Development of a fully characterized Total PAPP-A chemiluminescence assay for maternal serum evaluation

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ABSTRACT

Relevance: Maternal serum concentrations of pregnancy-associated plasma protein A (PAPP-A, EC 3.4.24.79, papalysin-1) are used to predict occurrence of Down syndrome. During pregnancy, PAPP-A is produced in high concentrations by the trophoblast and released into maternal circulation. In pregnancy, PAPP-A primarily circulates as 500-kDa heterotetrameric 2:2 complex with the proform of eosinophil major basic protein (proMBP), which inhibits the proteolytic activity of PAPP-A. Dimeric PAPP-A is the only active form and proteolyses IGFBP-4 and IGFBP-5. Significant amounts of active PAPP-A is reported at gestational ages between seven and thirteen weeks.

Methodology: We have developed a well characterized two-step sandwichtype enzymatic microplate glow based chemiluminescence assay to measure PAPP-A levels in the maternal serum. The assay measures PAPP-A in 50 µL of serum sample (diluted 100 folds in sample diluent) against heterotetrameric PAPP-A calibrators (25-5000 ng/mL). The antibody pair used in the assay measures dimeric PAPP-A and PAPP-A/proMBP complex in almost equal concentration and does not cross-react with proMBP, PAPP-A2 and MMP-9 at twice the physiological concentrations.

Validation Total imprecision calculated on 3 samples over 10 runs, 4 replicates per run, using CLSI EP5-A guidelines was 3.1% at 249ng/mL, 4.3% at 476ng/mL and 5.7% at 732.5ng/mL. The limit of detection calculated using six serum samples in the range of 3.95-159.15ng/mL over 10 runs is 7.73ng/mL. The functional sensitivity of the assay at 15% CV was 11.65ng/mL. Dilution studies showed an average recovery of 96-109%. The median PAPP-A value on a first trimester samples (n=50) was 643.68ng/mL. When potential interferents (hemoglobin, triglycerides and bilirubin) were added at two times their physiological concentration, PAPP-A concentrations were within $\pm 10\%$ of the control.

Conclusions: A quantitative, robust and fully characterized microplate PAPP-A chemiluminescence assay has been developed to measure PAPP-A in maternal serum. The approximate median PAPP-A levels found in a first trimester maternal serum can be measured with < 7 % CV using this assay. The performance of the assay is acceptable for investigation of clinical utility in a variety of maternity related disorder.

INTRODUCTION

Circulating PAPP-A forms:

Disulfide-bound homodimer of 400 kDa.

- ☐ Early pregnancy, In tissue culture; linflammation, Renal impairment, Asthma, Lung cancer
- ☐ in vitro IGFBP-4 AND IGFBP-5 Protease

Disulfide-bound heterodimer of 500 kDa 2:2 complex with proMBP In pregnancy.

□ No Proteolytic activity. PAPP-A subunits in PAPP-A/proMBP complex is inhibited by proMBP

