Development of a fully characterized picoPAPP-A chemiluminescence assay for male and female serum evaluation

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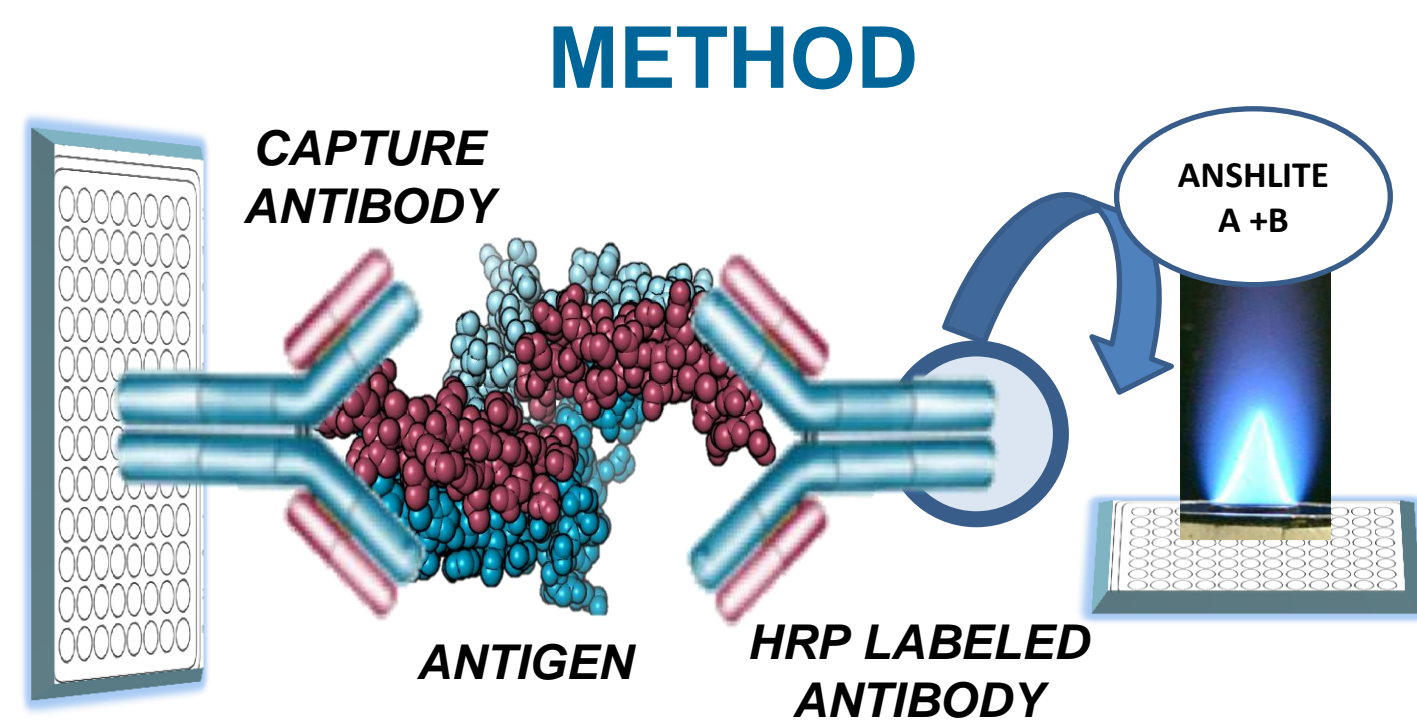
ABSTRACT

Relevance: Pregnancy-associated plasma protein A (PAPP-A) is a large placenta-derived glycoprotein. During pregnancy it is produced in high concentrations by the trophoblast and released into maternal circulation. In addition to trophoblasts, PAPP-A expression has been reported in various tissues, including endometrium, testis, atherosclerotic arteries, kidney, bone, colon, and other adult and fetal tissue. PAPP-A is potentially proatherosclerotic and has been proposed as a new marker of inflammation, as high serum PAPP-A levels are observed in patients with renal impairment, asthma, lung cancer, unstable angina, etc. Studies suggest that the PAPP-A form in non-pregnant females and males is dimeric and is not complexed with proMBP and proteolyses IGFBP-4 and IGFBP-5.

