Validation of a New Lipoprotein(a) Particle Number ELISA

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ABSTRACT

Background: Measurement of lipoprotein(a) particle number [Lp(a)-P] is important in CHD risk assessment. Unfortunately, turbidimetric assays for Lp(a) mass are biased due to apo(a) size isoforms. Therefore, we have developed an ELISA for Lp(a)-P without isoform bias. Methods: Limit of the Blank (LOB) was determined by measuring delipidated human serum 50X over 5 days (LOB=mean+1.654 SD). Limit of Detection (LOD) was determined by measuring 2 serum samples with low Lp(a) ~50X over 5 days (LOD=LOB+1.654 SD). LOQ was the lowest concentration with <20% CV. Precision was assessed by measuring10 replicates of 5 serum pools over 5 plates. Linearity was assessed by intermixing low and high Lp(a) pools. Accuracy and bias vs. apo(a) size isoforms was assessed using 20 samples from Northwest Lipid and Diabetes Research Laboratory (Seattle, WA) with reference method assigned concentrations and known kringle 4 type 2 repeats by linear regression analysis. Results: The LOB and LOD were 1 and 13 nmol/L, respectively. The LOQ was equal to the LOD. Imprecision at the extreme low and high ends of the analytical range were 12.1% and 11.4%, respectively. Results were linear from ~2 to 500 nmol/L. Lp(a)-P was highly correlated to the reference method (r=0.971) with an average bias of 34 nmol/L, which was corrected by adjusting the master calibrator assigned values. There was no statistically significant association between bias and the number of kringle 4 type 2 repeats. Conclusion: The Sun Dx Lp(a)-P ELISA is sensitive, precise, and linear over a wider analytical range than most Lp(a) assays, and is strongly correlated to the reference method. Importantly, the assay shows no bias due to apo(a) size isoforms.

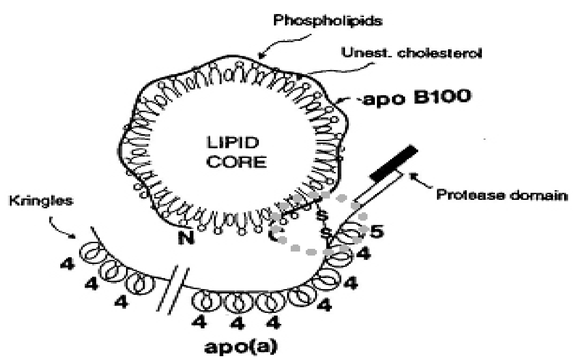
METHODS

In the Sun Diagnostics Lipoprotein(a) assay, sample is diluted with buffer, transferred to the microtiter plate (coated with anti-apo(a) monoclonal antibody) along with calibrators and quality controls, and incubated for one hour at room temperature. The plate is then washed and anti-apoB-HRP conjugate is added. After incubation at room temperature for one hour the plate is again washed and substrate is added to activate the HRP. The reaction is stopped after 30 minutes and absorbance is read at 500 nm. Lipoprotein (a) concentration is determined from a standard curve. Validation experiments generally followed CLSI guidelines. Limit of the Blank (LOB) was determined by measuring delipidated human serum 50X over 5 days (LOB=mean+1.654 SD). Limit of Detection (LOD) was determined by measuring 2 serum samples with low Lp(a) ~50X over 5 days (LOD=LOB+1.654 SD). LOQ was the lowest concentration with <20% CV. Precision was assessed by measuring10 replicates of 5 serum pools over 5 plates. Linearity was assessed by intermixing low and high Lp(a) pools. Accuracy and bias vs. apo(a) size isoforms was assessed using 20 samples from Northwest Lipid and Diabetes Research Laboratory (Seattle, WA) with reference method assigned concentrations and known kringle 4 type 2.