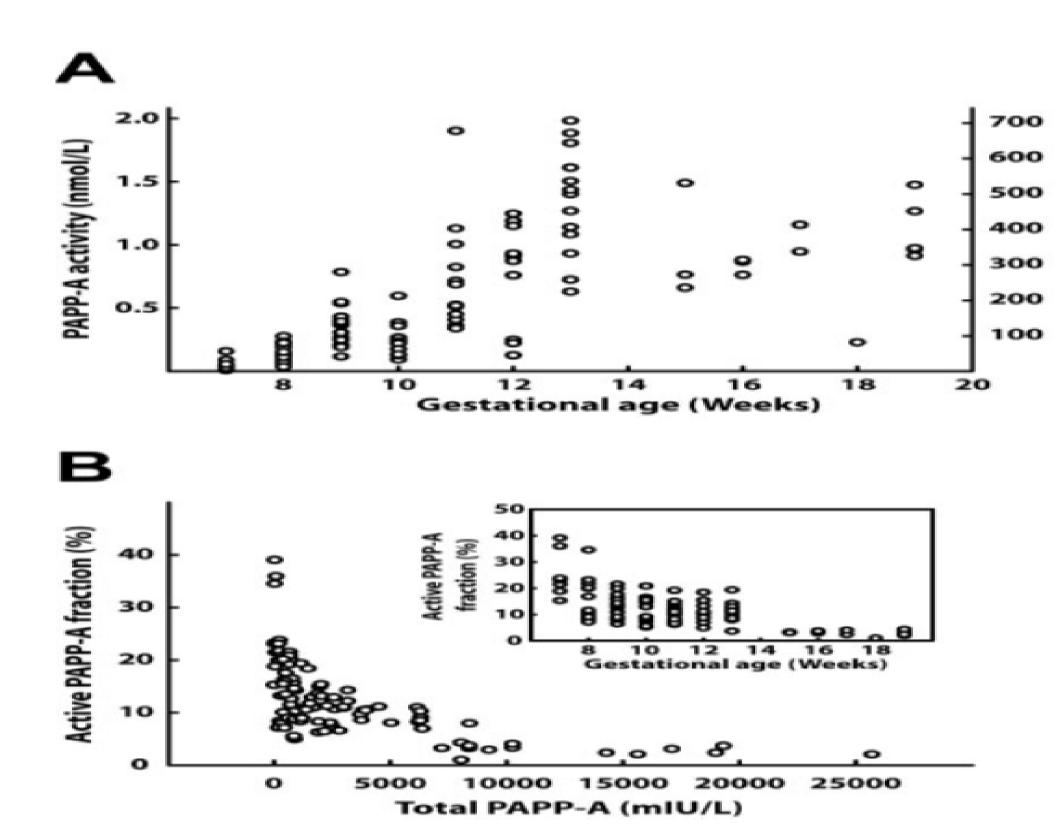
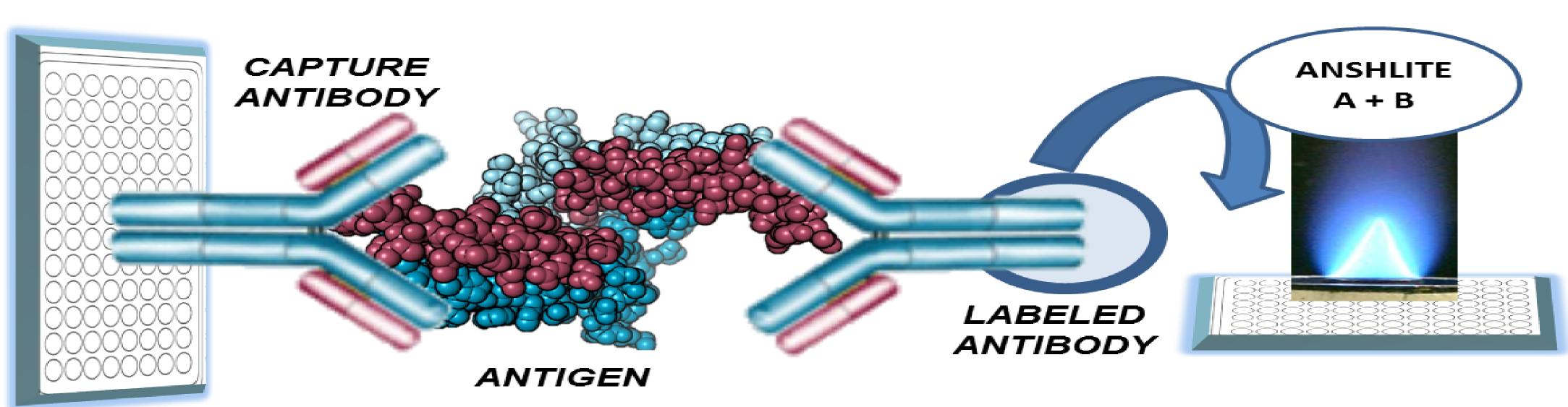


