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On the cusp of global lipoprotein(a) standardization

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Abstract: Lipoprotein(a) (Lp(a)) has the reputation of being the most misunderstood metric in laboratory medicine. The unique apolipoprotein(a) (apo(a)) in Lp(a) is very heterogenous, the kringle IV domain of apo(a) being formed by 12–50 kringles due to 3 to ~40 KIV_2 repeats. The variable number of repeated identical KIV_2 domains causes KIV_2-dependent antibodies to form different amounts of immunocomplexes with apo(a), leading to higher recovery for larger and lower recovery for smaller apo(a) particles than the calibrator. Consequently, the required identity between the analyte in samples and in assay calibrator(s), which is at the basis of any immunoassay, cannot be accomplished in the case of Lp(a). Global Lp(a) standardization was first attempted in the nineties by an IFCC Working Group on Lp(a) Standardization using an ELISA-based reference measurement procedure (RMP) with monoclonal anti-apo(a) antibodies against unique epitopes. WHO-IFCC reference material (RM), named SRM2B, was established with apo(a) expressed in molar units. Currently, a 2nd generation, ISO 15193 compliant, IFCC-endorsed multiplex RMP based on quantitative Mass Spectrometry (MS) has been developed. Traceability to SRM2B is maintained using a value transfer protocol that assigned values to commutable serum-based secondary RMs. ISO 15194 compliant serum-based RMs are currently available. A network of three calibration laboratories runs the harmonized apo(a) RMP. Equipped with a *state-of-the-art* calibration hierarchy for Lp(a) and using a 2-step approach, it is prime time for global Lp(a) standardization to ensure effective implementation of Lp(a) clinical guidelines and refined cardiovascular precision diagnostics.

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Chronicle of Lp(a) test standardization since its discovery

Lipoprotein(a) (Lp(a)), an LDL-like lipoprotein particle with a unique and highly heterogenous apolipoprotein(a), was originally discovered by the geneticist Kare Berg in 1962. Lp(a) is currently considered a highly relevant, genetically determined cardiovascular risk factor, linked to both atherosclerotic cardiovascular disease (ASCVD) and aortic stenosis [1]. The unique apolipoprotein (a) in Lp(a) has a plasminogen-like kringle IV domain formed by 12–50 kringles due to a variable number of KIV_2 repeats (3–40 copies). Beyond the KIV_2 size polymorphism, huge genetic variation is described [2] as well as high N- and O-glycosylation [3]. The impact of apo(a) heterogeneity on accuracy of Lp(a) test results runs like a red thread through this chronicle.

Five milestones and three major eras of Lp(a) test standardization can be identified in Figure 1. In the period since Lp(a) discovery till the turn of the millennium, local standards and assay dependent reference values were the norm and international standardization efforts were not yet in place. Initially, measurement procedures (MPs) were manual, often homemade, such as radial immunodiffusion (RID), rocket electrophoresis, radio-isotopic IRMA methods and ELISAs. Later on automation entered medical labs and immunonephelometric and immunoturbidimetric-based MPs were introduced on either batch or random access analyzers. MPs often used polyclonal anti-apo(a) antibodies that variably cross-reacted with KIV_2 repeats in apo(a), depending on the entire application design and platform used. The consequent impact of apo(a) isoform dependent tests on Lp(a) accuracy (shown in Figure 2) [4], led to inconsistent associations with clinical outcome. An absolute nadir were the absent associations between Lp(a) levels and the risk of future MI, stroke or peripheral vascular disease in three consecutive reports on the Physician Health Study [5–7]. Hence, after the original hype on investigating Lp(a)'s

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clinical utility, researchers lost interest in Lp(a) for more than a decade due to these "negative" outcome studies.

