

DEVELOPMENT OF A NEW LIQUID STABLE IMMUNOTURBIDIMETRIC ASSAY KIT FOR THE DETERMINATION OF ADIPONECTIN IN SERUM

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Introduction

Adiponectin is one of the most abundant adipokines secreted by adipocytes and in humans the circulating level ranges from 5 to 30µg/ml, accounting for about 0.01% of total plasma protein, three times higher than the concentrations of other adipose tissue-derived hormones.¹ The level is decreased in obesity and related pathologies including type 2 diabetes and cardiovascular disease (CVD).^{2,4} High plasma levels of adiponectin are associated with a decreased risk of coronary heart disease, independently of other risk factors.³ Adiponectin protects cardiovascular health through its vasodilator, anti-apoptotic, anti-inflammatory and anti-oxidative activities in both cardiac and vascular cells.⁵

This study reports the development of a new immunoturbidimetric assay kit for the determination of adiponectin in human serum. This represents a useful analytical tool for clinical settings.

Methodology

The principle of the assay is immunoturbidimetric. A latex agglutination complex is formed between adiponectin and antibody coated latex particles. The evaluation of the analytical parameters was performed on the automated analyser Olympus AU640.

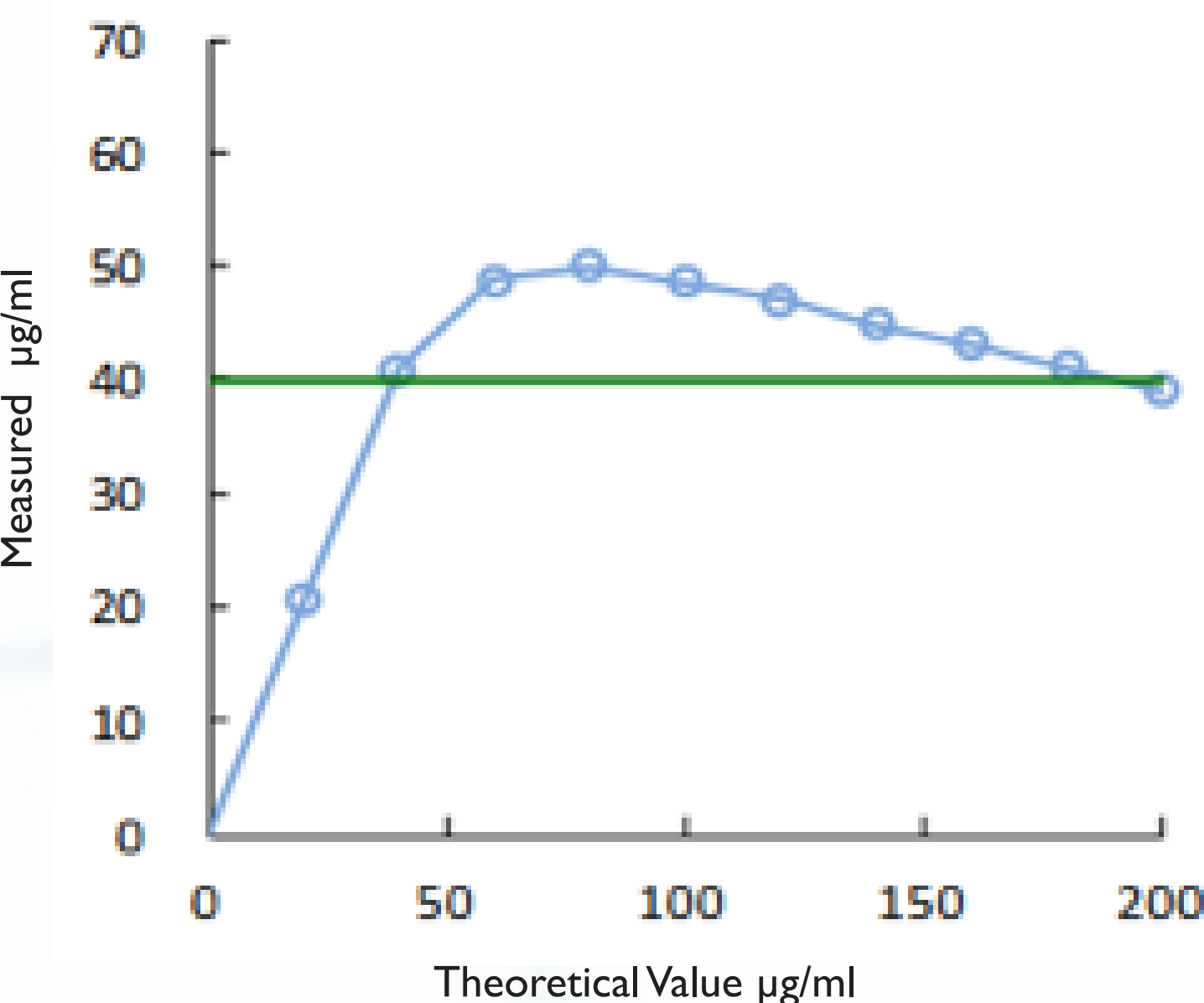
Within-run precision (n=10) was assessed by testing control samples at defined levels and was expressed as %CV. On-board and calibration stabilities were tested by storing the reagents uncapped on the analyser for a period of 30 days. Correlation studies were conducted using two commercially available immunoassays.