Fig. PAPP-A activity in weeks 7 to 19 of

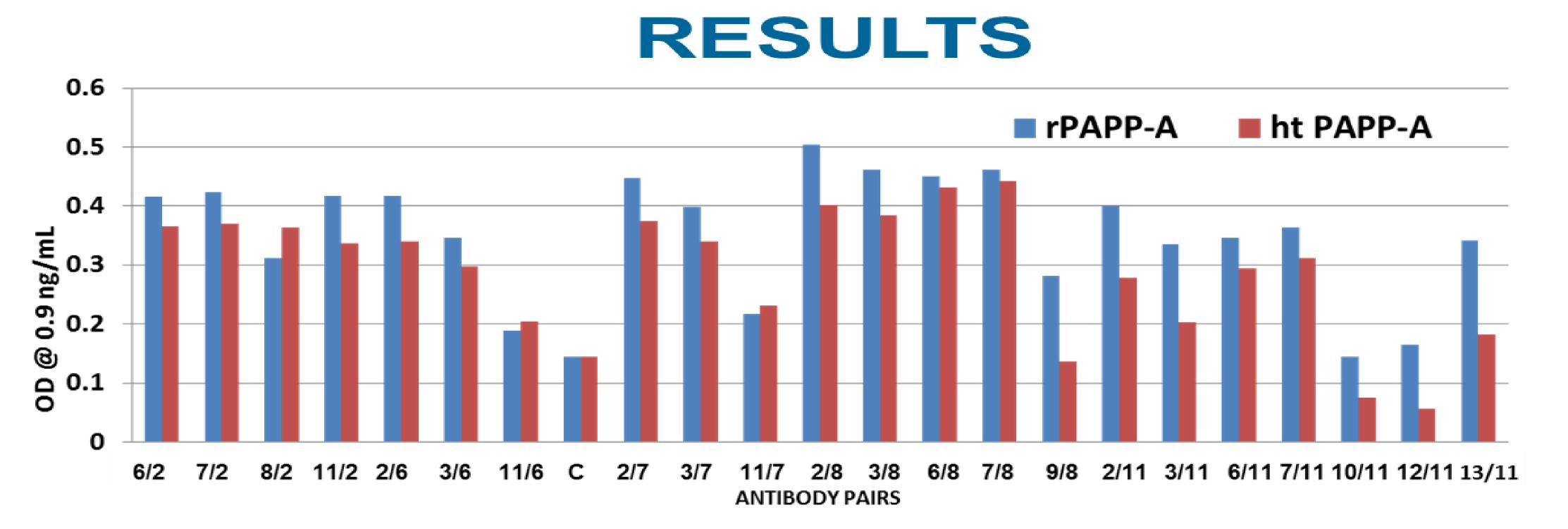
independent experiments. The inset shows

