Audit Diagnostics

CE

Lp(a) -TURBI

Lp(a)-turbilatex

Latex turbidimetry

Quantitative determination of Lipoprotein (a) (Lp(a))
IVD

Store 2 - 8ºC.

PRINCIPLE OF THE METHOD

The Lp(a)-turbilatex is a quantitative turbidimetric test for the measurement of Lp(a) in human serum or plasma.
Latex particles coated with antibodies anti-Lp(a) are agglutinated when mixed with samples containing Lp(a). The agglutination causes an absorbance change, dependent upon the Lp(a) contents of sample that can be quantified by comparison from a calibrator of known Lp(a) concentration.

CLINICAL SIGNIFICANCE

Lp(a) is a low density lipoprotein-like particle containing apolipoprotein B-100 disulphide-linked to one large glycoprotein called apolipoprotein (a). Many investigators have confirmed that a high Lp(a) concentration represents an indicator of risk for cardiovascular disease, especially when serum LDL-cholesterol or Apo B are elevated. The quantification of Lp(a) in serum or plasma is important for identification of individuals at risk for developing atherosclerosis.

REAGENTS

Diluent (R1)	Glycine buffer 50 mmol/L, pH 9.0. Sodium azide 0.95 g/L.
Latex (R2)	Latex particles coated with mouse monoclonal anti-human Lp(a), pH 8.2. Sodium azide 0.95 g/L.
Optional	Lp(a) Calibrator. Lp(a) Control.

CALIBRATION

The sensitivity of the assay and the target value of the calibrator have been standardized against an Internal Reference Material. It is not recommended the use of other commercially available Lp(a) calibrators.

PREPARATION

Calibration Curve Prepare the following Lp(a) calibrator dilutions in NaCl 9 g/L. Multiply the concentration of the Lp(a) calibrator by the corresponding factor stated in table bellow to obtain the Lp(a) concentration of each dilution.

Calibrator dilution	1	2	3	4	5
Lp(a) Calibrator (µL)	--	25	50	75	100
NaCl 9 g/L (µL)	100	75	50	25	-
Factor	0	0.25	0.5	0.75	1.0

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC and contaminations are prevented during their use. Do not use reagents over the expiration date.

Reagent deterioration: Presence of particles and turbidity.
Do not freeze; frozen latex and diluent could change the functionality of the test.

ADDITIONAL EQUIPMENT

- Thermostatic bath at 37ºC.
- Spectrophotometer or photometer thermostatable at 37ºC with a 570 nm filter (570 nm).

SAMPLES

Fresh serum or plasma. Stable 7 days at 2-8ºC or 3 months at –20ºC.
The samples with presence of fibrin should be centrifuged before testing.
Do not use highly hemolized or lipemic samples.

PROCEDURE

1. Bring the reagents and the photometer (cuvette holder) to 37ºC.
2. Assay conditions:
   Wavelength : 570 nm (540-600 nm)
   Temperature : 37 ºC
   Cuvette ligth path : 1cm
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:

R1: Diluent (µL)	800
R2. Latex (µL)	200
Sample or calibrator (µL)	15

5. Mix and read the absorbance after immediately and after 4 minutes (A_2) of the sample addition.

Audit Diagnostics has instruction sheets for several automatic analyzers.Instructions for many of them are available on request.

CALCULATIONS

Calibration curve (Note 1): Calculate the absorbance differences (A_2-A_1) of each Lp(a) calibrator and plot the values obtained against the Lp(a) concentration in a calibration curve. Lp(a) concentration in the sample is calculated by interpolation of its (A_2-A_1) in the calibration curve.
Conversion factor: mg/dL x 0.01 = g/L.

QUALITY CONTROL

Control Sera are recommended to monitor the performance of manual and automated assay procedures. It should be used the Audit Diagnostics Lp(a) Control.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES

Normal values up to 30 mg/dL. Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1. **Linearity:** Up to 90 mg/dL, under the described assay conditions. Samples with higher concentrations should be diluted 1/5 in NaCl 9 g/L and retested again. The linearity limit and measurement range depends on the sample to reagent ratio. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.
2. **Limit detection:** Values less than 1.5 mg/dL give non-reproducible results.
3. **Prozone effect:** No prozone effect was detected upon 250 mg/dL.
4. **Sensitivity:** Δ 6 mA. mg/dL.
5. **Precision:**

	Intra-assay (n=20)			Inter-assay (n=6)		
Mean (mg/dL)	4.62	12.35	24.33	31.57	39.10	54.33
SD	0.22	0.29	0.25	0.87	0.66	0.74
CV	4.74	2.33	1.05	2.77	1.70	1.37

6. **Accuracy:** Results obtained using this reagent (y) were compared to those obtained using a commercial ELISA reagent (x). 50 samples were assayed. The correlation coefficient (r) was 0.997 and the regression equation y = 1.062x + 2.021

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Hemoglobin (5 g/L), bilirrubin (20 mg/dL), plasminogen (680 mg/dL), ascorbic ac. (200 mg/dL), rheumatoid factors (100 IU/mL) and lipemia (20 g/L), do not interfere. Other substances may interfere[5].

NOTES

1. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

BIBLIOGRAPHY

1. Gaubatz JW et al. J Biol. Chem 1983; 258: 4582 – 4589.
2. Berg KA et al. Acta Pathol Microbiol Scand 1963; 59: 369-382.
3. Scanu AM et al. J Clin Invest 1990; 85: 1709-1715.
4. Frank S et al. Eur J Clin Invest 1996; 26: 109-114.
5. Young DS. Effects of drugs on clinical laboratory test, 4th ed. AACC Press, 1995.

Manufactured By: AUDIT DIAGNOSTICS
Business & Technology Park, Carrigtwohill, Co. Cork (Ireland)
Tel: 00353 - (0) 21 – 4533 652 *Fax:* 00353 - (0) 21 – 4533 653
E-mail: info@auditdiagnostics.ie *Website:* www.auditdiagnostics.ie