diluent. The % recovery on individual samples is represented in the following table.

Sample	Dilution Factor	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
1	1:10	8.134	N/A	N/A
	1:20	4.067	4.239	104
	1:40	2.034	2.126	105
	1:80	1.017	1.070	105
2	1:10	7.541	N/A	N/A
	1:20	3.771	3.808	101
	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
4	1:10	5.245	N/A	N/A
	1:20	2.623	2.693	103
	1:40	1.311	1.372	105
	1:80	0.656	0.824	126

Recovery:

Known amounts of PAPP-A2 were added to four serum samples containing different levels of endogenous PAPP-A2. The concentration of PAPP-A2 was determined before and after the addition of exogenous PAPP-A2 and the percent recovery was calculated.

Sample	Endogenous Conc.(ng/mL)	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
1	0.297	0.788	0.675	86
		1.179	0.978	83
2	0.302	0.793	0.699	88
		1.184	1.058	89
3	0.329	0.819	0.698	85
		1.208	1.009	84
4	0.53	1.009	0.934	93
		1.39	1.167	84

Analytical Specificity:

The antibody pair used in the PAPP-A2 ELISA measures PAPP-A2 and does not detect other similar molecules. The assay does not detect dimeric PAPP-A, STC-2, PAPP-A proMBP, PAPP-A-STC-2 complex and MMP-9.

Interference:

When potential interferents (hemoglobin, triglycerides, and bilirubin) were added at least at two times their physiological concentration to control sample, PAPP-A2 concentration were within ± 15% of the control as represented in the following table. This study was based on NCCLS EP7-P to serum matrix added.

Interferents	Analyte Conc. (mg/mL)	Non-spiked Sample Value (ng/mL)	Spiked Sample Value (ng/mL)	% Difference
Hemoglobin	1.35	0.221	0.229	3.62
		0.271	0.292	7.75
Triglycerides	11	0.221	0.213	-3.62
		0.358	0.343	-4.19
Bilirubin	0.6	0.214	0.225	5.14
		0.271	0.286	5.53

Expected Value:

These samples were analyzed using PAPP-A2 ELISA kit on site. The expected ranges for PAPP-A2 were calculated using 90-95% non-parametric estimation using Analyse-It® for Microsoft Excel.

Sample	Median Conc. (ng/ml)	2.5–97.5th Percentile Conc. (ng/ml)
Random First Trimester Pregnancy (N=50)	30.76	1.3-97.1
Random Second Trimester Pregnancy (N=65)	42.14	16.19-119.7
Random Third Trimester Pregnancy (N=36)	73.85	51.2-117.8

Note: *The values above are for reference only. It is recommended that each research organization should determine the reference values for their own study population.*

The expected ranges for PAPP-A2 in pediatric male samples in the age range of 3.0 – 18.0 years were calculated using 95% non-parametric estimation. A total of 389 samples in Pubic Hair Tanner stages 1 - 5 were evaluated using Analyse-It® for Microsoft Excel as seen in table 2 below.

Pubic Hair Tanner Stage	No of specimens (n)	Median Conc. (ng/mL)	PAPP-A2 (ng/mL) 95% CI
1	217	0.26	0.1 - 0.69
2	49	0.22	0.11 - 0.37
3	31	0.23	0.1 - 0.58
4	48	0.19	0.08 - 0.35
5	44	0.14	0.06 - 0.49

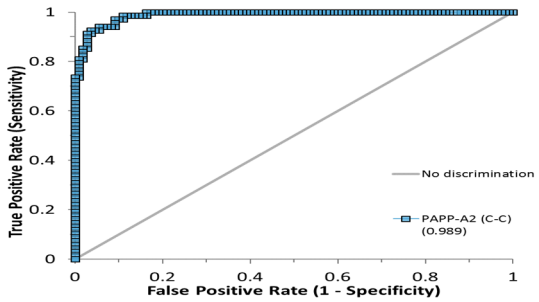
The expected ranges for PAPP-A2 in pediatric female samples in the age range of 2.4 – 18.0 years were calculated using 95% non-parametric estimation. A total of 423 samples in Breast Tanner stages 0 - 5 were evaluated using Analyse-It® for Microsoft Excel as seen in table 3 below.

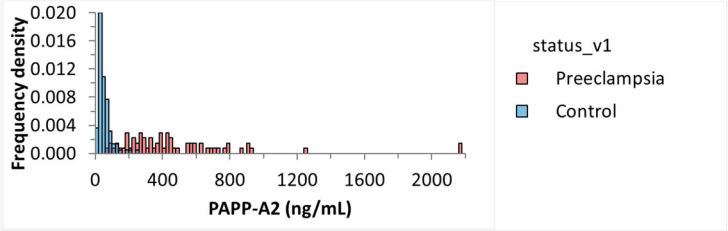
Breast Tanner Stage	No of specimens (n)	Median Conc. (ng/mL)	PAPP-A2 (ng/mL) 95% CI
0	14	0.32	0.1 - 0.71
1	174	0.28	0.12 - 0.81
2	60	0.25	0.11 - 0.83
3	57	0.14	0.09 - 0.42
4	49	0.17	0.07 - 0.31

NOTE: It is recommended that each laboratory should determine the reference range(s) for its own patient population. The results of this assay should be used in conjunction with other relevant and applicable clinical information.

Method Comparison:

A total of 178 pregnancy (18-34 wks.) serum samples from 110 controls and 68 preeclampsia subjects were analyzed. The ROC analysis showed an area under the curve of 0.99 and the frequency distributions of the two groups with respect to PAPP-A2 concentrations are shown in the figure below.





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