Methodology: We have developed a well characterized two-step sandwich-type enzymatic microplate glow based chemiluminescence assay to measure PAPP-A levels in serum. The assay measures PAPP-A in 50 μL of serum sample against dimeric PAPP-A calibrators (0.1-10ng/mL). The antibody pair used in the assay measures dimeric PAPP-A and PAPP-A/proMBP complex 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 12 runs, 4 replicates per run, using CLSI EP5-A guidelines was 3.01% at 0.979ng/mL, 1.41% at 1.45ng/mL and 2.86% at 3.02ng/mL. The limit of detection calculated using ten serum samples in the range of 0.045-4.16ng/mL over 12 runs is 0.03ng/mL. The functional sensitivity of the assay at 10% CV was 0.05ng/mL. Dilution studies showed an average recovery of 104-110%. The median PAPP-A value on random male and female samples (n=12) was 0.82ng/mL.

Conclusions: A quantitative, robust and fully characterized microplate PAPP-A chemiluminescence assay has been developed to measure PAPP-A in male and female serum. The approximate median PAPP-A levels found in a random male and female serum can be measured with < 5 % CV using this assay. The performance of the assay is acceptable for investigation of clinical utility in inflammation related disorders

INTRODUCTION

FIGURE 1. Serum PAPP-A levels in patients with lung cancer and control subjects.

FIGURE 2. Serum PAPP-A levels in control subjects and in patients with small cell carcinoma (SCC), epidermoid carcinoma (EC), and adenocarcinoma (AC).

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METHOD

RESULTS

Analytical Specificity: The antibody pair used in the picoPAPP-A CLIA measures equimolar concentrations of free and complexed PAPP-A to proMBP and does not detect proMBP). The picoPAPP-A calibrators are prepared using dimeric PAPP-A and calibrated to WHO (78/610) preparation. 1 ng/mL of purified rPAPP-A characterized by amino acid analysis is equal to 2.7 µIU/mL.

Limit of Detection: The lowest amount of PAPP-A in a sample that can be detected with a 95% probability (n=24) is 0.025 ng/mL. The value was determined by processing six serum samples in the range of 0.045 to 1.6 ng/mL. Four runs per day were performed over three days with all samples run in duplicate per run.

Limit of Quantitation: The estimated minimum dose achieved at 5% total imprecision is 0.1 ng/mL. The value was determined by processing ten samples in the range of 0.045 to 4.16 ng/mL with a minimum of twelve runs and three days in duplicates (n=24) following CLSI EP17 guidelines.

Imprecision: Reproducibility of the picoPAPP-A CLIA was determined on three serum pools. Serum pools were run in replicates of four per assay and twelve runs (n=48).

Mean Conc.		Within run		Between run		Total	
Sample	(ng/mL)	SD	% CV	SD	% CV	SD	% CV
Pool-1	0.979	0.026	2.64%	0.014	1.45%	0.029	3.01%
Pool-2	1.451	0.015	1.03%	0.014	0.96%	0.020	1.41%
Pool-3	3.021	0.076	2.53%	0.041	1.35%	0.087	2.86%

Cross Reactivity and Interference:

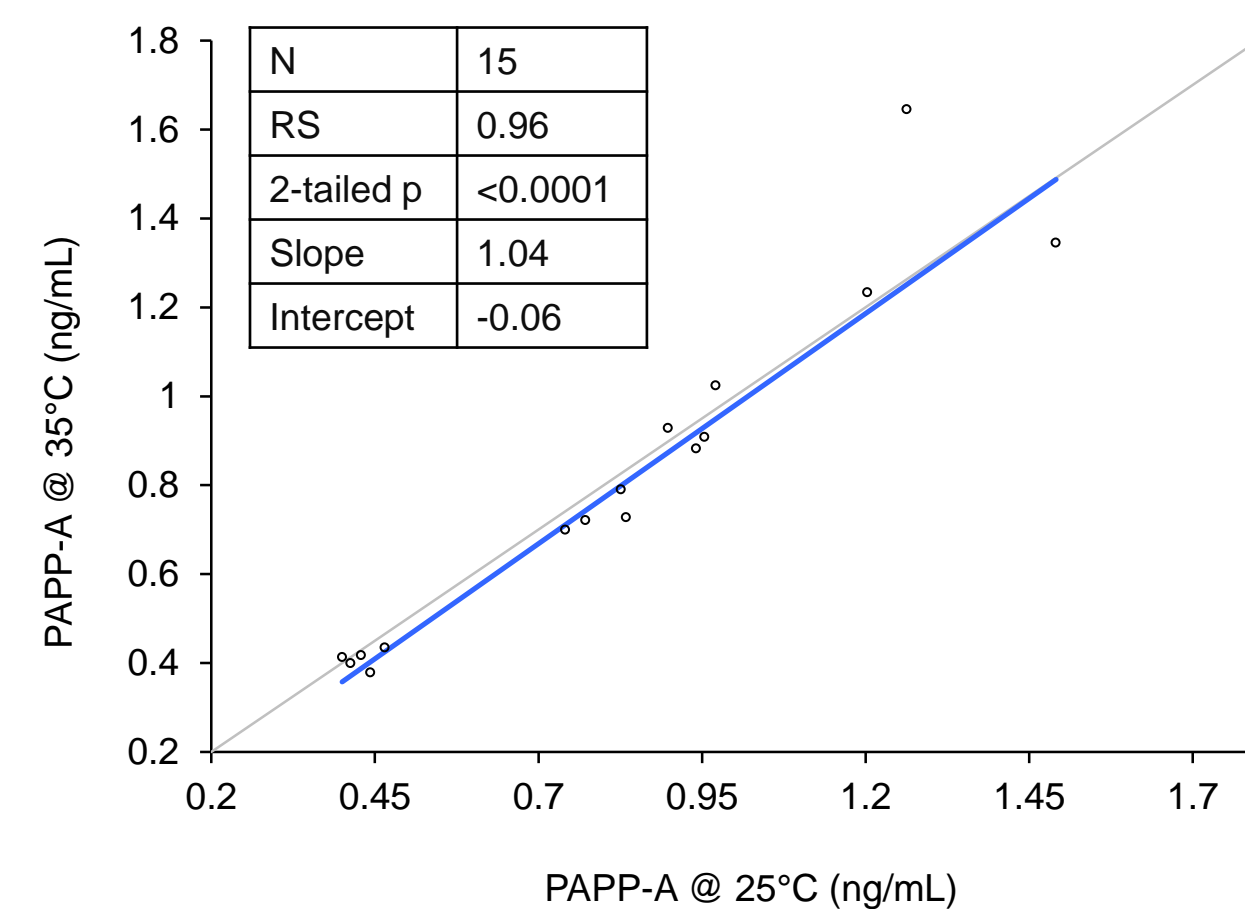| S. No	Analyte	Conc. (ug/mL)	% Difference to Control
1	PAPP-A2	0.05	ND
2	ProMBP	0.05	ND
3	MMP-9	0.05	ND
4	Hemoglobin	1350	0.22
5	Triglyceride	5000	2.93
6	Bilirubin	600	1.57

Linearity of Dilution: Three serum samples were diluted in calibrator A.

