RX SERIES

LATEX ENHANCED IMMUNOTURBIDIMETRIC ASSAY FOR THE MEASUREMENT OF LIPOPROTEIN (A) ON THE RX MONACO ANALYSER
INTRODUCTION

What is Lipoprotein(a)?
Lipoprotein(a), also known as Lp(a) or lipoprotein-little-a, are particles that are made in the liver and found in the blood plasma that transport cholesterol, phospholipids, triglycerides and apolipoproteins. The amount that is present in your body is mainly determined by genetics and varies widely hence, the levels of Lp(a) can vary up to 1000-fold between individuals. Lipoprotein is assembled from a cholesterol rich LDL particle and two protein molecules; including apolipoprotein apo(a) and apolipoprotein B100. Although the size of apolipoprotein B100 is the same for everyone the size of apo(a) is genetically determined and differs between individuals and can vary widely.

Having a high level of Lp(a) (greater than 500mg/L or 120nmol/L) increases your risk of cardiovascular disease (CVD). Levels of Lp(a) do not change throughout life and are usually unaffected by lifestyle or the environment. According to the British Heart Foundation, an average of 420 people die from CVD each day which equates to one death every three minutes.

What is Lp(a) used for?
Although having some cholesterol and Lp(a) in your blood is normal, elevated concentrations of Lp(a) are a risk factor for coronary heart disease (CHD), a heart attack, peripheral vascular disease, aortic stenosis, blood clots and stroke. Therefore measuring Lp(a) offers the possibility of early diagnosis and risk of CVD. It has been estimated that one in five people globally have a high level of Lp(a) and remain unaware despite high Lp(a) being a common condition. Additionally around 30% of those diagnosed with Familial Hypercholesterolemia have high levels of Lp(a). If a parent has been diagnosed with high Lp(a) then their children will have a one in two chance of inheriting it also. An Lp(a) test is recommended for families where heart attacks and stroke are common, especially at a young age and where no additional risk factors are known to be a contributing factor.

Yet despite the above evidence and the recommendation of screening for elevated Lp(a) from the European Atherosclerosis Society, measurement of Lp(a) is not conducted in clinical practice. This is largely due to the major challenge associated with Lp(a) measurement and the size variation of apo(a) with Lp(a). Dependent upon the size of apo(a) in the assays calibrator, many assays under or overestimate apo(a) size in the patient sample which can lead to patient misclassification.

The Randox assay is one of the only methodologies on the market that detects the non-variable part of the Lp(a) molecule and therefore suffers minimal size related bias; providing accurate and consistent results. The Randox Lp(a) kit is standardised to the WHO/IFCC reference material SRM 2B and is closest in terms of agreement to the ELISA reference method.

Clinical significance of Lp(a)
Lp(a) determination is intended for use in conjunction with clinical evaluation, patient risk assessment and other lipid tests to evaluate disorders of lipid metabolism and to assess coronary heart disease risk in specific populations. Evidence suggests that between 60-70% of African populations are estimated to have between 250-300mg/L levels of Lp(a) putting them at greater risk of developing CVD compared to Chinese and Japanese populations; where only 30% of the population is estimated to have between 250-300mg/L levels of Lp(a).
Scientific Study
This study reports the development of an immunoturbidimetric assay kit with enhanced precision and accuracy for the measurement of Lp(a) in human serum and plasma applied to the fully automated bench top/floor standing analyser RX monaco. The assay is applied to the fully automated RX series analysers and is also suitable for use on many mainstream clinical chemistry platforms. This is of value as an accurate, stable and convenient tool for the determination of Lipoprotein(a) using these automated systems.

Methodology
Agglutination occurs due to an antigen-antibody reaction between Lp(a) in a sample and anti-Lp(a) antibody adsorbed to latex particles. This is detected as an absorbance change at 700 nm proportional to the concentration of Lp(a) in the sample. Concentrations are calculated from a multi-point calibration. On-board and calibration stabilities were tested by storing the reagents uncapped on the analyser for a period of 28 days. Within-run and total precision were assessed by testing serum samples at defined medical decision levels, 4 replicates twice a day for 20 days. Correlation studies were conducted using another commercially available clinical chemistry system.