INTRODUCTION

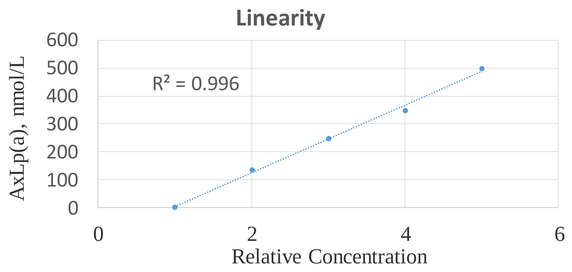
Lipoprotein(a), discovered in 1963 [1], is an LDL-like particle formed by the covalent addition of apolipoprotein(a) [apo(a)], to apolipoprotein B (apo B) of LDL (Fig. 1) [2,3]. Most studies find Lp(a) to be a significant, independent predictor of cardiovascular disease [4], and although the strength of association appears to be modest, the relative risk associated with Lp(a) may be attenuated by poor quality assays, especially inaccuracies associated with the use of antibodies that recognize kringle 4 type 2 repeats. Lipoprotein(a) concentration in serum is largely genetically determined [5], and therefore serum concentrations remain relatively constant over time. The atherogenicity of Lp(a), like LDL, is attributed to its deposition in atherosclerotic plaque and, to a prothrombotic effect related to apo(a)'s structural similarity to (and inhibition of) plasminogen [5]. Lp(a) also accumulates oxidized phospholipids, and like oxidized LDL, may be taken up by macrophages and contribute to foam cell formation [6]. Apo(a) contains an inactive protease domain, one copy of kringle V (KV), and multiple copies of kringle IV (KIV) [5]. The KIV domains of apo(a) are classified as ten different types. These variable numbers of KIV-2 result in a significant size heterogeneity in the apo(a) protein, with up to 51 repeats and a variable molecular weight of 300 to 800 kDa [5,7]. The structural homology of Lp(a) and plasminogen and the repeating KIV-2 subunits presents a challenge for the accurate measurement of Lp(a). Immunoassays are widely used for measurement of Lp(a), but unfortunately, current immunoassays are influenced by apo(a) size polymorphism. The best design for an immunoassay is a sandwich-type ELISA using monoclonal or polyclonal anti-apo(a) antibodies for "capture and monoclonal or polyclonal anti-apo B for detection. This method is more specific than ITA or INA because it includes two antibodies, and the use of apo B for detection eliminates the problem of bias due to apo (a) size polymorphisms.

FIGURE 1: STRUCTURE OF LIPOPROTEIN(A)



RESULTS

The LOB and LOD for the Lp(a)-P ELISA is 1 and 13 nmol/L, respectively. The LOQ is equal to the LOD: 13 nmol/L (5 mg/dL). The imprecision at the extreme low end of the analytical range was 12.1%; the imprecision in the midrange of the assay varied from 6.9% to 7.7%. The analytical measurement range of the assay is 13 to 500 nmol/L (5 to 208 mg/dL). We have decided to limit the assay to 400 nmol/L (167 mg/dL). Interferences from bilirubin, triglycerides, and hemolysate were evaluated and no interference (>10% bias) was observed at unconjugated or conjugated bilirubin levels up to 34 mg/dL and triglyceride concentrations up to 950 mg/dL. A sample with hemoglobin concentration of 735 mg/dL was associated with a 14% bias. Plasminogen up to 275 mg/dL and apolipoprotein B up to 130 mg/dL did not influence the Lp(a) assay.

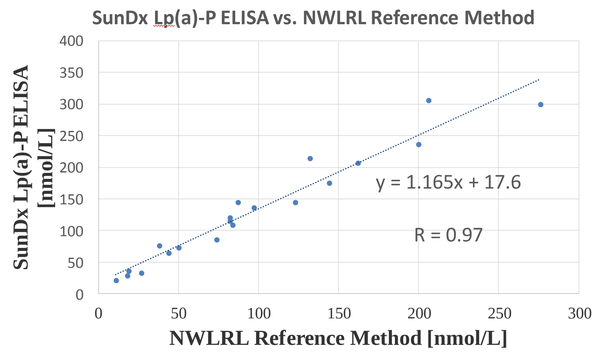


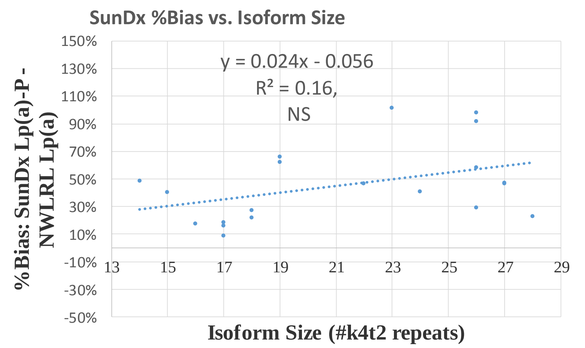
Level	Mean	Expected	Bias nmol/L	%Bias	SD	%CV
1	1.8	5.0	-3.2		0.37	
2	135.5	125.6	9.8	7.84%	8.76	6.46%
3	247.5	246.3	1.1	0.47%	16.95	6.85%
4	348.0	367.0	-19.1	-5.19%	32.15	9.24%
5	499.0	487.7	11.3	2.31%	18.13	3.63%

CONCLUSIONS

- The Sun Dx Lp(a)-P ELISA is sensitive, precise, and linear over a wider analytical range than most Lp(a) assays (13-500 nmol/L).
- SunDx Lp(a)-P ELISA is strongly correlated to Reference Method (r= 0.971)
- SunDx has a positive bias vs. Reference Method with an average bias of 34 nmol/L. This was easily corrected by adjusting the assigned master calibrator values.
- There is no statistically significant association between bias and the number of kringle 4 type 2 (k4t2) repeats (Sun Dx versus NWLRL).

RESULTS





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