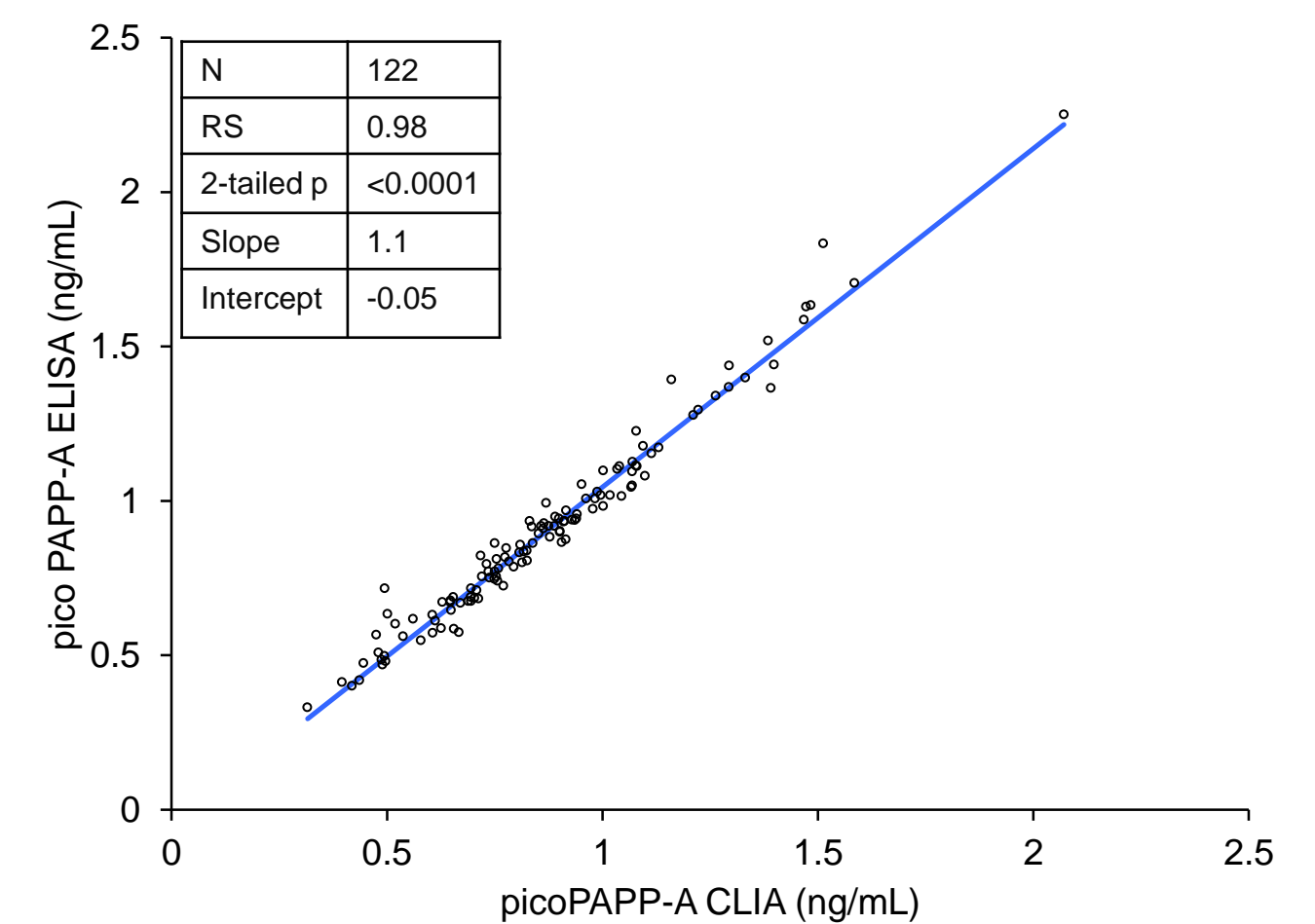| Sample	Dilution Factor	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
1	Neat	3.922	N/A	N/A
	1:2	1.961	2.079	106
	1:4	0.981	1.077	110
	1:8	0.490	0.551	112
	1:16	0.245	0.274	112
2	Neat	3.855	N/A	N/A
	1:2	1.928	1.991	103
	1:4	0.964	1.017	105
	1:8	0.482	0.526	109
	1:16	0.241	0.256	106
3	Neat	3.568	N/A	N/A
	1:2	1.784	1.878	105
	1:4	0.892	0.938	105
	1:8	0.446	0.491	110
	1:16	0.223	0.238	107

Spike Recovery: Four serum samples containing different levels of endogenous PAPP-A were spiked with different amounts of rPAPP-A antigen.