To unravel the inconsistent clinical findings, influential researchers decided to develop a higher order Reference Measurement System for Lp(a) to be assured about test standardization with resulting accuracy and comparability of Lp(a) test results across IVD-manufacturers. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), through its Working Group on Lipoprotein(a), together with research institutions and several diagnostic companies, succeeded in developing an ELISA-based reference measurement procedure (RMP) with monoclonal anti-apo(a) antibodies against unique epitopes and an international reference material that was intended for the transfer of the Lp(a) concentration to manufacturers' master calibrators [8–11]. The assigned unitage of 0.1071 nmol of lipoprotein(a) per vial SRM2B is traceable to the consensus reference method for lipoprotein(a) and enabled conformity by diagnostic companies to the European Union's Directive on *In-Vitro Diagnostic* Medical Devices for metrological traceability of calibrator materials. In combination with the ELISA-based RMP available at NWLRL, Seattle, Washington, USA a Reference Measurement System (RMS) was developed as *gold standard*, unaffected by apo(a) heterogeneity and KIV_2 size polymorphism and faulty assumptions regarding Lp(a) mass levels. Around the millennium turn calibration services were offered by the single calibration lab at NWLRL, led by Santica Marcovina. In essence, by performing split-sample comparisons, IVD-manufacturers were guided to (re)standardize their Lp(a) test to this higher order RMS.

Using the first established Lp(a) RMS, Rifai and collaborators reanalyzed the specimens from the Physician Health Study with the gold standard RMS from NWLRL and found that median Lp(a) in cases were significantly higher than in controls, whereas no significant differences were found