Results

Sensitivity and linearity

Adiponectin assay	
Sensitivity (µg/ml)	Linearity (µg/ml)
0.4	40.0

Prozone was not observed up to 200 µg/ml



Recovery

Sample	Adiponectin		
	Target (µg/ml)	Mean concentration (µg/ml)	% Recovery
Control Sample	5.2	4.9	95
Control Sample	16.3	16.4	101
Sample 1	0.0	0.1	-
Sample 2	5.0	4.8	96
Sample 3	10.0	10.1	101
Sample 4	20.0	19.9	100
Sample 5	40.0	40.1	100

On-board stability

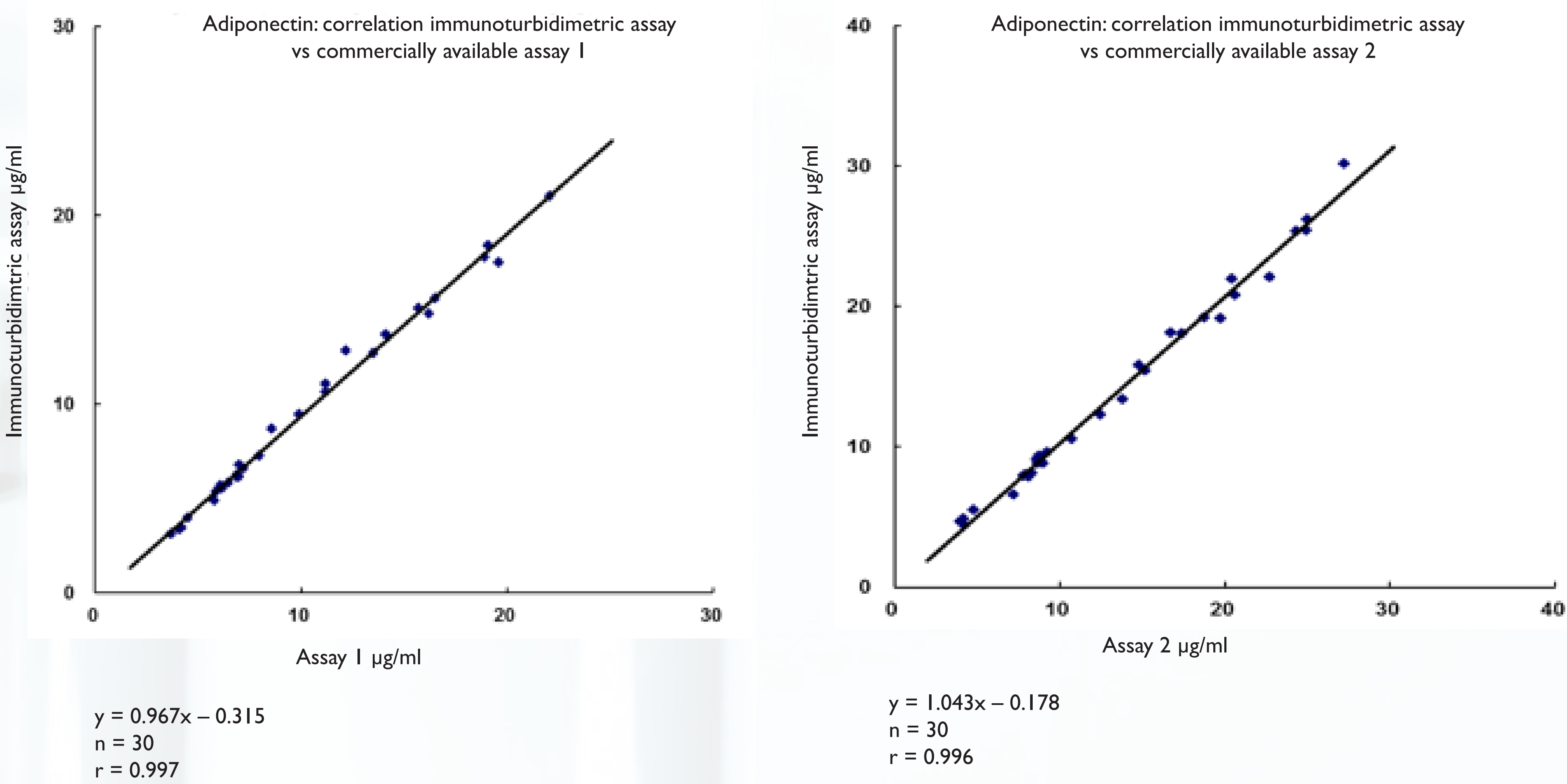
The reagents presented an on-board stability and calibration frequency of 30 days

Correlation with other commercially available assays

In the correlation studies 30 serum patient samples were tested, linear regression on the resulting data generated r values of 0.996 and 0.997.

Precision

Within-run precision	
Sample	
Control level 1 (µg/ml)	Control level 2 (µg/ml)
4.8	16.3
4.9	16.5
5.0	16.5
4.9	16.4
5.0	16.4
4.9	16.4
4.9	16.5
4.9	16.5
4.8	16.4
5.0	16.4
Mean 4.92 SD 0.08 %C.V. 1.6	Mean 16.43 SD 0.08 %C.V. 0.5



Conclusion

The developed immunoturbidimetric assay kit exhibits high accuracy, reproducibility and correlates favourably with other methodologies. It presents the added advantage of using liquid reagents with good stability. This represents an improvement for use in the accurate and reliable determination of adiponectin in human serum with applications for other automated analysers.

References
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