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METHOD

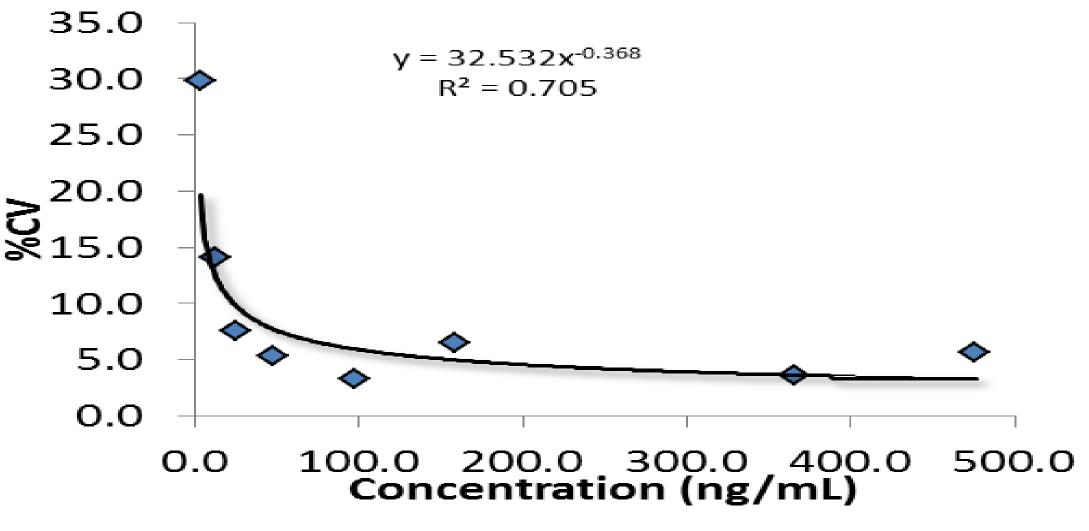


Antibody pair used in this assay binds to the human PAPP-A



Limit of Detection: The lowest amount of PAPP-A in a sample that can be detected with a 95% probability (n=20) is 7.73 ng/mL. The value was determined by processing six serum samples in the range of **3.95** to **159.15 ng/mL**. Two assay runs per day were performed over five days with all samples run in duplicate per run.