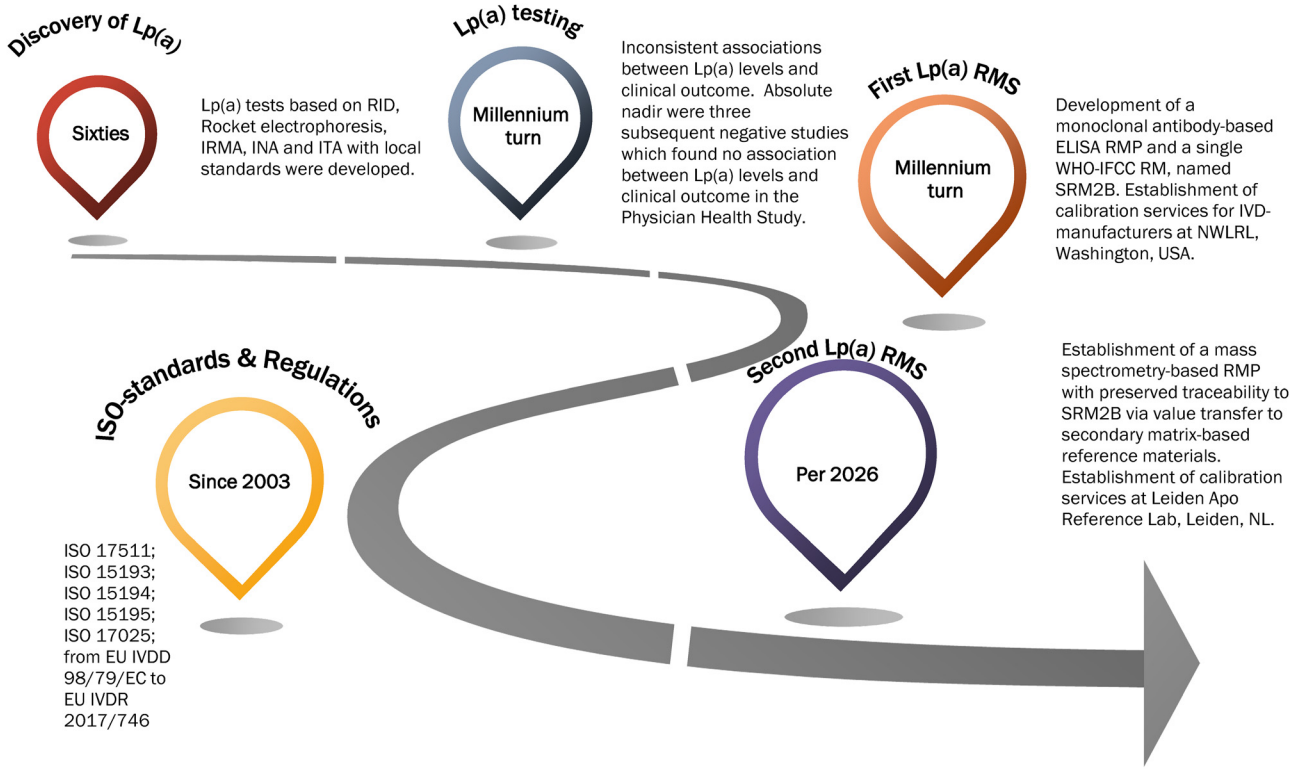


Figure 1: Infographic timeline with milestones in Lp(a) test standardization since its discovery. First, local standards were used for quantifying Lp(a) in serum/plasma. As the lab medicine and clinical communities were faced with inconsistent findings on the clinical utility of Lp(a), a first Reference Measurement System was developed by an IFCC Working Group on Lp(a) standardization led by professor Marcovina from NWLRL, Seattle, Washington, USA. The goal was to ensure accurate measurement of Lp(a) by addressing all the challenges of the KIV_2 isoform heterogeneity. Adoption by IVD-manufacturers was partial, likely due to the confusion on its clinical relevance. As it has become clear nowadays that there is an unequivocal causal relation with clinical outcome and because Lp(a) lowering medications are looming, Lp(a) testing is currently undergoing a *Renaissance* as it is understood that accurate Lp(a) test results are key for refined CVRM. Since 2017, another IFCC Working Group on Apolipoprotein Standardization by Mass Spectrometry has developed a successor Reference Measurement System which also allows concomitant apoA-I and B_{total} standardization among other apolipoproteins (the latter is not in the scope of this paper). CVRM: cardiovascular risk management; EU IVDD: European Union *In-Vitro* Diagnostic Directive; EU IVDR: European Union *In-Vitro* Diagnostic Regulation; RM: reference material; RMP: reference measurement procedure.

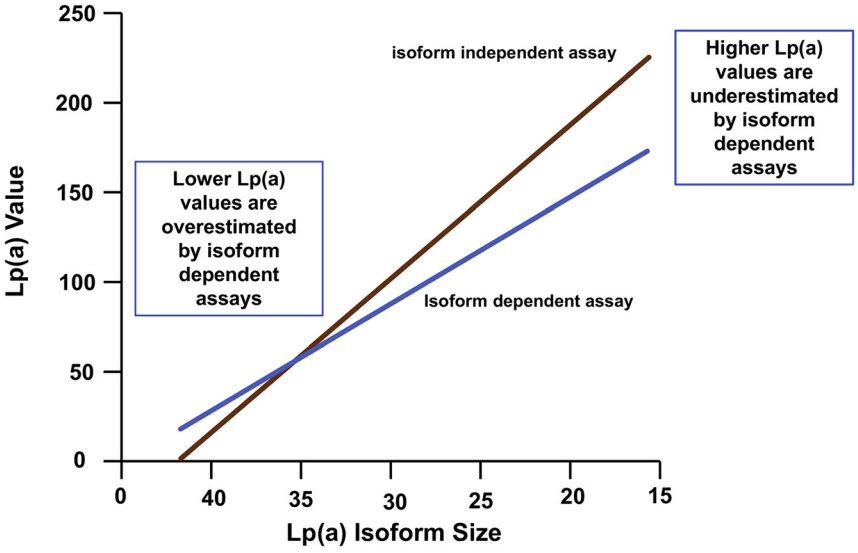


Figure 2: Theoretical relationship of Lp(a) values according to isoform size in isoform-independent and isoform-dependent assays in case of a 1-point calibration strategy. Lp(a) values are inversely related to apo(a) isoform size, with large isoforms being associated with lower levels and vice versa. Irrespective of the expression of Lp(a) values (nmol/L or mg/dL), isoform-dependent assays tend to overestimate low Lp(a) values and underestimate high Lp(a) values. Lp(a), lipoprotein(a). Source: Tsimikas et al. [4].

