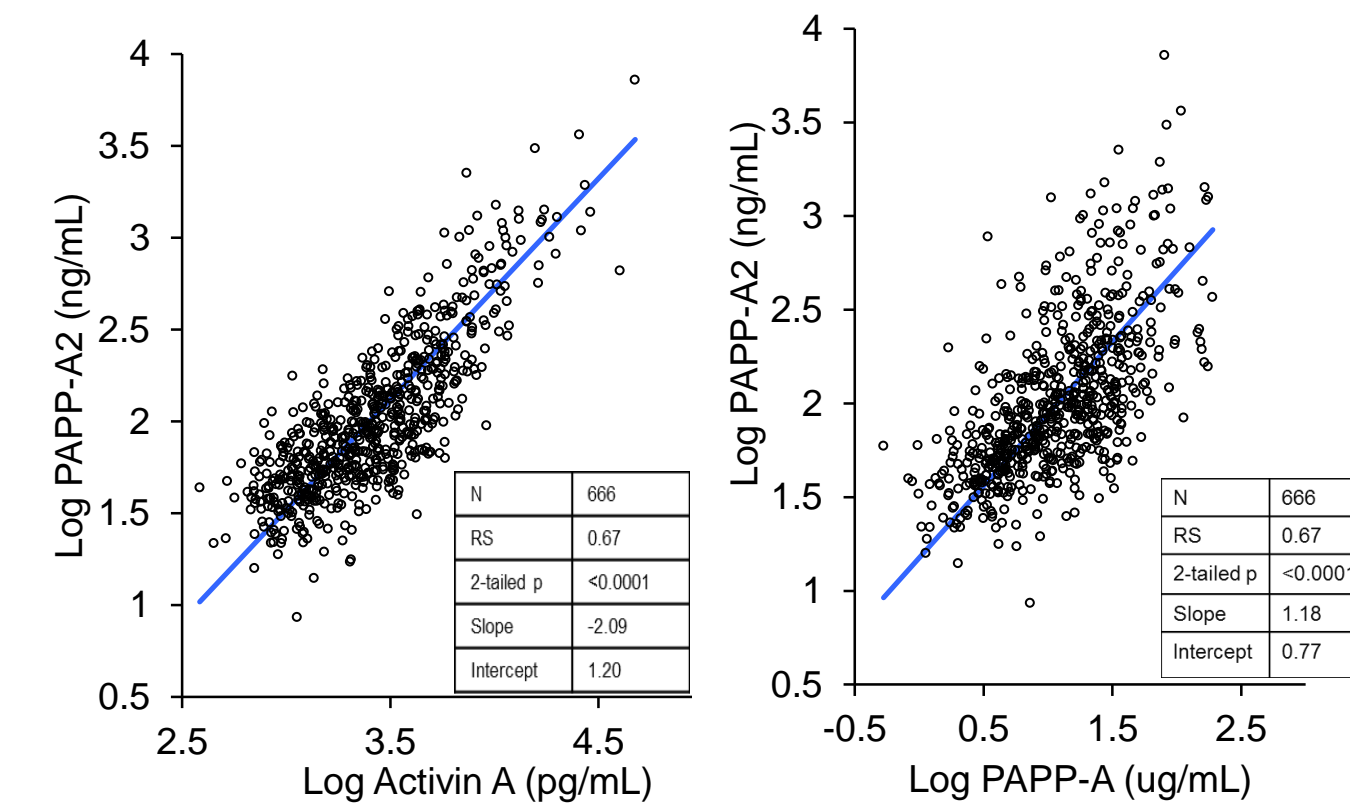
**Spike Recovery:** Serum sample were spiked with rPAPP-A2

Sample	Endogenous conc. (ng/mL)	Expected conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
1	0.252	0.76	0.689	92
		1.138	1.038	91
2	0.320	0.811	0.692	85
		1.2	1.022	85
3	0.4551	0.938	0.856	91
		1.323	1.215	92