determined samples in the range of 3.95-2087.9 over ten runs and five days in duplicates (n=20) following CLSI EP17 guidelines.



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

Sample	Mean Conc.	Within	n Run	Betwee	n Run	Tota	al
	(ng/mL)	SD	%CV	SD	%CV	SD	%CV
Pool-1	249.00	7.81	3.14%	0.00	0.00%	7.81	3.14%
Pool-2	476.04	29.19	3.98%	11.87	1.62%	31.51	4.30%
Pool-3	732.48	22.57	4.74%	15.23	3.20%	27.23	5.72%

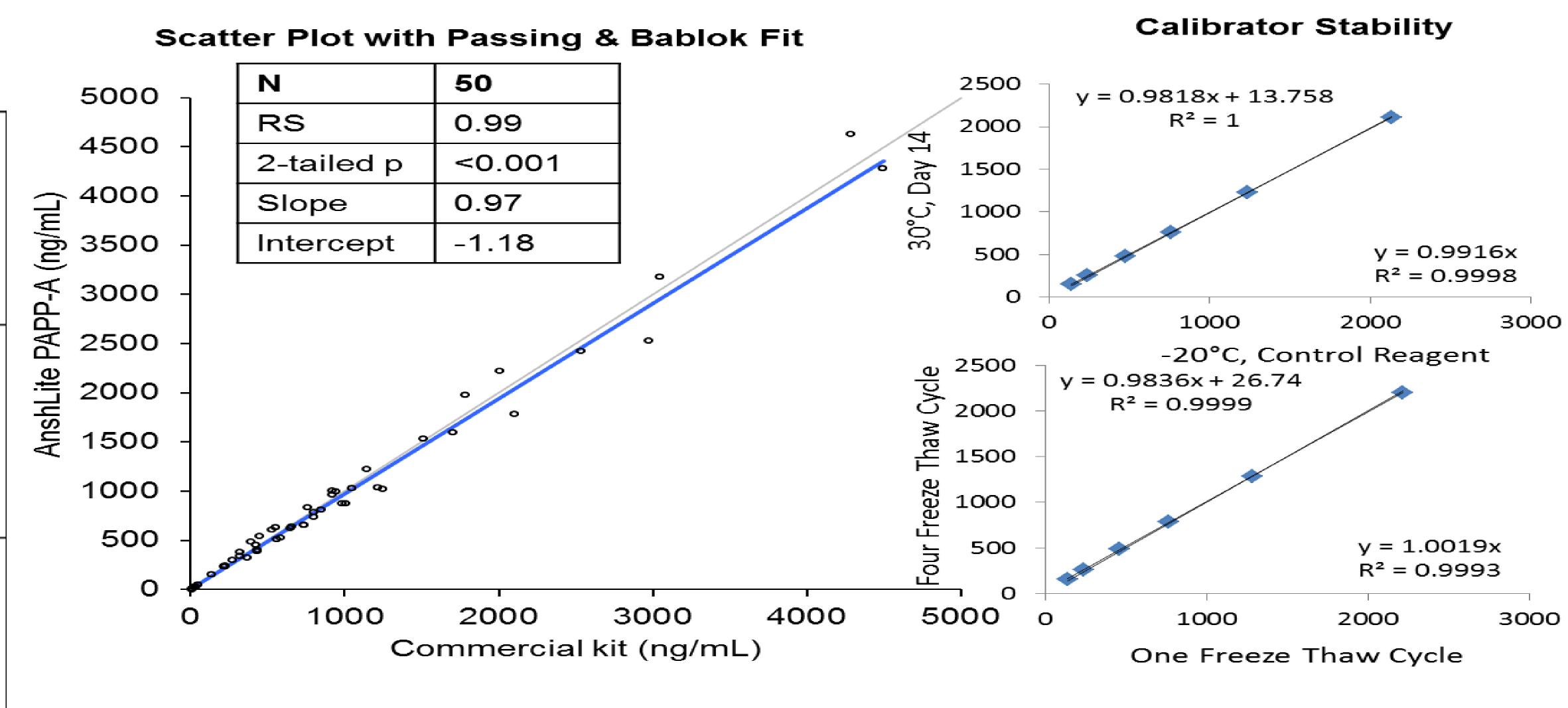
Linearity of Dilution: First Trimester Pregnancy sera diluted in calibrator A/Sample diluent.

