

RANDOX

LIPOPROTEIN(A) [LP(A)]



OVERCOMING THE CURRENT CHALLENGES IN LP(A) TESTING

- 01** BACKGROUND

- 02** PHYSIOLOGICAL SIGNIFICANCE
 - A. LIPOPROTEIN(A) ANATOMY
 - B. APOLIPOPROTEIN(A) ANATOMY

- 03** FREQUENCY OF TESTING

- 04** CLINICAL UTILITY OF LIPOPROTEIN(A)

- 05** WHO / IFCC REFERENCE MATERIAL

- 06** CURRENT CHALLENGES IN LP(A) MEASUREMENT

- 07** HOW RANDOX CAN OVERCOME THE CURRENT CHALLENGES IN LP(A) MEASUREMENT

- 08** MEASURING IN NMOL/L

- 09** LP(A) IN COVID-19

- 10** RANDOX LP(A) PRODUCT OVERVIEW

- 11** CONCLUSION

- 12** REFERENCES

Overcoming the Current Challenges in Lp(a) Testing

1. BACKGROUND

Cardiovascular disease (CVD) is the number one cause of death globally, with more people dying from CVD than any other illness globally. In 2016, 17.9 million people died from CVD, representing 31% of all global deaths. Of these, 85% were attributed to myocardial infarction (heart attack) and cerebrovascular accident (CVA) (stroke). In 2015, 17 million premature deaths in those under 70 years of age were non-communicable, 37% of these deaths were attributed to CVD. CVD is preventable by addressing behavioural risk factors including: diet, physical activity and tobacco use. It is imperative that CVD is detected as early as possible to enable the early implementation of treatment plans ¹.

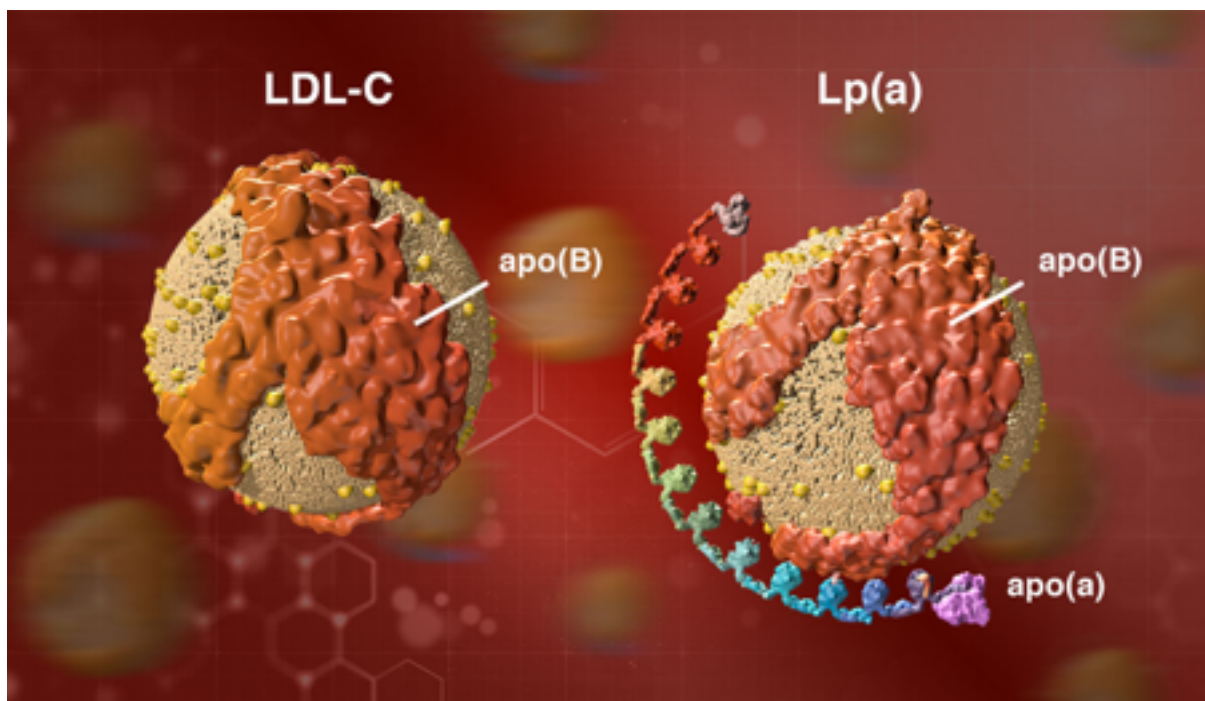
Lipoprotein(a) [Lp(a)] is an independent genetic risk factor of CVD. One in five (20%) of the population have elevated Lp(a) levels from birth, and most don't know they have it. Lp(a) collects in the arteries, gradually narrowing them, limiting blood supply to vital organs, including: the brain, kidneys and heart, increasing the risk of peripheral artery disease, stroke and myocardial infarction ².

