Assay kit for the assessment of Lp(a) levels in serum or plasma with minimum apo (a) size related bias

Lp(a) is a complex macromolecule synthesized in the liver, it presents similarities with Low Density Lipoprotein (LDL) as it possesses a cholesterol-phospholipid core and a closely associated protein, apoB100. Lp(a) differs however from LDL in that each lipoprotein particle contains one copy of the glycoprotein apolipoprotein (a) [apo (a)] covalently bound to apoB100 by a single disufide bond [1]. Studies indicate the association of elevated levels of plasma Lp(a) and risk of coronary heart disease [2,3]. A study reported that elevated plasma Lp(a) and small apolipoprotein (a) increased the risk of recurrent arterial ischemic stroke in children [4]. Moreover, the structural similarities between apo(a) and plasminogen highlighted the importance of Lp(a) in both atherosclerosis and thrombogenesis [5-7]. The intra and inter-individual size heterogeneity of apo(a) is genetically determined, this size variation constitutes a challenge for the immunochemical measurement of Lp(a) in plasma. The use of a 5-point calibrator which take into account the heterogeneity of Lp(a) for each of the levels would reduce result discrepancies. This study reports the performance characteristics of an immunoturbidimetric assay for the determination of Lp(a) in serum or plasma with minimum apo(a) size related bias.

Methodology

In this immunoturbidimetric assay agglutination occurs due to an antigen-antibody reaction between Lp(a) in a sample and anti-Lp(a) antibody adsorbed to latex particles. The agglutination is detected as an absorbance change at 700 nm proportional to the concentration of Lp(a) in the sample. The reagents are stable and ready to use. Lp(a) Calibrator Series, Lp(a) Control and Lipid Controls were used (Randox Laboratories Limited, Crumlin, UK). The assay is applicable to a variety of clinical chemistry analysers, for the results of this study the RX Daytona Plus analyser was used (Randox Laboratories Limited, Crumlin, UK).

Limit of Quantitation (mg/dL)	3.00
Limit of Detection (mg/dL)	2.57
Limit of Blank (mg/dL)	0.45

Table 1 Lp(a) immunoturbidimetric assay on the RX Daytona Plus: sensitivity.

Results

Assay range

This immunoturbidimetric assay presented a reportable range of 3 to 106 mg/dL.

Sensitivity

The Limit of Quantitation (LOQ), the Limit of Detection (LOD) and the Limit of Blank were determined consistent with CLSI guidelines EP17-A (table 1).

Prozone

Antigen excess effects were not noted until Lp(a) levels approached 493 mg/dL.

Within run and total precision

Within run precision and total precision, expressed as CV(%), were <4.00 and <4.5 respectively (Table 2).

Serum/plasma comparison

The assay was used to compare serum to plasma samples (n=56) collected into tubes containing Li heparin, Na heparin, Na EDTA, K EDTA or citrate. The data was subjected to linear regression analysis and in all cases the correlation coefficient (r) was > 0.996.

Mean (mg/dL)	Within run precision			Total precision		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
	17.4	28.3	72.5	17.4	28.3	72.5
SD	0.636	0.665	1.54	0.725	0.920	3.01
CV (%)	3.66	2.35	2.12	4.17	3.25	4.15
n	79	80	80	79	80	80

Table 2 Lp(a) immunoturbidimetric assay on the RX Daytona Plus: precision.

Conclusion

The immunoturbidimetric assay reported here for the determination of Lp(a) in serum/plasma with minimum apo (a) size related bias showed optimal analytical performance. The assay utilises ready- to –use stable reagents which facilitates the application in test settings by simplifying the experimental procedure and reducing handling errors. Its applicability to different automated analysers ensures the reliability, the accuracy of the measurements and facilitates the testing procedure. This represents an excellent analytical tool to facilitate clinical investigation.

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