

## Latex immunoassay of human serum Lp(a\*) lipoprotein

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**Summary** A sensitive latex immunoassay for human serum lipoprotein Lp(a\*) is based on direct agglutination by Lp(a\*) of latex particles coated with specific antibody. The agglutination is quantified by turbidimetry using a photometer at 360 nm. The stabilization of antibody-coated latex particles by bovine serum albumin occurs under well-defined conditions (pH, concentration of bovine serum albumin, and antibody loading of latex particles). The standard curve of serum lipoprotein Lp(a\*) ranges from 0.05 to 1.15 mg/l. Inter- and intra-assay coefficients of variation were less than 8% and 3%, respectively. Results were well correlated with those obtained by electroimmunodiffusion ( $r = 0.98$ ,  $n = 108$ ). — **Vu-Dac, N., A. Chekkor, H. Parra, P. Duthilleul, and J.-C. Fruchart.** Latex immunoassay of human serum Lp(a\*) lipoprotein. *J. Lipid Res.* 1985. 26: 267-269

**Supplementary key words** turbidimetry • electroimmunodiffusion

Interest in the Lp(a\*) lipoprotein, a variant of apolipoprotein B (apoB)-containing lipoproteins, has been stimulated by reports indicating that Lp(a\*) represents an independent risk factor for ischemic heart disease (1-5). A number of different approaches have been utilized to measure quantitatively the occurrence of Lp(a\*) in plasma samples, including immunoassays (4-9) and quantitating the electrophoretic band corresponding to Lp(a\*) (10). Based on these studies, Lp(a\*) has been reported to vary from 5 mg/l as measured by radioimmunoassay (4) to more than 2,000 mg/l (11).

In this report, we propose a new method, based on agglutination of latex particles, which does not require the use of radioisotopes and offers at least the same precision, specificity, and sensitivity as RIA.

## MATERIALS AND METHODS

### Antibodies

Sheep antiserum against the Lp(a\*) specific polypeptide (apo Lp(a\*), lot 2421/012) was obtained from Immunodiagnostika, Vienna, Austria. Desalting of antiserum was performed on Trisacryl GF05 [Ind. Biologique Française (IBF), Villeneuve La Garenne, France] equilibrated with 0.025 M Tris-0.035 M NaCl buffer, pH 8.80. Immunoglobulins were then isolated by ion exchange chromatography on DEAE-Trisacryl (IBF, France) equilibrated with the same buffer. This fraction was dialyzed against 0.1 M  $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$  buffer, pH 7.40, containing 7.6 mM  $\text{NaN}_3$ . By double immunodiffusion, the IgG fraction was monospecific for Lp(a\*) and gave no cross-reactivity with low density lipoproteins (LpB) or other serum apolipoproteins.

### Latex particles

Polystyrene latex particles, 0.77  $\mu\text{m}$  in diameter, (Estapor K 109, lot 616) were obtained as a 10% suspension (100 g/l) by Rhône Poulenc, Aubervilliers, France.

### Standard and serum samples

Lp(a\*) antigen was prepared by preparative electrophoresis according to Desreumaux et al. (12). Standard Lp(a\*) serum (lot 2900/021) was from Immunodiagnostika. Serum samples were collected from 108 apparently healthy men, 20 to 65 years of age. Dilutions were made in a solution containing 0.15 M NaCl, 7.6 mM  $\text{NaN}_3$ , and 0.014 mM BSA (Sigma, St. Louis, MO).

### Latex immunoassay

*Coating of latex particles with the immunoglobulins.* Seventy-five micrograms of sheep immunoglobulins against apo Lp(a\*) were diluted in 0.4 ml of 0.1 M  $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$  buffer, pH 7.4, and incubated with 50  $\mu\text{l}$  of the 10% latex suspension at room temperature for 1 hr. The latex particles were then precipitated by centrifugation (3,000 g, 15 min, 20°C), washed twice with 1 ml of 0.15 M NaCl con-

taining 7.6 mM  $\text{NaN}_3$ , and resuspended in 1 ml of this solution.

**Stabilization of the coated latex particles.** Immunoglobulin-coated latex particles are unstable and tend to agglutinate spontaneously. Introduction of negatively charged bovine serum albumin (BSA) on the latex surface eliminates nonspecific agglutination. After vigorously vortexing for 2 min, antibody-coated latex particles are added to 3.5 ml of 0.01 M phosphate buffer, pH 6.0, containing 0.15 M NaCl, 7.6 mM  $\text{NaN}_3$ , and 0.014 mM BSA. The stabilized latex particles can be used for at least 5 months when stored at 4°C. Fifteen to 30 min before use, 0.5 ml of 1 M glycine buffer, pH 10, containing 1.7 M NaCl, 76 mM  $\text{NaN}_3$  is added and the mixture is vigorously vortexed for 2 min.

#### Assay procedure

Fifty microliters (50  $\mu\text{l}$ ) of the standard or sample dilutions and 50  $\mu\text{l}$  of the stabilized latex suspension (1 g/l) are mixed and incubated for 45 min at 37°C in a shaking water bath (150 cycles/min, 6 cm amplitude). After addition of 2 ml of a solution containing 0.15 mol of NaCl and 1 ml of Tween 20 (Merck, R.F.A.) per liter, the agglutination is quantified by turbidimetry at 360 nm, and expressed by difference of absorbance between the blank and the assay.

#### Comparison method

Electroimmunoassay of  $\text{Lp(a}^*)$  was performed according to Kostner et al. (3).