2. PHYSIOLOGICAL SIGNIFICANCE OF LP(A)

A. Lipoprotein(a) Anatomy

Lp(a) is described as “the most complex and polymorphic of the lipoprotein particles”. Lp(a) is composed of a lipoprotein particle similar in composition to low-density lipoproteins (LDL) ³. The main difference between LDL and Lp(a) is that Lp(a) contains an additional protein, apolipoprotein (a) [apo(a)]. A single copy of apo(a) is covalently bonded to the apoB-100 by a single disulphide bridge, which differentiates Lp(a) from LDL as indicated in Fig 1 ^{3, 4}. The chemical weight of Lp(a) is believed to range between 1.05 to 1.21 g/ml and the chemical composition is believed to be: 30% to 36% protein; 26% to 35% cholesteryl esters; 22% to 23% phospholipid; 7% to 10% free cholesterol; and 3% to 9% triglycerides ⁴.

Fig 1: LDL-C vs Lp(a) Anatomy

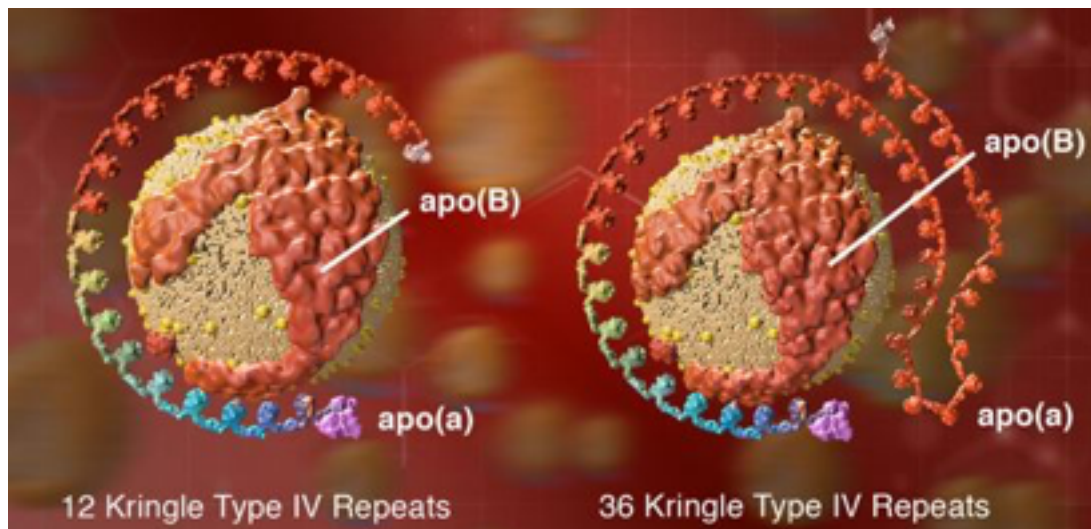


B. Apolipoprotein(a) Anatomy

Apo(a) is comprised of five cysteine-rich domains called kringles, which are coded by the LPA gene, located on the long (q) arm of chromosome 6 at positions 26 and 27 (6q26-27), and the plasminogen (PLG) gene, also located on the long (q) arm of chromosome 6 at position 26 (6q26) ^{4,5,6}. The PLG gene is believed to encode 5 kringles (types I to V) as well as an active protease domain. Conversely, kringles IV and V and an inactive protease domain are present in apo(a) ³.

