REF: K – 9520T

CRM® *diagnostic systems*

Lp(a) [Lipoprotein (a)]

In vitro diagnostic reagents for the quantitative determination of Lipoprotein (a) [Lp(a)] in serum by means of particle-enhanced turbidimetric immunoassay.

Diagnostic Relevance

Lipoprotein (a) [Lp(a)] was initially though to be a genetic variant of low density lipoprotein (LDL). Lp(a) is a low density lipoprotein-like particle containing apolipoproteín B-100 disulphide-linked to one large glycoprotein called apolipoprotein (a). Apolipoprotein (a) has been shown to have a considerable degree of homology with human plasminogen. The characteristic feature of lipoprotein (a) is that it is distinct from all other serum proteins and apolipoproteins. This protein is believed to be inherited as an autosomal dominant trait and appears to be insensitive to either diet, lifestyle or most hypolipidaemic drugs.

Since its discovery by Berg in 1963, there has been a considerable rise in interest, not only in specialized research centres but also in clinical routine laboratories, in the accurate measurement of lipoprotein (a) in blood. This interest was stimulated by reports indicating that levels above 0,2 – 0,3 g/L, present in approximately 25 % of the population, are associated with an increased risk of coronary heart disease. Many investigators have confirmed that a high lipoprotein(a) concentration represents an indicator of risk for cardiovascular disease, especially when the serum LDL-cholesterol or apo B are elevated. Therefore a convenient and reliable method for the quantitation of Lp(a) in serum or plasma is important for identification of individuals at risk for developing atherosclerosis.

Principle

The Lp(a) test is based upon the reactions between Lp(a) in the sample and latex-covalently bound antibodies against human Lp(a). Lp(a) values are determined turbidimetrically using fixed-time measurement with sample blank correction. The relationship between absorbance and concentration permits a multipoint calibration with a measuring range between 0 and 800 mg/L. The measuring temperature is 37ºC. The assay can be performed on different instruments allowing turbidimetric measurements at 500 to 600 nm.

Reagents

Each Lp(a) kit contains :

A.- Buffer - 30 ml of Glycine buffer, pH: 8,0, containing protein stabilizers and 0,09 % sodium azide as preservative.

B.- Latex reagent – 4,0 ml of a suspension of latex microparticles covalently bound antibodies against human Lp(a) in a glycine buffer (0,1 M, pH: 8,2), containing NaCL (0,15M) and bovine serum albumin (0,5%). Preservative: Sodium azide 0,075%.

Precautions

For in vitro diagnostic use only. Do not pipette by mouth. Reagents containing sodium azide must be handled with precaution. Sodium azide can form explosive azides with lead and copper plumbing. Since absence of infectious agents cannot be proven, all specimens and reagents obtained from human blood should always be handled with precaution using established good laboratory practices.

Disposal of all waste material should be in accordance with local guidelines.

As with other diagnostic tests, results should be interpreted considering all other test results and the clinical situation of the patient.

Materials required but not provided

Automatic analyzer. Saline solution. Calibrator. Controls.

Storage and Stability

The Lp(a) reagents should be stored tightly capped at +2..+8ºC when not in use. **Do not freeze**. Reagents in the original vials are stable to the expiration date on the vial label when capped and stored at +2...+8ºC. Immediately following the completion of an assay run, the reagent vials should be capped until next use in order to maximize curve stability. Once opened the reagent can be used within 1 month if stored tightly closed at +2...+8ºC after use.

The Lp(a) buffer reagent should be clear and colourless. Any turbidity may be sign of deterioration and reagent should be discarted.

The Lp(a) latex reagent should have a white, turbid appearance free of granular particulate. Visible agglutination or precipitation may be a sign of deterioration, and the reagent should be discarted.

Specimens

Serum specimens should be collected by venipuncture following good laboratory practices. Lp(a) remain stable for 14 days at +2…+8ºC. if the test should be performed later, it is recommended to freeze the serum. Lipemic specimens, or turbid specimens, must be clarified before the assay by high-speed centrifugation (10 min at approx. 15.000 rpm).

Procedure

The reagents are ready to use as supplied. Latex reagent should be gently shaken before each use.