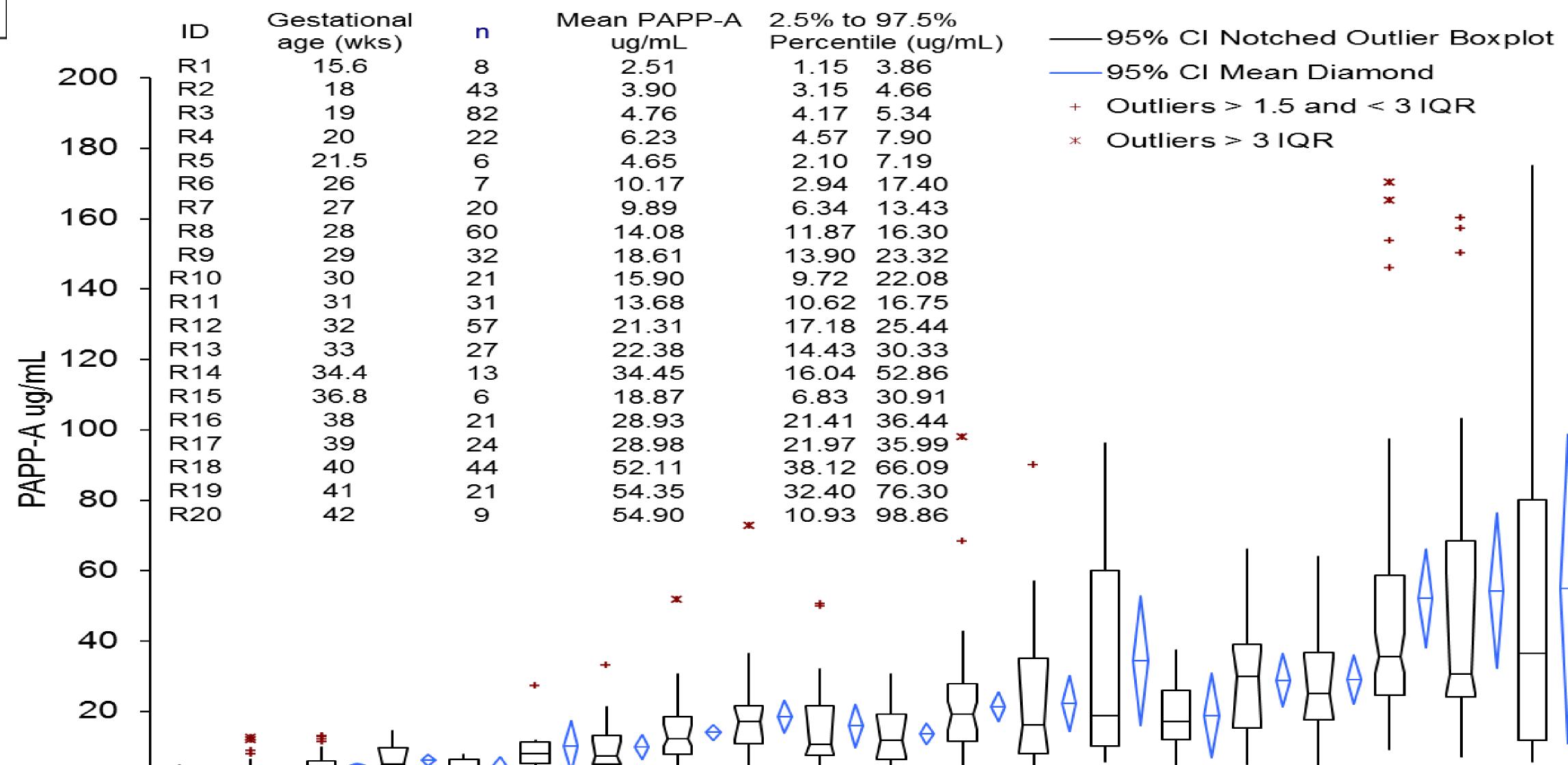
SAMPLE	Dilution Factor (1:X)	Expected Value (ng/mL)	Observed Value (ng/mL)	% Recovery
	1:25	2117.41	N/A	N/A
	1:50	1:50 1058.71 1083.05	102	
1	1:100	529.35	559.73	106
'	1:200	264.68	259.76	98
	1:400	132.34	139.94	106
	1:800	66.17	70.85	107
	1:25	2665.12	N/A	N/A
	1:50	1332.56	1306.36	98
2	1:100	666.28	684.25	103
_	1:200	333.14	352.75	106
	1:400	166.57	179.71	108
	1:800	83.28	85.21	102
	1:25	3977.77	N/A	N/A
	1:50	1988.88	1971.15	99
2	1:100	994.44	989.43	99
3	1:200	497.22	519.24	104
	1:400	248.61	264.51	106
	1:800	124.30	132.95	107

Cross Reactivity and Interference: Cross reactivity was studied by adding the following compounds (1-5) to the PAPP-A calibrator A (0 ng/mL) and the interference was studied by spiking the following compounds (6-8) in serum samples at the concentration listed below.

S.	Interferents	Analyte Conc.	% Difference to	
No	Interierents	Arialyte Conc.	Control	
1	PAPP-A2	50 ng/mL	ND	
2	proMBP	50 ng/mL	ND	
3	MMP-9	50 ng/mL	ND	
4	PAPP-A proMBP Complex	50 ng/mL	100	
5	Dimeric PAPP-A	50 ng/mL	100	
6	Hemoglobin	9.3 mg/mL	-1.3	
7	Triglycerides	51 mg/mL	6.8	
8	Bilirubin	4.2 mg/mL	-3.1	

Method Comparison





CONCLUSIONS

- ☐ A sensitive, reliable and easy-to-run microplate PAPP-A assay has been developed to measure total PAPP-A (equimolar concentrations of dimeric and hetero- tetrameric PAPP-A) in pregnancy serum.
- ☐ The approximate median PAPP-A 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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