LIPOPROTEIN (a)

INTENDED USE
Immunoturbidimetric assay for the quantitative in vitro determination of Lipoprotein (a) in human serum or plasma.

Cat. No. LPA400

1. Buffer 1 x 20 ml
2. Latex 1 x 10 ml

CLINICAL SIGNIFICANCE(1)
The [Lp(a)] test system is intended to measure lipoprotein (a) in serum. Lipoprotein (a) measurements are used to evaluate disorders of lipid metabolism and to assess coronary heart disease in specific populations.

PRINCIPLE(2)
Agglutination occurs due to an antigen-antibody reaction between Lp(a) in a sample and anti-Lp(a) antibody adsorbed to latex particles. This agglutination is detected as an absorbance change at 700 nm proportional to the concentration of Lp(a) in the sample.

Note: This product is licensed from Denka Seiken.

SAMPLE COLLECTION AND PREPARATION(2)
Collect serum using standard sampling tubes and plasma using tubes containing Li heparin, Na heparin, Na EDTA, K EDTA.

SAMPLE STORAGE AND STABILITY
The samples should be analyzed immediately or stored up to 14 days at 2 - 8°C. For delayed testing, store at −20°C or - 70°C. Freeze thaw cycling is not recommended.

REAGENT COMPOSITION

<table>
<thead>
<tr>
<th>Contents</th>
<th>Initial Concentration of Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1. Buffer Solution</td>
<td>0.17M</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.08M</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>0.05M</td>
</tr>
<tr>
<td>Sodium ethylenediamine</td>
<td>0.09% w/v</td>
</tr>
<tr>
<td>Sodium azide</td>
<td>0.09% w/v</td>
</tr>
<tr>
<td>R2. Latex Reagent</td>
<td>0.17M</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.17M</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>0.1M</td>
</tr>
<tr>
<td>Suspension of latex particles coated with anti-lp(a) antibodies</td>
<td>0.5%</td>
</tr>
<tr>
<td>Sodium azide</td>
<td>≤0.09% w/v</td>
</tr>
</tbody>
</table>

SAFETY PRECAUTIONS AND WARNINGS
For in vitro diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Human source material from which this product has been derived has been tested at donor level for the Human Immunodeficiency Virus (HIV 1, HIV 2) antibody, Hepatitis B Surface Antigen (HBsAg) and found to be NON-REACTIVE. FDA approved methods have been used to conduct these tests. However, since no method can offer complete assurance as to the absence of infectious agents, this material and all patient samples should be handled as thought capable of transmitting infectious diseases and disposed of accordingly.

Material Safety Data Sheets are available on request.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS

1. Buffer
Buffer is ready for use and is stable up to the expiration date when stored at 2 to 8°C protected from light. Once opened the contents are stable for 30 days on board the analyzer at approximately 10°C.

2. Latex Reagent
Latex Reagent is ready for use and stable up to the expiration date when stored at 2 to 8°C protected from light. Once opened the contents are stable for 30 days on board the analyzer at approximately 10°C. Invert several times before use, avoiding the formation of foam.

R1 = Buffer Solution
R2 = Latex Reagent

MATERIALS PROVIDED
Buffer Solution
Latex Reagent

MATERIALS REQUIRED BUT NOT PROVIDED
MedTest DX Lipoprotein (a) Calibrator Set Cat. No. LPAC400
MedTest DX Direct Lipid Control Level 1, Cat. No. 3001
MedTest DX Direct Lipid Control Level 3, Cat. No. 3003

CALIBRATION
The use of MedTest DX Lipoprotein(a) Calibrator is recommended for calibration. Saline is used as S1 and Cal 1-5 as S2-S6. A multi point calibration is recommended every 14 days or when reagent lot is changed.

This assay uses an exponential calculation and no reagent blank. Ensure that on the Calibration Checks screen the following are selected for this test:

Reagent Blank Measurement
- Disable reagent blank

QUALITY CONTROL
MedTest DX Direct Lipid Controls, Levels 1 and 3 are recommended for quality control to monitor accuracy and precision. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:
1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water, Contaminants, i.e. bacterial growth, may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiration date of kit and contents
6. Contact MedTest DX Technical Services at (800) 757-5313.

INTERFERENCE
The following analytes were tested up to the noted levels and did not cause interferences:

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Interference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intralipid®</td>
<td>5%</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>35 mg/dl</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>1040 mg/dl</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>50 mg/dl</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>493 mg/dl</td>
</tr>
<tr>
<td>Plasminogen</td>
<td>200 mg/dl</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>200 mg/dl</td>
</tr>
</tbody>
</table>
**NORMAL RANGE**

**ADULTS**

The above reference range was established based on a sample of 96 Caucasian individuals comprising 49 males (age range 17-90 years; mean = 55 years) and 47 females (age range 13-84 years; mean = 55 years) resident in Northern Ireland. The population tested was an ambulatory population with no history of coronary disease. Results showed a mean Lp(a) value of 18.5 mg/dl for males and 20.6 mg/dl for females. Reference ranges have not been established for this assay for different ethnic populations or disease states.