Follow the instructions of the operator's manual to load the cartridge, technique programation, calibration, sample measurement and control.

Calibration. Quality control

Standardization: use CRM® *diagnostic systems* Calibrators. The method was standardized against a highly purified material .

The Lp(a) concentration of the Calibrator and Control is given on the label. Prepare the following dilutions of the calibrator using saline solution:

1; $^1/_2$; $^1/_4$; $^1/_8$, $^1/_{16}$ saline

The standard dilutions are to be used for measurement within 4 hours.

This curve is stored in memory by the analyzer and recalled for later use. Calibration curves are stable for up to 14 days, after which a new curve must be generated. Additionally, recalibration must be performed whenever reagent lots are changed or QC so indicate.

For quality control use CRM® *diagnostic systems* Control or another suitable control material. The control intervals and limits must be adapted to the individual laboratory requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits. Control must be assayed and evaluated as for patient samples.

Calculation

The turbidimetric analysers automatically calculate the Lp(a) concentration of each sample.

Reference Values

Values < 300 mg/L are within the normal range.

This data must be interpreted as a guide. Each laboratory should establish its own reference intervals.

Assay range and Sensitivity

The assay range is established from 0 to 1200 mg/L

Calculating the mean plus 3SD of twenty replicates of zero standard resulted in a lower limit of detection of <5 mg/L.

Specificity

The assay is specific for Lp(a) determination. For interference studies we followed the procedure of Glick. We assessed the effect of bilirrubin and hemoglobin by adding known amounts of these substances to a baseline serum pool containing 500 mg/L of Lp(a). Interference from lipemia was assessed by adding various amounts of a 20 % intralipid (Kabivitrum. Stockholm, Sweden) solution to the same pool.

Due to the assay measuring principle (wavelength 600 nm), no endogenous interferences by billirubin (up to 600 µmol/L), haemoglobin (up to 10 g/L) and intralipid (up to 12,5%) occurred.

Other substances can interfere. For a comprehensive review of interfering substances, refer to the publication by Young.

Assay Precision

Intra-assay coefficients of variation (CV) for three pooled samples (Lp(a) values ranging from 150 to 720 mg/L) were between 3,4 and 4.7 %. Daily calibrated inter-assay CVs were between 3,45 and 5,1 %.

Assay Linearity

Linearity was evaluated using serial dilutions, prepared with saline solution, of two different samples, which contained values of Lp(a) in the range of analysis. Linear regression values of Lp(a) mg/L vs concentration yielded correlation coefficients, r> 0.996, for all samples. Within the assay's measuring range, the deviations of measurement from theoretical values did not exceed the 10 % level. In addition, the system did not show prozone phenomenon at least up to 2250 mg/L.

Method comparison

80 samples were correlated with a commercial ELISA immunoassay. When comparing the results by lineal regression the result was: y=1,133x+4,3 and r=0,957

Analytical characteristics have been obtained in a single experiment in a Cobas-Mira plus analyser. As is well known the analytical characteristics of a clinical chemistry reagent depend on both the reagents and the instrument used. Multicenter studies indicate important differences in analytical characteristics among similar instruments. Therefore, the data expressed in the present document should be interpreted as a guide example.

Literature

Berg K. A new serum type system in man: The Lp-system. Acta Pathol. Microbiol. Scand. 1963;59:369-82.

Gaubatz JW, Heideman C, Gotto A MJr., Morriset JD, Dahlen G.H. Human plasma lipoprotein (a): Structure and properties. J.Biol. Chem.1983;258: 4582-89.

Scanu A M, Fless G M. Lipoprotein (a). J. Clin. Invest.1990; 85:1709-15.

Sandkamp M, FunkeH, Schulte H, Kohler E, Assmann G. Lipoprotein (a) is an independent risk factor for myocardial infarction at a young age. Clin Chem.1990;36: 20-3.

Rosengren A, Wilhelmsen L, Eriksson E, Risberg B, Wedel H.Lipoprotein (a) and coronary heart disease: A prospective case-control study in a general population sample of middle age men. Br. Med. J.1990; 301: 1248-51.

