

### Intended Use

The Lp(a) reagent set is an *in vitro* diagnostic test for the quantitative determination of lipoprotein(a) [Lp(a)] in human serum and plasma samples. The measurement of Lp(a) is useful in assessing lipid metabolism disorders and atherosclerotic cardiovascular disease in specific populations, when used in conjunction with clinical evaluation and other lipoprotein tests.

### Summary and Explanation

Lp(a) was discovered by K. Berg in 1963.<sup>1</sup> Lp(a) is similar to low-density lipoprotein (LDL) in lipid composition but differs in protein profile. The structural component of Lp(a) distinguishing it from LDL and implicating it in the coronary disease process is apolipoprotein(a) [apo(a)], a highly glycosylated protein attached by disulfide bond to apolipoprotein B100 [apo B100].<sup>2-4</sup> apo(a) has a high degree of structural homology with plasminogen that is a key zymogen of the coagulation cascade.<sup>5</sup> The similarity of structural components of Lp(a) to LDL and to plasminogen suggests that Lp(a) may be associated with atherosclerosis and/or thrombosis.<sup>6</sup> Although there is a lack of consistency in the conclusions of the studies about the contributory role of Lp(a) to coronary heart disease, it is widely accepted that Lp(a) is an important risk factor that may contribute to coronary artery disease independently or cooperatively with other risk factors.<sup>7</sup> While the wide differences in Lp(a) levels seen among individuals are largely due to hereditary factors,<sup>8</sup> the identification of individuals at risk through diagnostic screening should nevertheless be useful in alerting them to the need to eliminate or control other high risk factors when possible.

Lp(a) values should be interpreted in conjunction with clinical evaluation and other lipoprotein tests when assessing atherosclerotic cardiovascular disease in specific populations.

### Principle of the Method

This reagent set is a latex-enhanced immunoturbidimetric *in vitro* diagnostic assay. Lp(a) in the sample binds to the specific anti-Lp(a) antibody, which has been adsorbed to latex particles, and agglutinates. The agglutination is detected as an absorbance change when read on an automated chemistry analyzer. The magnitude of the change in absorbance is proportional to the quantity of Lp(a) in the sample. The actual concentration is then determined by interpolation from a calibration curve prepared from calibrators of known concentrations.

**IMPORTANT: Read all instructions before starting this test.**

### Materials Provided

1. R1: Glycine buffer solution
2. R2: 0.4% w/v suspension with latex particles sensitized with anti-Lp(a) antibodies

### Equipment and Materials Required but Not Provided

1. Automated Chemistry Analyzer
2. Physiological Saline
3. Quality Control Materials

### Storage and Handling

Store reagents at 2 to 8 °C, while protecting from light.

The reagents can be used any time before the expiration date indicated on the box label.

### Warnings and Precautions

1. FOR IN VITRO DIAGNOSTIC USE ONLY
2. Calibrators prepared from human sera have been tested as negative for HBs antigen, HIV 1/2 and HCV antibodies. As no test method can assure the complete absence of HIV, hepatitis B virus, hepatitis C virus or other infectious agents, patient samples and human based reagents should be handled with care and treated as potentially infectious and biohazardous.
3. Calibrators and reagents contain less than 0.1 w/v% sodium azide as a preservative. As sodium azide may react with lead and copper piping to form explosive metal azides, the calibrators and the reagents should be disposed by flushing with copious amounts of water.
4. All solutions supplied with the test kit should be handled carefully and disposed of properly.

### Test Procedure

#### Cautions

1. It is recommended that each laboratory determine calibration frequency. Calibration is recommended after reagent lot change and as required following quality control procedures.
2. Samples containing high levels of Lp(a) (above the assay range) should be diluted with physiological saline and retested.
3. After measurements are taken, reagent bottles should be capped and kept at 2 to 8 °C.
4. Do not use reagents after expiration date on the label.

### Sampling and Sampling Method

1. After sampling, the test should be performed without delay. If the test cannot be done immediately, the sample should be placed in a tightly sealable container and stored at -20° C or below.
2. For serum samples, after the blood has clotted thoroughly, the sample is centrifuged and the serum is separated from blood cells and fibrins.
3. Plasmas collected with Disodium EDTA, Dipotassium EDTA, Sodium Heparin, Lithium Heparin or Citric acid can be used for the assay in addition to serum samples.

### Reagent Preparation

1. No pretreatment is required for reagents or sample.
2. Calibrators are provided in 5x1ml set. (Pointe Scientific Lp(a) Calibrator Set).
3. Physiological saline may be required to dilute high Lp(a) samples. For physiological saline, dissolve 0.9g sodium chloride in distilled water and bring to a final volume of 100 mL.

### Assay Procedures

Lp(a) should be measured according to the specific application parameters the specific chemistry analyzer. Below is a general example of the assay procedure and the specific application parameters for the Hitachi 917 analyzer.

1. Incubation of 5.6 µL sample with 180 µL R1 at 37°C for 5 minutes.
2. Addition of 60 µL R2.
3. Reading of an absorbance change at 700nm for 2.5 minutes, 20 seconds after the addition of R2.
4. Calculation of Lp(a) value with the read absorbance change by interpolation from a calibration curve prepared with calibrators of known concentrations.