Sample	Endogenous Conc. (ng/mL)	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
1	0.696	1.139	1.069	94
		1.542	1.368	89
		1.910	1.689	88
2	0.707	1.149	1.082	94
		1.552	1.380	89
		1.919	1.709	89
3	0.769	1.209	1.123	93
		1.609	1.436	89
		1.973	1.763	89
4	0.797	1.235	1.218	99
		1.633	1.501	92
		1.997	1.823	91

Thermal Stability: picoPAPP-A assay sample and calibrator parallelism were studied at 25ºC and 35ºC incubated temperature.
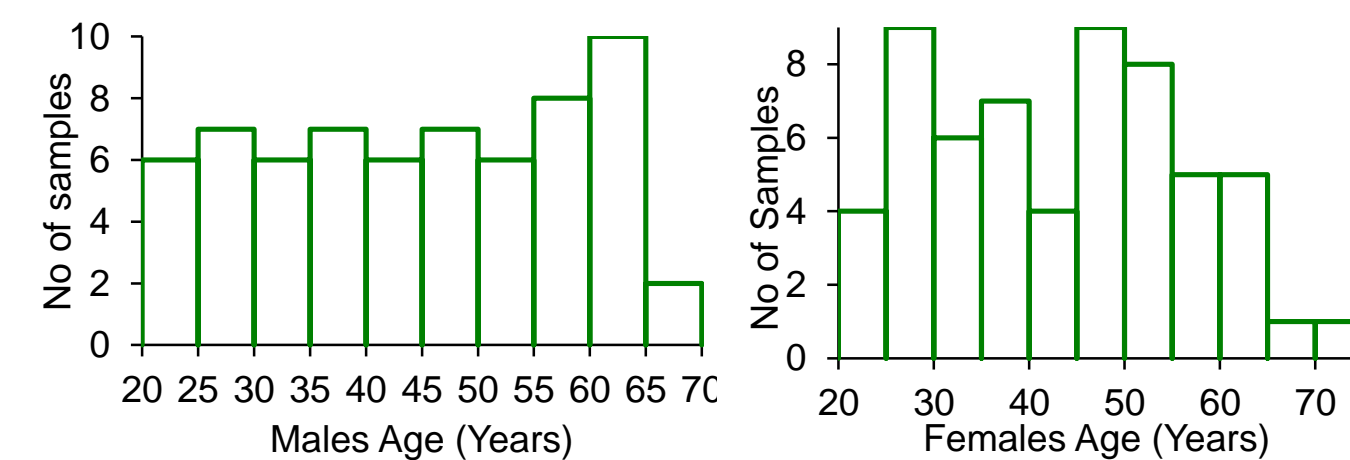