Young DS. Effects of Drugs on Clinical Laboratory Test. 5th Edition, AACC Press, 2000.

Sonderdruck aus DG Klinische Chemie Mitteilungen 1995; 26: 207 – 224.

Significados de los símbolos indicados en las etiquetas. Explanation of symbols used on labelling. Explication des symbols figurant sur les etiquettes. Spiegazione dei simboli utilizzati sull'eticheta. Significado dos símbolos indicados nas etiquetas. Erläuterung der symbole auf den etiketten.

							REAG	CAL	Buffer	LYOPH	Conc.	Control H / Control L
Fecha de Caducidad Expirate Date Date de Péremption Data di Scadenza Data Expiração Verwendbar bis	Temperatura de almacén Storage Temperature Temperature de Conservation Temperatura de Conservazione Temperatura de Conservação Lagertemperatur	Número de Lote Lot Number Número de Lot Numero di Lotto Número de Lote Chargen-Nr	Para Diagnóstico In Vitro For In Vitro Diagnostic Usage In Vitro Per Uso Diagnostico In Vitro Utilizar em Diagnostico In Vitro In Vitro Diagnosticum	Número de catálogo Catalog Number Numéro de catalogue Numero di catalogo Número de catálogo Katalognummer	Conformidad Europea European Conformity Conformité aux normes européennes Conformità europea Comformidade com as normas europeias CE-Konformitätskennzeichnung	Fabricado por Manufactured by Fabriqué par Fabbricato da Fabricado por Hergestellt	Reactivo Reagent Réactif Reagenti Reagente Reagenz	Calibrador Calibrator Calibrateur Calibratore Calibrador Kalibrator	Tampón Buffer Tampon Tampone Buffer Puffer	Liofilizado Lyophilised Lyophilisé Liofilo Liofilizado Lyophilisiert	Concentración Concentration Concentration Concentrazione Concentraçao Konzentration	Control Alto / Control Bajo Control High / Control Low Contrôle élevé / Contrôle Bas Controllo Alto / Controllo Basso Controlo Alto / Controlo Baixo Kontrolle Hoch / Kontrolle Niegrid

CE

Lipoprotein (a) [Lp(a)] / Lipoproteína (a) [Lp(a)]
Procedure for / Procedimiento para
COBAS MIRA PLUS

GENERAL	
Measurement mode:	Absorb
Reaction mode:	D-R-S-SR1
Calibration mode:	LOGIT/LOG5
Reagent blank:	REAG/DIL
Cleaner:	NO
Wavelenght:	600 nm
Decimal position:	1
Units:	mg/L
ANALYSIS	
Diluent Name:	Saline
Factor:	NO
Time:	NO
STD:	MAIN INDIRECT
Main STD(***):	mg/L
POS(**):	
FACT. STD-1: 1.0	2: 2.0
3: 2.0	4: 2.0
5: 2.0	6: 0.0
7: NO	8: NO
Post. Dil. Factor:	2.00
Conc. factor:	NO
SAMPLE	Vol: 3.0 µL Cycle: 3
DILUENT	Name: H_2O Vol: 10 µL
REAGENT	Vol: 225 µL Cycle: 1
START REAGENT	Vol: 40 µL Cycle: 1
DILUENT	Name: H_2O Vol: 10.0 µL

CALCULATION	
Sample limit:	NO
Reaction Direction:	INCREASE
Check:	ON
Antigen excess	NO
Conversion factor:	1.00000
Offset:	0.00000
Normal range low:	0.0 mg/L
Normal range high:	300 mg/L
Number of steps:	1
Calc. Step A:	ENDPOINT
Reading first:	T2
Reading last:	15
CALIBRATION	
Calibr. Interval:	ON REQUEST
Reagent blank:	
Reag. Range low: NO	High: NO
Blank range low: NO	High: NO
STANDARDS:	
1: ***	2:
3:	4:
5:	6:
7: NO	8: NO
REPLICATE:	SINGLE
Deviation:	NO
Correction std:	NO

(**) Select one position on the standard rack
(***) Standard value on the label / insert

Revision 2010/09