Apo(a) is described as polymorphic as the kringle type IV undergoes duplication, coding for 10 kringle IV types (KIV-1 through KIV-10) (Fig 2). "All kringles contain approximately 80 amino acids and are stabilized by three intrachain disulphide bonds, giving them a characteristic triple look structure". All kringles, except for KIV-2, are present as single copies. KIV-2 is present in multiple copies, ranging from 2 to >40, within the apo(a) proteins of distinct sizes. The number of KIV-2 repeats determines the size of the apo(a) isoform, thus heterogeneously affecting the size of the apo(a) protein and the plasma levels of Lp(a). An inverse correlation between the size of the apo(a) isoform and plasma concentrations of Lp(a) has been identified ⁴

Fig 2: Kringle variation in Lp(a) molecules



3. FREQUENCY OF TESTING

Lp(a) is believed to remain relatively stable over its lifespan as Lp(a) is predominantly genetically determined. Lp(a) testing is believed to be more cost effective in comparison to genetic testing at this time. A single Lp(a) test is believed to be sufficient to improve the accuracy of cardiovascular risk assessment, however, qualification levels that are close to the action thresholds should be considered on a case-by-case basis ⁷.

Repeat testing can be initiated if a secondary cause is suspected or therapeutic measures to lower Lp(a) levels have been instigated ⁷. The once-only recommendation for Lp(a) testing is consistent with the recently published European Guidelines ⁸, however HEART UK ⁷, recommend that Lp(a) is measured in specific cohorts as opposed to all adults. The European guidelines aim to identify those at a very high risk (Lp(a) levels >430nmol/l), however, HEART UK argues that the risk conferred by Lp(a) occurs at a much lower threshold. Consequently, the HEART UK approach is to measure Lp(a) in specific populations and manage Lp(a) associated risk in those with levels >90nmol/l ⁷.

4. CLINICAL UTILITY OF LIPOPROTEIN(A)

A. Federal Practitioner (2019): Lipoprotein(a) elevation: A new diagnostic code with relevance to service members and veterans ⁹

A USA study prospectively examined the clinical utility of Lp(a) with incident CVD in three cohorts of women from primary prevention: the Women's Health Study (WHS) (n=24,558), Women's Health Initiative Observational Study (WHIO) (n=1,989), the Justification for Use of Statins in Prevention (JUPITER) (n=2,569) and men from JUPITER (n=5,161). In the three cohorts of women, Lp(a) was associated with CVD in those with elevated total cholesterol levels ⁹.

B. *European Heart Journal* (2017): Lipoprotein(a) and the risk of cardiovascular disease in the European population: results from the BiomareCaRE consortium ¹⁰

A European study prospectively analysed 56,804 participants from 7 prospective population-based cohorts, based on the Biomarkers for Cardiovascular Risk Assessment in Europe (BiomarCaRE) across Europe with a maximum follow-up of 24 years. Regional differences in Lp(a) concentrations within the European populations were observed. Elevated Lp(a) levels were robustly associated with an increased risk for major coronary event (MCE) and CVD, particularly in those with diabetes ¹⁰.

C. *Lipids in Health and Disease* (2016): The relationship between Lp(a) and CVD outcomes: a systematic review ¹¹

A systematic literature review of 2,850 records up to December 2018 were examined. Of these, 60 studies were included in the review. Of the 39 primary prevention studies and seven high risk primary prevention studies included, a statistically significant correlation between increased Lp(a) levels and an increased risk of future cardiac events ¹¹.

D. *Circulation* (2013): Associations between lipoprotein(a) levels and cardiovascular outcomes in African Americans and Caucasians: The atherosclerosis risk in communities (ARIC) study ¹²

A USA study prospectively evaluated 3,467 African Americans and 9,851 Caucasians to determine the associations between Lp(a) and incident CVD events in the ARIC study as Lp(a) is not considered a risk factor for CVD in African Americans. The study concluded that Lp(a) levels were positively associated with CVD events in both African Americans and Caucasians. Larger ranges of Lp(a) concentrations were observed in African Americans compared to Caucasians ¹².