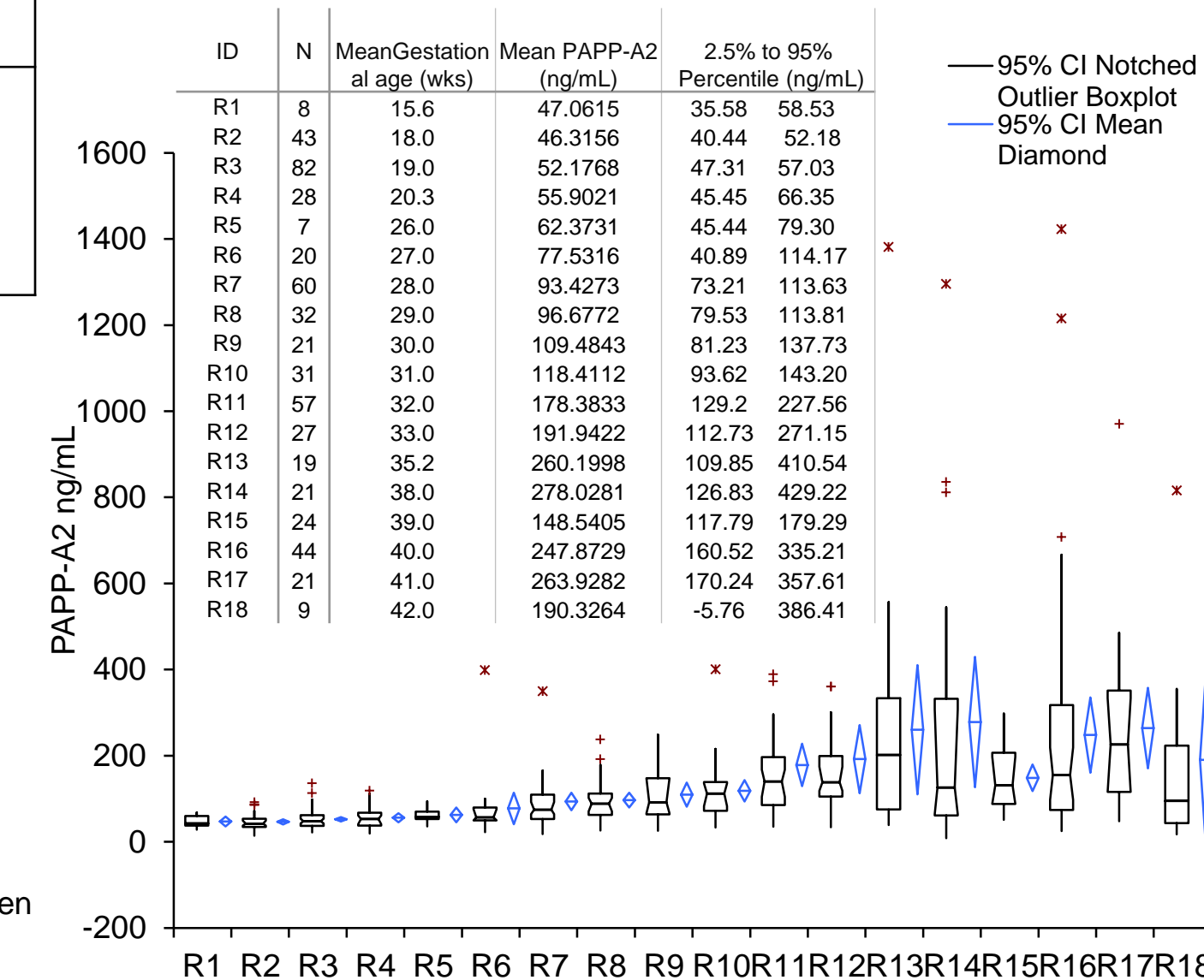
**Sample Stability:** Fresh drawn samples were compared to frozen specimens.



## Analyte Comparison



## Reference Range



## 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 <7% CV using this assay.

The assay has shown excellent analytical performance and is suitable for studies in the area of pregnancy related complications.

## ACKNOWLEDGEMENTS

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