# Lipoprotein(a) Reagent Set

## Application parameters for Hitachi 917 analyzer

Hitachi 917 analyzer	
Temperature:	37°C
ASSAY CODE	2 POINT END-10
ASSAY POINT	19-34
SAMPLE VOLUME	5.6 (μL)
R1 VOLUME	180 (μL)
R2 VOLUME	60 (μL)
WAVELENGTH	--- /700
CALIB. METHOD	SPLINE-6-6
UNITS	mg/dL

## Establishing a calibration curve

Prepare a multi-point calibration curve using the Lp(a) Calibrator Set according to the specific application parameters.

The Lp(a) concentration of the calibrators is given on each calibrator label. Note: Values for the calibrators in nmol/L are also available by contacting the Pointe Scientific, Inc. Technical Service department.

## Quality Control

It is recommended that commercially available controls with known concentrations be included in all assays.

## Assay Range

The Lp(a) Reagent Set has a measurable range from 2.0 mg/dL to 80.0 mg/dL.

2.0 mg/dL was obtained by extrapolating +2.6SD value of the zero standard (saline) to the plot of the observed mean concentrations and  $\pm 2.6$ SD of serially diluted low Lp(a) pool. The linearity was assessed using equally spaced serial dilutions of Lp(a) control. Comparison of the observed concentrations to the concentrations calculated from the linear regression equation showed bias within plus or minus 5% up to a dilution over 80 mg/dL.

## Performance

The following performance data were obtained from testing on Hitachi 917 analyzer using appropriate application parameters and calibration instructions for Hitachi 917.

## Sensitivity

When saline is used as a sample, the range of absorbance change per minute (at 700 nm) is from - 0.0050 to 0.0050. When standard solution containing 40 mg/dL Lp(a) is used as a sample, the range of absorbance change per minute (at 700 nm) is from 0.0300 to 0.0600.

## Specificity

When sera containing known levels of Lp(a) in the assay range were measured, the values obtained for the sera were in the range of the known concentrations, plus or minus 10%.

## Within-run precision

Within-run study was performed using two levels of control material.

## Control level I

N=10  
Mean=21.91 mg/dL  
SD=0.438  
CV=2.00%

## Control level II

N=10  
Mean=54.67 mg/dL  
SD=0.686  
CV=1.26%

## Between-run precision

Between-run study was performed using two levels of control material and three pooled samples on 20 days with 2 runs per day.

	Control-N	Control-AN	Pool-1	Pool-2	Pool-3
n	40	40	40	40	40
Mean	18.7	41.5	8.4	26.5	66.3
SD	0.40	0.44	0.19	0.29	0.66
CV	2.12%	1.06%	2.22%	1.11%	0.99%

## Lower Detection Limit

Lower limit of detection is 2 mg/dL.

## Interference

Hemoglobin (up to 500 mg/dL), conjugated bilirubin (up to 30 mg/dL), unconjugated bilirubin (up to 30 mg/dL), plasminogen (up to 200 mg/dL), apolipoprotein B (up to 200 mg/dL) and triglycerides (up to 1500 mg/dL) do not interfere with Lp(a) determination by this test.

No substantial apo(a) size dependency was observed with the Lp(a) assay when calibrated with the IFCC proposed reference material (PRM) in molarity unit.<sup>9</sup>

## Sample types

Plasma samples drawn with each of EDTA, Heparin and Citrate and serum samples from 50 subjects were evaluated on Hitachi 917 Automated analyzer using this Lp(a) reagent and calibrators. Results of the comparison study showed that serum and plasma samples can provide substantially the same Lp(a) values.

	Serum	EDTA (2Na)	EDTA (2K)	Heparin (Na)	Heparin (Li)	Citrate
<b>Median</b> (mg/dL)	13.5	13.0	13.2	11.1	11.0	12.7
<b>Mean</b> (mg/dL)	16.44	16.00	16.21	15.22	14.51	15.90
<b>Minimum</b> (mg/dL)	2.0	1.7	1.8	2.3	2.0	2.0
<b>Maximum</b> (mg/dL)	81.1	78.8	79.5	78.3	77.3	79.4
<b>SD</b>	15.1	14.7	14.8	14.5	14.5	14.5
<b>Serum vs. Plasma</b>						
<b>Slope</b>	-	0.972	0.981	0.958	0.956	0.963
<b>Intercept</b>	-	0.023	0.085	-0.522	-1.199	0.065
<b>r</b>	-	0.999	0.999	0.996	0.996	0.999

## Correlation

This Lp(a) assay was compared to a another commercially available Lp(a) turbidimetric assay using 105 male and female human serum samples ranging from 6.9 to 72.6 mg/dL. The linear regression analysis showed:  
 $y = 0.978x - 3.143$   $r = 0.919$

### Expected Values

30 mg/dL was used as a cut-off point or threshold value in some previous studies.<sup>10, 11</sup> A study of 190 samples obtained from an ambulatory population with no history of CAD (120 males and 70 females) was performed and found a comparable normal range (4.0 to 27.4 mg/dL). Mean age of the males was 33.6 (ranged from 20 to 51) and mean age of the females was 28.8 (ranged from 21 to 43). There was no substantial difference in Lp(a) levels between the males and the females. Means and SDs were 15.5 mg/dL and 21.6 mg/dL in the males and 18.3 mg/dL and 17.6 mg/dL in the females, respectively.

Reference ranges have not been established for this assay for different ethnic populations or disease states. Since Lp(a) levels are largely influenced by hereditary factors and vary with ethnic population, it is recommended that each laboratory establish its own expected values.

Lp(a) values should be interpreted in conjunction with clinical evaluation and other lipoprotein tests when assessing atherosclerotic cardiovascular disease in specific populations.

### References

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