RESULTS
Sensitivity and linearity

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Assay Sensitivity</th>
<th>Assay Linearity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp(a)</td>
<td>6.18 mg/dL</td>
<td>103 mg/dL</td>
</tr>
</tbody>
</table>

Prozone was not observed up to 493 mg/dL

Reagent on-board stability

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>n</th>
<th>Mean</th>
<th>Within-Run Precision</th>
<th>Total Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen Type</td>
<td>n</td>
<td>Mean</td>
<td>Within-Run Precision</td>
<td>Total Precision</td>
</tr>
<tr>
<td>QC Level 1</td>
<td>80</td>
<td>17.71</td>
<td>0.49</td>
<td>2.8</td>
</tr>
<tr>
<td>Patient sample</td>
<td>80</td>
<td>24.00</td>
<td>0.98</td>
<td>4.1</td>
</tr>
<tr>
<td>Serum pool</td>
<td>80</td>
<td>29.25</td>
<td>0.6</td>
<td>2.0</td>
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<tr>
<td>Calibrator LS</td>
<td>80</td>
<td>72.29</td>
<td>1.27</td>
<td>1.8</td>
</tr>
</tbody>
</table>
Correlation
This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:
\[ Y = 0.97X + 0.59 \]
and a correlation coefficient of \( r = 1.00 \)

40 patient samples were analysed spanning the range 6.51 mg/dL to 101.97 mg/dL.
The Randox 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.

Correlation Lp(a) assay on RX monaco vs available clinical chemistry system

Interference
The analytes below were tested up to the following levels and were found not to interfere at Lp(a) concentrations of 16 mg/dL and 40 mg/dL.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>16 mg/dL</th>
<th>40 mg/dL</th>
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</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>1000 mg/dL</td>
<td>1000 mg/dL</td>
</tr>
<tr>
<td>Free Bilirubin</td>
<td>60 mg/dL</td>
<td>60 mg/dL</td>
</tr>
<tr>
<td>Conjugate Bilirubin</td>
<td>60 mg/dL</td>
<td>60 mg/dL</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>2000 mg/dL</td>
<td>2000 mg/dL</td>
</tr>
<tr>
<td>Intralipid®</td>
<td>2000 mg/dL</td>
<td>2000 mg/dL</td>
</tr>
</tbody>
</table>
**FINDINGS**

The Randox Lp(a) immunoturbidimetric assay applied to the bench top/floor standing RX monaco analyser, exhibits high sensitivity and reproducibility with the added advantage of using reagents with good stability. This is of value in the accurate determination of this analyte in human serum/plasma for clinical and research applications. The Randox Lp(a) immunoturbidimetric assay is also available on all fully automated RX analysers including the RX imola, RX daytona+ and RX modena.

Further information on the assay and RX analyser used within this study

**Lp(a) features:**
- Sample type - Suitable for use with serum and plasma
- Immunoturbidimetric method - Making it simple and quick to perform
- Liquid ready-to-use reagents - For ease of use and convenience
- Excellent stability - All reagents are stable to expiry date when stored at +2-8°C or 28 days on board the analyser at approximately 10°C.
- Extensive measuring range 3-90 mg/dl - Ensuring concerning levels of Lipoprotein(a) are comfortably detected
- Superior 5-point calibrator available - Providing a complete diagnostic testing package

**Advantages of the RX analysers for testing:**
- Low water consumption
- User friendly software
- High throughput of tests including ISE (analyser dependent)
- Low sample volume required
- STAT sample functionality
- Dual 5 speed stirrers optimized for each chemistry reaction
- Reagent micropipette with liquid level sensor and crash detection
- Liquid level sensor, crash, bubble and clot detection
REFERENCES