5. WHO / IFCC REFERENCE MATERIAL

The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), through its Working Group on Lp(a) and together with research institutions and several diagnostic companies recommends that laboratories use assays which do not suffer from apo(a) size-related bias ¹³.

The 'IFCC SRM 2B' was accepted by the WHO Expert Committee on Biological Standardisation as the First WHO / IFCC International Reference Reagent for Lp(a) to ensure conformity by diagnostic companies to the European Union's Directive on In vitro Diagnostic Medical Devices for the metrological traceability of calibrator materials ¹³.

6. CURRENT CHALLENGES IN LP(A) MEASUREMENT

The biggest challenge that exists surrounding Lp(a) measurement is the heterogeneity of the apo(a) isoforms, resulting in the underestimation or overestimation of Lp(a) concentrations ⁷.

In immunoassays, the variable numbers of repeated KIV-2 units in Lp(a) act as multiple epitopes. This is where standardisation across calibrators is vital. Unless the calibrants have the same range of isoforms as test samples, those with higher numbers of the KIV-2 repeat, will represent with an overestimation in Lp(a) concentrations and those with smaller numbers of the KIV-2 repeat, will represent with an underestimation. The smaller isoforms are strongly associated with higher Lp(a) concentrations. Lack of standardisation of the calibrant would result in an underestimation of Lp(a) associated CVD risk. It is important to note that an Lp(a) immunoassay employing isoform insensitive antibodies does not exist ⁷.

The gold standard Lp(a) method is the Northwest Lipid Metabolism and Diabetes Research Laboratory (NLM DRL) method which employs an isoform insensitive antibody and is meticulously calibrated with well characterised material, however, this test is not commercially available ⁷.

Lp(a) assays that are standardised to the WHO/IFCC reference material, transferring values from mg/dl to nmol/l are more uniformed. The Denka Seiken Lp(a) assay is the most reliable commercially available Lp(a) assay, because ⁷:

A. The isoform size variations are reduced as a range of calibrators from separate pools of serum were used which covered a range of Lp(a) concentrations.

B. The isoform size and concentrations are inversely correlated better matching calibrants with test samples.

C. Methods based on the Denka Lp(a) assay, calibrated in nmol/l and traceable to WHO / IFCC reference material gave acceptable bias compared with the NLM DRL gold standard method.

7. HOW RANDOX CAN OVERCOME THE CURRENT CHALLENGES IN LP(A) MEASUREMENT

We must acknowledge that there are competitors in the market that also offer the Lp(a) reagent and calibrator. However, Randox utilise the Denka Seiken assay and Randox are the first diagnostic manufacturer to develop a five-point calibrator, enabling Randox to overcome the current challenges in Lp(a) measurement, leading the market to Lp(a) testing standardisation.

1. The Reagent

No commercially available assay employs isoform insensitive antibodies.

Whilst not commercially available, the North West Lipid Metabolism and Diabetes Research Laboratory (NLMDRL) is the gold standard method that utilises an isoform insensitive antibody. NLMDRL have confirmed that Denka Seiken reagents are the most reliable commercial assays available.

Randox utilise the Denka Seiken method in the development of the Randox Lp(a) assay.

2. The Calibrator

Due to the variability of the isoform apo(a) sizes, it is vital that the calibrators utilised cover the full range of Lp(a) concentrations.

The calibrators must be prepared from separate pools of serum.

The calibrator must be able to cover low level isoform size to high level isoform size.

Randox offer a dedicated five-point calibrator traceable in nmol/l of Lp(a) values to the WHO/IFCC reference material, accurately reflecting the heterogeneity of the apo(a) isoforms.

