An enzymatic Lp-PLA$_2$ assay for fully automated analysis: A valuable supplementation to currently used cardiovascular risk assessment

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BACKGROUND

Lipoprotein-associated phospholipase A_2 (Lp-PLA$_2$) is a Ca^{2+}-independent serine lipase. The enzyme is produced by macrophages and is mainly expressed in atherosclerotic lesions. Lp-PLA$_2$ is primarily associated with low-density lipoprotein (LDL) and responsible for the hydrolysis of oxidized phospholipids, resulting in the production of pro-inflammatory and pro-atherogenic mediators. Lp-PLA$_2$ is a useful plasma biomarker associated with cardiovascular disease and enables a more pecise identification of vulnerable plaques.

Here we present a liquid-stable, ready-to-use reagent for determining the activity of Lp-PLA$_2$ on fully automated systems. Enzyme activity in sample material is determined after hydrolysis of a Lp-PLA$_2$ specific substrate. Resulting product can be quantified at 415 nm.

METHODS

Evaluation of the test has been carried out on Hitachi 917 system. All reagents, calibrators and controls were from DiaSys Diagnostic Systems GmbH. Performance data were determined for 3 different sample materials (serum, EDTA and-heparin plasma). Analytical performance was established according to the CLSI protocol. Method comparisons were performed against competitor test on Hitachi 917. Data have been evaluated by using regression analysis according to Passing and Bablok.

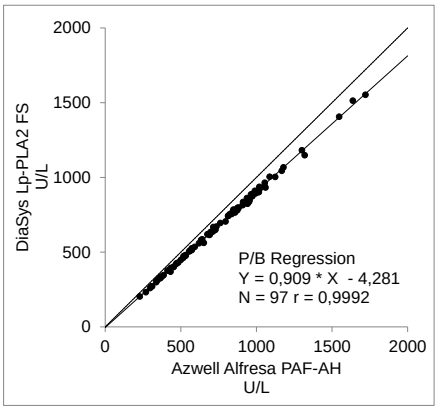

Figure 1: Method comparison using serum samples

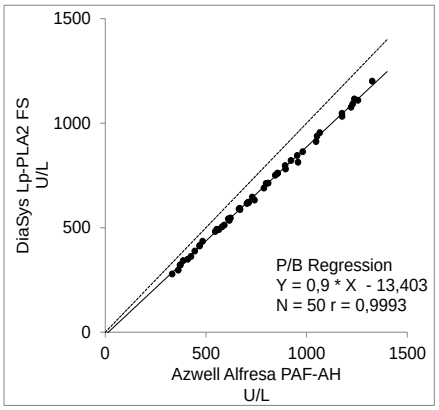

Figure 2: Method comparison using EDTA samples

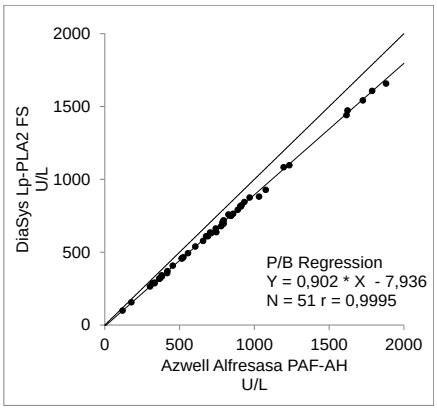

Figure 3: Method comparison using heparin samples

RESULTS

A method comparison of Lp-PLA$_2$ FS with 97 native samples against a competitor test demonstrated excellent correlation [r = 0.999; Passing/Bablok: y = 0.909 x – 4.28 U/L] (Figure 1). Lp-PLA$_2$ shows outstanding intra-assay precision with a CV of <0.72% (Table 1). Bilirubin, hemolysis or lipemia showed no significant interference (Table 2).

Intra-assay precision (n=20)	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	318	2.02	0.63
Sample 2	633	4.40	0.69
Sample 3	1113	7.98	0.72
Total precision CLSI (n=80)	**Mean [U/L]**	**SD [U/L]**	**CV [%]**
Sample 1	314	4.80	1.53
Sample 2	625	10.0	1.61
Sample 3	1105	13.3	1.20

Table 1: Precision (intra-assay and total precision according to CLSI)

Interferences	Lp-PLA$_2$ [U/L] normal range	Lp-PLA$_2$ [U/L] pathological range	Analyte [mg/dL]
Bilirubin conj.	467	932	50
Bilirubin unconj.	466	934	50
Lipid	435	909	2000
Hemolysis	516	1019	1000
Ascorbic acid	432	843	50

Table 2: Interferences tested with normal and pathological samples

CONCLUSIONS

DiaSys Lp-PLA$_2$ FS assay shows outstanding performance characteristics for recommended sample material. The test correlates well to available assays in the market. The new DiaSys Lp-PLA$_2$ FS reagent provides rapid, accurate and convenient measuring of Lp-PLA$_2$ in human samples on any clinical chemistry analyzer. Determination of Lp-PLA$_2$ allows a more precise assessment of cardiovascular risks.

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