<table>
<thead>
<tr>
<th>Lipoprotein(a) In 96 N.Ireland Individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>SD from Mean</td>
</tr>
<tr>
<td>Male mean = 18.5  SD = 23.8</td>
</tr>
<tr>
<td>Female mean = 20.6  SD = 24.9</td>
</tr>
</tbody>
</table>

Lp(a) concentrations have been shown to be genetically determined and to vary with ethnic populations. One study carried out in the United States showed that mean plasma levels of Lp(a) were approximately twice as high in African people or people of African descent compared to levels in Caucasians. Also, the distribution of Lp(a) is less skewed in African people or people of African descent than in Caucasians. Other studies have also shown no difference in Lp(a) levels between men (mean = 14 mg/dl) and women (mean = 15 mg/dl). Levels of Lp(a) have been shown not to differ significantly between pre- and post menopausal caucasian women.

It is recommended that each laboratory establish its own reference range to reflect the age, race, sex, diet and geographical location of the population.

**ASSAY RANGE**

The range of this assay is approximately 2 - 90 mg/dl depending on the lot specific value of the calibrator in use. Samples with levels of Lp(a) greater than the high calibrator value should be diluted with distilled water and re-assayed. The result should be multiplied by the appropriate factor.

**NB.** It is recommended that the results falling below the concentration of the lowest calibrator be reported as less than the concentration of the lowest calibrator.

**PROZONE EFFECTS**

Antigen excess effects are not noted until levels approach 300 mg/dl.

**SENSITIVITY**

The minimum detectable concentration with an acceptable % coefficient of variation was determined as 2 mg/dl.

**SPECIFIC PERFORMANCE CHARACTERISTICS**

The following performance characteristics were obtained using a Hitachi 717 analyzer.

**PRECISION**

<table>
<thead>
<tr>
<th>Within run precision</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (mg/dl)</td>
<td>21.0</td>
<td>51.5</td>
<td>83.05</td>
</tr>
<tr>
<td>SD</td>
<td>0.342</td>
<td>0.789</td>
<td>1.99</td>
</tr>
<tr>
<td>CV(%)</td>
<td>1.63</td>
<td>1.53</td>
<td>2.40</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Between run precision</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (mg/dl)</td>
<td>24.19</td>
<td>32.56</td>
<td>50.48</td>
</tr>
<tr>
<td>SD</td>
<td>0.75</td>
<td>1.15</td>
<td>1.43</td>
</tr>
<tr>
<td>CV(%)</td>
<td>3.11</td>
<td>3.52</td>
<td>2.83</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

**METHOD COMPARISON (SERUM)**

(i) The MedTest DX method (Y) was compared to another commercially available method (X). Thirty eight patient samples with values spanning the range 0 – 71.5 mg/dl were tested. Linear regression analysis of the data resulted in the following equation: Y = 0.83x + 2.13 with a correlation coefficient of r = 0.75.

The MedTest DX Lp(a) test kit shows minimum apo(a) size related bias. Size heterogeneity of apo(a) can affect to varying degrees the outcome of other commercially available kits.

**SERUM/PLASMA COMPARISON**

The MedTest DX Lp(a) test kit was used to compare serum samples (X) to plasma samples (Y) collected into tubes containing Li heparin, Na heparin, Na EDTA, K EDTA or citrate. 56 samples were tested. The data was subjected to linear regression analysis.

**RESULTS**

- **(i) Serum/Plasma (Li heparin):**
  - Sample range: 2 - 77.3 mg/dl
  - Linear regression analysis: \( y = 0.956x + 1.199 \)
  - Correlation coefficient: 0.996

- **(ii) Serum/Plasma (Na heparin):**
  - Sample range: 2.3-78.3 mg/dl
  - Linear regression analysis: \( y = 0.958x - 0.522 \)
  - Correlation coefficient: 0.996

- **(iii) Serum/Plasma (Na EDTA):**
  - Sample range: 1.7-78.8 mg/dl
  - Linear regression analysis: \( y = 0.972x + 0.023 \)
  - Correlation coefficient: 0.999

- **(iv) Serum/Plasma (K EDTA):**
  - Sample range: 1.8-79.5 mg/dl
  - Linear regression analysis: \( y = 0.981x + 0.085 \)
  - Correlation coefficient: 0.999

- **(v) Serum/Plasma (Citrate):**
  - Sample range: 2.0-79.4 mg/dl
  - Linear regression analysis: \( y = 0.963x + 0.065 \)
  - Correlation coefficient: 0.999
LIMITATIONS
1. Performance of this assay was not tested with age-matched pairs in a diseased population.
2. Normal range values for this assay have not been established for African-American populations.
3. This assay has not been tested for interference by Statin Therapy.
4. Intake of alcohol, aspirin, niacin and estrogen supplements have the potential of causing a misrepresentation of the true LP(a) concentrations.

REFERENCES