8. MEASURING IN NMOL/L

Traditionally Lp(a) has been measured in mg/dl, a measurement that indicates the amount of a substance in a specific amount of blood i.e. the mass. Lp(a) expressed in mass units (mg/dl) encompasses the mass of the ENTIRE particle, comprising of:

- Apo(a)
- ApoB-100
- Cholesterol
- Cholesterol esters
- Phospholipids
- Triglycerides

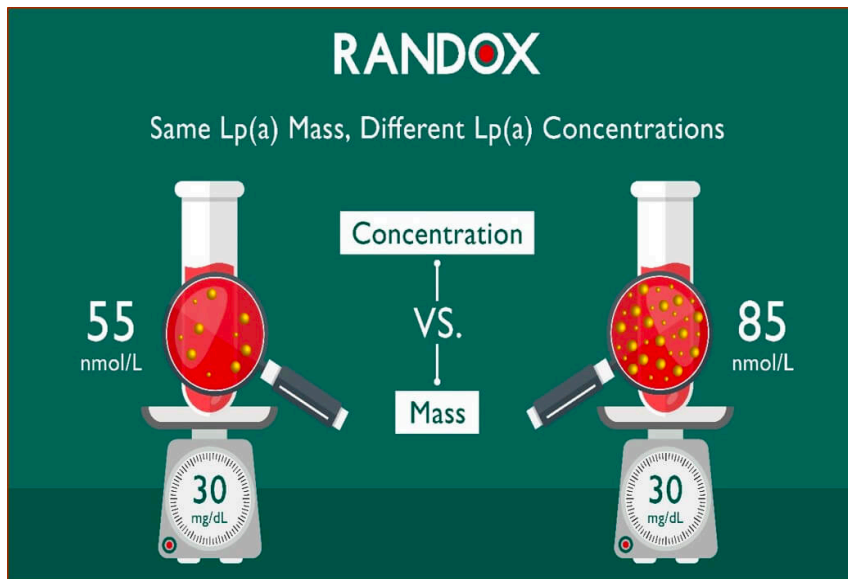
Because of the heterogeneity of the apo(a) size / presence and the lack of standardisation in calibration, mass measurement can be inaccurate.

nmol/l (nanomoles per litre): a mole is the amount of a substance that contains a large number of molecules or atoms. A nanomole is one-billionth of a mole. So, this is known as molecular units.

nmol/L focuses specifically on the number of particles of Lp(a) in the blood.

The molar units reflect the measure of the number of circulatory Lp(a) particles & because the particles are so variable in size it is the most scientifically correct way to express the level of Lp(a) in human serum.

Fig 3: Two samples with the same Lp(a) mass, but different Lp(a) concentrations ⁶



9. LP(A) IN COVID-19

Lp(a) has been identified to play a role in COVID-19. Those with either baseline elevated Lp(a) or those whose Lp(a) levels increased following infection from COVID-19, or both, may be at a significantly increased risk of developing thromboses. Elevated Lp(a) levels may cause acute destabilisation of pre-existing but quiescent, atherosclerotic plaques, which could induce an acute myocardial infarction and stroke ¹⁴.

10. RANDOX LP(A) PRODUCT OVERVIEW

The Randox Lp(a) assay utilises the Denka Seiken method in the development of the Lp(a) assay, the most reliable commercial assay available and therefore suffers minimal size related bias, providing more accurate and consistent results.

- Dedicated five-point calibrator with accuracy-based assigned target values in nmol/l are provided and standardised to the WHO/IFCC reference material (IFCC SRM 2B) and so is closest in terms of agreement to the NLM DRL reference method and so accurately reflects the heterogeneity of the apo(a) isoforms present in the general population.
- Measuring units are available in nmol/l which is more uniformed.
- Immunoturbidimetric method is highly sensitive and specific for the detection of Lp(a) in serum and plasma.
- Excellent correlation coefficient of $r=0.995$ when compared against other commercially available methods.
- Excellent within run precision of $<2.54\%$.
- Liquid ready-to-use format for convenience and ease-of-use.
- Dedicated Lp(a) control available offering a complete testing package.
- Applications available detailing instrument-specific settings for the convenient use of the Randox Lp(a) assay on a variety of clinical chemistry analysers.