## RESULTS

Using standard  $\text{Lp(a}^*)$  serum dilutions ranging from 0.05 to 1.15 mg/l a curvilinear relationship was seen (Fig. 1). A change of absorbance of 300 milliabsorbance units ( $\text{mA}^*$ ) was highly acceptable for this type of assay. A post-zone phenomenon, due to excess of antigen and resulting in a decrease of agglutination, was seen at  $\text{Lp(a}^*)$  levels higher than 11.5 mg/l. Because the sera were routinely diluted 1,000-fold, in practice this effect need not be considered.

Standard curves obtained with purified  $\text{Lp(a}^*)$  and with standard  $\text{Lp(a}^*)$  serum were similar. Because of the known instability of  $\text{Lp(a}^*)$  in purified form, we used a commercially available serum as a secondary standard.

Intra-assay precision was estimated by assaying 30 replicate samples of two pooled sera with low (120 mg/l) and high (540 mg/l)  $\text{Lp(a}^*)$  concentration in one run, respectively. The coefficients of variation were 2.6% and 3.0%.

Inter-assay imprecision was evaluated with the same samples on 8 consecutive working days and resulted in coefficients of variation of 5.6% and 7.8%.

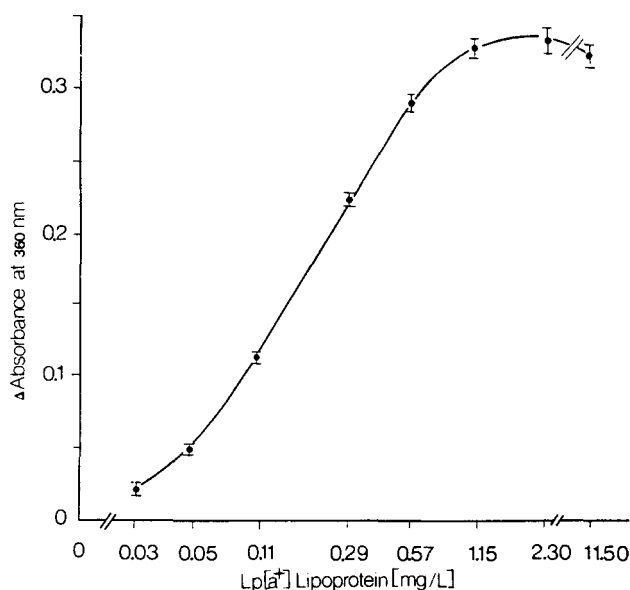


Fig. 1 Standard curve of human serum lipoprotein  $\text{Lp(a}^*)$ . Agglutination was expressed by difference of absorbance at 360 nm between the blank and the assay.

Results obtained in 108 serum samples by the latex immunoassay correlated well with those obtained by electroimmunoassay performed according to Kostner et al. (3) (Fig. 2).

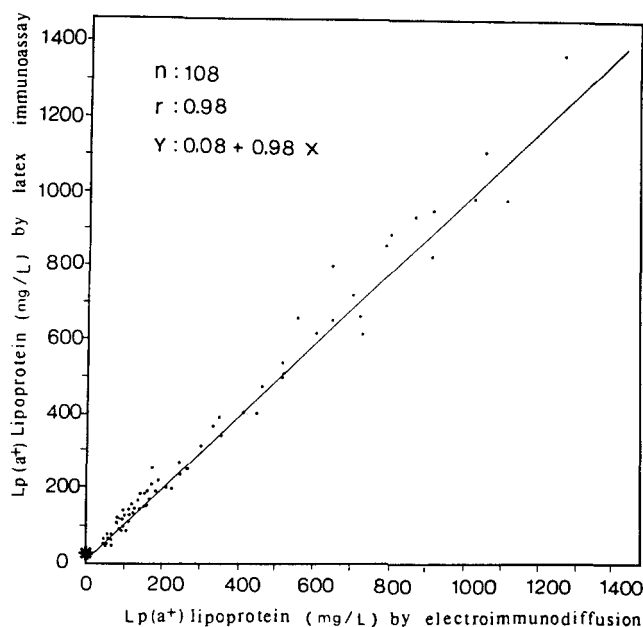


Fig. 2 Correlation between lipoprotein  $\text{Lp(a}^*)$  in serum as measured by electroimmunodiffusion and latex immunoassay. The equation of the continuous line is  $y = 0.08 + 0.98 x$ . (\*)  $\text{Lp(a}^*)$  concentration ranging from 11 to 40 mg/l by latex immunoassay. These levels cannot be measured by electroimmunodiffusion because of its low sensitivity (40 mg/l).

## DISCUSSION

Even though Lp(a\*) has been linked to atherosclerosis and ischemic heart disease, the usually low serum concentration of this lipoprotein makes it somewhat difficult to quantitate. Recently, in order to develop quantitative assays based on latex agglutination, several evaluation procedures such as nephelometry (13), particle counting (14, 15), and turbidimetry (15, 16) have been proposed. Our study has shown that latex immunoassay provides a specific and highly sensitive method for measuring Lp(a\*). The precision and sensitivity of our method equalled that reported for radioimmunoassay and allow estimation of Lp(a\*) in lipoprotein fractions or in experimental situations where small concentrations need to be measured.

A main difficulty of latex immunoassay is to control the stability (or nonspecific agglutination) of the antibody-coated particles, since this influences specificity of the assay. Maximum stability was obtained when the antibody-coated particles were stabilized with BSA (0.014 mM, pH 6.0). Final optimum pH for incubation with antigen must be pH 9.80. Latex reagent prepared under these conditions could be used at least 5 months when stored at 4°C.

Simplicity and reproducibility seem to be the factors of choice for the latex immunoassay for screening of large numbers of individuals for the presence of pathological values of lipoprotein Lp(a\*). ■■

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