

## A SIMPLE VISUAL IMMUNOASSAY (VIA) FOR THE SEMIQUANTITATIVE DETERMINATION OF Lp(a) BLOOD LEVELS

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### INTRODUCTION

Lipoprotein (a) (Lp(a)) has been identified as an independent risk factor for coronary artery disease, stroke and peripheral atherosclerosis (1).

Individuals with serum Lp(a) levels above 300 mg/L are considered to have a two-fold increased risk for suffering myocardial infarction (2). Lp(a) is a low-density lipoprotein-like particle characterized by the presence of apolipoprotein (a) [apo(a)] linked to apolipoprotein B. Apo(a) is strikingly homologous to plasminogen. There are several isoforms of apo(a) which have different molecular sizes (3). Different commercial immunoassay kits for measuring Lp(a) are available, like enzyme immunosorbent-linked assays (ELISA), immunoradiometric assays (IRMA), and electroimmunoassays (EID) (4). All of them are time-consuming and require specialized equipment. We describe here the development of a visual reading immunoassay (VIA) as an alternative for the semi-quantitative determination of Lp(a) levels in blood.

### EXPERIMENTAL PROCEDURES

Slide-like, opaque-white polystyrene supports with 12 low-depth wells each, of the type used by the AuBIO-DOT™ (CIGB, Havana) technology, were sensitized by coating with a monoclonal antibody (Mab) to apo(a). Samples of serum, plasma or whole blood, appropriately diluted, were incubated in the wells, and the captured Lp(a) particles recognized by a second Mab to apo(a), conjugate to colloidal gold. The reaction was finally amplified with physical developers based on silver ions, rendering an insoluble product of metallic darkish-black color, proportional to the amount of Lp(a) in the samples. Three standard samples of human serum with Lp(a) con-

centrations of 100, 300 and 900 mg/L were simultaneously analyzed. The intensity of the color developed for each sample was compared with those of the standard by a simple visual inspection. The samples were classified in two categories: those with Lp(a) levels below 300 mg/L and those with Lp(a) levels equal or higher than 300 mg/L.

### RESULTS AND DISCUSSION

The Lp(a) levels obtained in the samples were not affected by plasminogen or apoB concentrations up to 2 mg/mL (5 and 3 times higher than normal plasminogen or apoB serum levels, respectively). The method was precise: the results were the same for each sample analyzed 9 times in the same assay, and 10 times in the independent assays. Of 92 serum samples studied by the VIA and a Mab-ELISA developed in our laboratory, 88 were correctly classified according to Lp(a) levels by our visual system. Three groups of samples with different apo(a) isoforms were analyzed by our method, by EID (10 samples), an IRMA kit from Kabi Pharmacia (7 samples), and an ELISA from Organon technika (15 samples). All of them were correctly classified by our method. The immunoassay described here is useful for the semi-quantitative determination of Lp(a) levels, can be done in only 40 minutes without any specialized equipment, and the slides can be stored indefinitely as a permanent record of results.

### REFERENCES

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