11. CONCLUSION

The heterogeneity of the apo(a) isoforms within the Lp(a) molecule results in the underestimation or overestimation of Lp(a) concentrations. This is the biggest challenge surrounding Lp(a) testing ⁶. Randox utilise the Denka Seiken and Randox are the first diagnostic manufacturer to develop the five-point calibrator, enabling Randox to overcome the current challenge in Lp(a) measurement, leading the market to Lp(a) testing standardisation.

12. REFERENCES

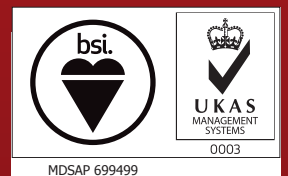
1. World Health Organization (WHO). Cardiovascular diseases (CVDs). [https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-\(cvds\)](https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)) (accessed 24 February 2020).
2. Lipoprotein(a) Foundation. Understand Inherited Lipoprotein(a). <https://www.lipoproteinafoundation.org/page/UnderstandLPA> (accessed 6 March 2020).
3. Marcovina SM, Albers JJ. Lipoprotein (a) measurements for clinical application. *Journal of Lipid Research* 2019; 57(): 526-537.
4. Nordestgaard BG, Chapman MJ, Ginsberg HN. Lipoprotein (a): EAS Recommendations for Screening, Desirable Levels and Management, 1st ed. Dorset: Sherborne Gibbs Limited; 2012.
5. Bermudez V, Rojas J, Salazar J, Bello L, Anez R, et al. Variations of Lipoprotein(a) Levels in the Metabolic Syndrome: A Report from the Maracaibo City Metabolic Syndrome Prevalence Study. *Journal of Diabetes Research* 2013; 2013(416451): 1-12. <https://www.hindawi.com/journals/jdr/2013/416451/> (accessed 29 November 2019).
6. Genetics Home Referencing. PLG gene. <https://ghr.nlm.nih.gov/gene/PLG> (accessed 29 November 2019).
7. Cegla J, Dermot R, Neely G, France M, Ferns G, et al. HEART UK consensus statement on Lipoprotein(a): A call to action. *Atherosclerosis* 2019; 291(): 62-70.
8. McGill University Health Centre. What is high lipoprotein(a), and should I be concerned?. <https://medicalxpress.com/news/2017-02-high-lipoproteina.html> (accessed 17 December 2019)
9. Engler RJM, Brede E, Villines T, Vernalis MN. Lipoprotein(a) Elevation: A New Diagnostic Code with Relevance to Service Members and Veterans. *Federal Practitioner* 2019; 36(7): 19-31.
10. Waldeyer C, Makarova N, Zeller T, Schnabel RB, Brunner FJ, et al. Lipoprotein(a) and the risk of cardiovascular disease in the European population: results from the BiomarCaRE consortium. *European Heart Journal* 2017; 38(32): 2490-2498.
11. Forbes CA, Quek RGW, Deshpande S, Worthy G, Wolff R, et al. The relationship between Lp(a) and CVD outcomes: a systematic review. *Lipids in Health and Disease* 2016; 15(95): .
12. Virani SS, Brautbar A, Davis BC, Nambi V, Hoogeveen RC, et al. Associations Between Lipoprotein(a) Levels and Cardiovascular Outcomes in African Americans and Caucasians: The Atherosclerosis Risk in Communities (ARIC) Study. *Circulation* 2012; 125(2): 241-249.
13. Dati F, Tate JR, Marcovina SM, Steinmetz A, et al. First WHO/IFCC International Reference Reagent for Lipoprotein(a) for Immunoassay-Lp(a) SRM 2B. *Clinical Chemistry and Laboratory Medicine* 2004; 42(6): 670-676.
14. Moriarty PM, Gorby LK, Stroes ES, Kastelein JP, Davidson M, et al. Lipoprotein(a) and Its Potential Association with Thrombosis and Inflammation in COVID-19: a Testable Hypothesis. *Current Atherosclerosis Reports* 2020; 22(48): 1-8.



NOTES:

A series of horizontal dotted lines for writing notes.

RANDOX
REAGENTS



Copyright © 2019 Randox Laboratories Ltd. All rights Reserved. VAT number: GB 151682708. Product availability may vary from country to country. Some products may be for Research Use Only. For more information on product application and availability, please contact your local Randox Representative