LIPOPROTEIN (a) [Lp(a)]
Mono-Reagent Procedure

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

Background
Lipoprotein (a) [Lp(a)] was initially thought to be a genetic variant of low density lipoprotein (LDL). Lp(a) is a low density lipoprotein-like particle containing apolipoprotein B-100 disulfide-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.

Test Principle
This Lp(a) test is based upon the reactions between Lp(a) in the sample and latex-covalently bound rabbit antihuman Lp(a) antibodies. Lp(a) antibodies are determined photometrically.

Reagents
Buffer
Glycine buffer, pH: 8.0, containing protein stabilizers and 0.09 % sodium azide as preservative.

Latex reagent
a suspension of latex microparticles covalently bound antibodies against human Lp(a) in a glycine buffer (0.1 M, pH: 8.2), containing NaCl (0.15 M) and bovine serum albumin (0.5%). Preservative: Sodium azide 0.075%.

Calibrator
Human - based reference fluid. Preservative: sodium azide, 0.075%.

All raw materials of human origin used in the manufacture of this product should be handled as though capable of transmitting infectious diseases.

Precautions and Warnings
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.

Material Required
Spectrophotometric analyzer.
Saline solution.

Storage and Stability
The Lp(a) reagents should be stored tightly capped at (2 - 8 °C) 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 - 8 °C). 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 - 8 °C) after use.

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

Specimen Collection and Preparation
Serum specimens should be collected by venipuncture following good laboratory practices. Lp(a) remain stable for 14 days at (2 - 8 °C). 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).

Reagent Preparation
Working Reagent is prepared with 1 part of Latex Reagent and 7 parts of Buffer Reagent. Prepare a fresh Working Reagent based on its workload. Shake gently the reagents before pipetting.

Procedure

| Wavelength | 600 nm |
| Temperature | 37° C |
| Cuvette | 1cm light path |

Bring the reagents at 37º C and pipette:

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Sample</th>
<th>Blank</th>
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<tbody>
<tr>
<td>4 µl</td>
<td>500 µl</td>
<td>500 µl</td>
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</table>

Mix and measure absorbance immediately (A1) for calibration. Lp(a) sample absorbance (A2) after incubation.

Calculation
Plot the calibration curve and the sample concentration is obtained by interpolation the sample absorbance (A2-A1) in the calibration curve.

If is an one point calibration:

(As-A1)sample - (As-A1)blank x Calibrator concentration

*(As-A1)calibrator - (As-A1)blank*
Linearity

Up to 800 mg/L.

Calibration and Quality Control

<table>
<thead>
<tr>
<th>Calibrator 1</th>
<th>100 µl of Spectrum LP(a) Calibrator*</th>
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<tbody>
<tr>
<td>Calibrator 2</td>
<td>100 µl of Calibrator 1 + 100 µl of Saline Solution</td>
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<tr>
<td>Calibrator 3</td>
<td>100 µl of Calibrator 2 + 100 µl of Saline Solution</td>
</tr>
<tr>
<td>Calibrator 4</td>
<td>100 µl of Calibrator 3 + 100 µl of Saline Solution</td>
</tr>
<tr>
<td>Calibrator 5</td>
<td>100 µl of Saline Solution</td>
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[*] See values on the label or on the insert. Multiply by the appropriate factor.

For quality control use Spectrum Control or other 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.

Expected Values

Values < 300 mg/L are within the normal range. This data must be interpreted as a guide.

Each laboratory should establish an expected range for the geographical area in which it is located.

References


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

ORDERING INFORMATION

<table>
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<th>CATALOG NO.</th>
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<td>50 test</td>
</tr>
<tr>
<td>550 002</td>
<td>100 test</td>
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