N	15
RS	0.96
2-tailed p	<0.0001
Slope	1.04
Intercept	-0.06

Method Comparison

N	122
RS	0.98
2-tailed p	<0.0001
Slope	1.1
Intercept	-0.05

Reference Range

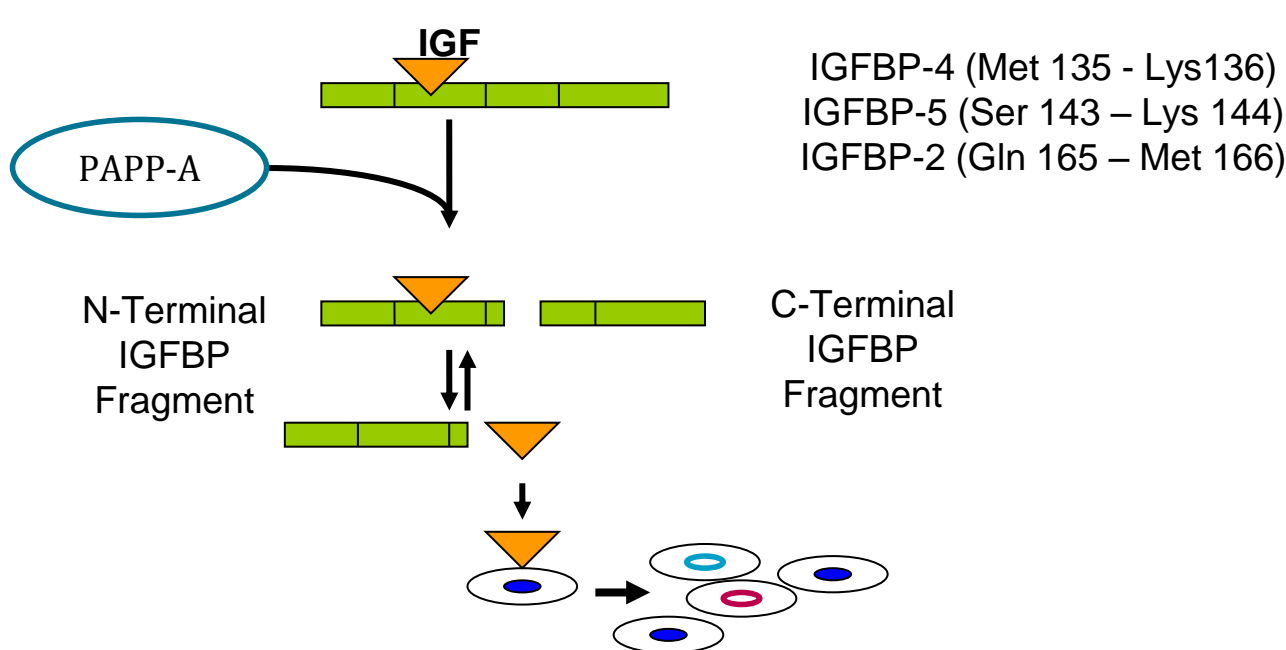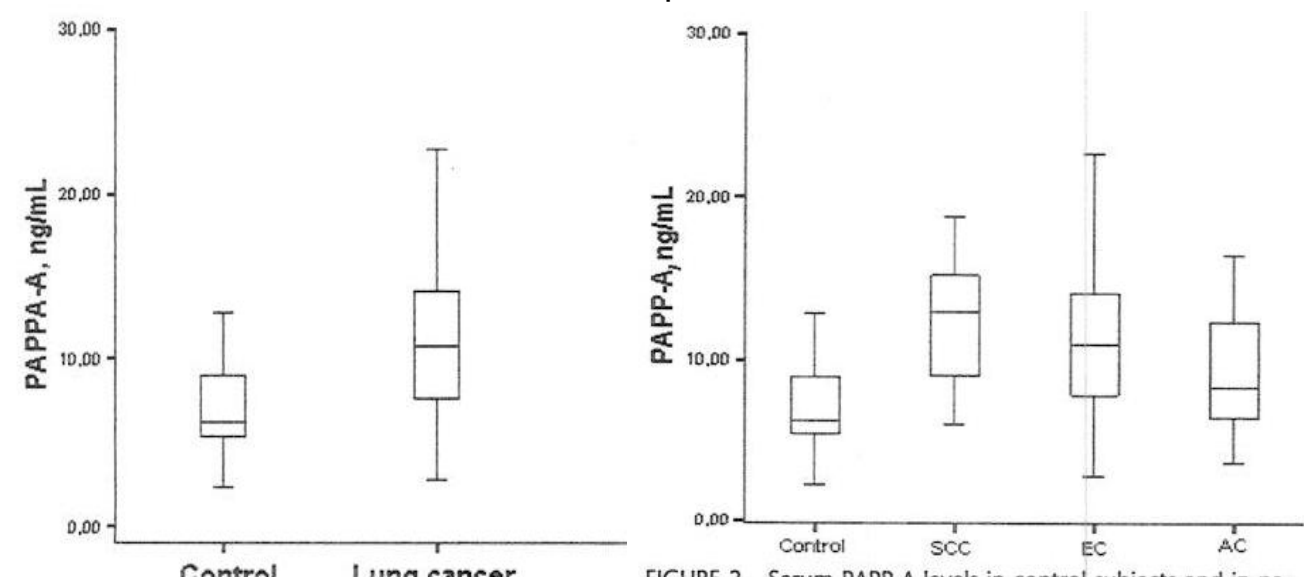| Sample	Median Age (Years)	Median Conc. (ng/mL)	2.5–97.5th Percentile Conc. (ng/mL)
Random Male (N=65)	45	0.94	0.61-1.98
Random Female Samples (N=59)	43	0.717	0.36-1.34

CONCLUSIONS

☐ A sensitive, reliable and easy-to-run microplate picoPAPP-A assay has been developed. The approximate median PAPP-A levels found in healthy population can be measured within <5% CV using this assay.

☐ The assay has shown excellent analytical performance and is suitable for research studies in patients with renal impairment, asthma, lung cancer and other disorders associated with inflammation.

ACKNOWLEDGEMENTS