between cases and controls when using the commercial immunonephelometric assay [12]. Irrefutable proof revealed that Lp(a) test inaccuracy in the immunonephelometric assay had obscured the true relationship between Lp(a) levels and CVD. In Figure 2 the mitigating impact of apo(a) size polymorphism on Lp(a) levels is visualized in case of isoform-dependent Lp(a) tests using a one point calibration strategy [4]. The effect of apo(a) size polymorphism occurs irrespective of the use of mass or molar units.

Some *In-Vitro Diagnostic* (IVD)-manufacturers were early adopters and applied for Lp(a) certification by the NWLRL calibration lab. These IVD-manufacturers started making their Lp(a) test results traceable to the WHO-IFCC reference material SRM2B, including reporting in molar units. Other IVD-manufacturers continued manufacturing their existing Lp(a) immunoassays based on polyclonal antibodies that cross-reacted with apo(a) KIV_2 repeats and reporting in Lp(a) mass concentrations. As interest in Lp(a) testing faded into the background, adoption and implementation by IVD-manufacturers of this first generation RMS was only partially accomplished and non-equivalence and method dependency of Lp(a) results persisted until now, further bringing along inaccurate results that variably distort the associations of Lp(a) levels with clinical performance and outcomes. Note that also the calibrator choices and kit design, the limited measuring ranges and the read out choices on the analyzer platforms were insufficiently considering the impact of the apo(a) size polymorphism and particle size in specimens respectively calibrators on the Lp(a) recovery of the unknown samples [13].

Renewed interest in Lp(a) arose the last decade since epidemiological and genetic studies strongly supported a causal and direct, continuous association in all racial populations between serum Lp(a) levels and risk of ASCVD and aortic valve stenosis. Currently, Lp(a) is considered an independent genetic cardiovascular risk factor, mostly unaffected by lifestyle, and remaining stable over a lifetime. In the case of serum/plasma Lp(a) testing accurate Lp(a) levels are important for risk stratification as high Lp(a) levels double or triple the risk of heart attack or stroke [14]. Lp(a) testing is recommended for guiding clinical decisions by identifying patients who may benefit from more aggressive existing LDL-c lowering therapy even if LDL-c is normal, and for supporting decisions on PCSK9 inhibitors in high-risk individuals. Lp(a) testing is nowadays encouraged for first-degree relatives of individuals with elevated Lp(a), aiding early detection and prevention [15, 16]. Once-in-a-lifetime testing is also recommended by the USA National Lipid Association [17] and by European clinical guidelines [14–16] for all adults, especially important in case of premature ASCVD, familial hypercholesterolemia and in case of a strong family history of heart disease. Recently, the Safe Hearts Plan was launched in the EU, the first ever comprehensive EU approach to tackling this immense public health challenge (dfb60cde-21a5-426d-8616-e394a326abc2_en).

Moreover, interest in Lp(a) testing is substantially fueled due to the fact that pharma-companies have promising Lp(a)-lowering medications in their pipelines. Phase 2 and 3 clinical trials are ongoing and it is anticipated that the Lp(a)-lowering effects with genetically interfering drugs (ASOs, siRNAs) or muvalaplin are impressive and effective by preventing apo(a) protein expression or Lp(a) assemblage, respectively [18–20]. Consequently, Lp(a) is again in the center of both preventive and curative cardiovascular risk management, which explains why it arose as a phoenix from its ashes.

To accomplish global Lp(a) standardization, a 2nd IFCC working group was established in 2017 with a broader scope. The goal was to establish a multiplexed RMP, for continued global standardization of apo(a)/Lp(a) and other clinically relevant apolipoproteins such as apoA-I and $apoB_{total}$, and emerging apoCs, apoE and apoE phenotypes. Only apo(a)/Lp(a) standardization is covered in this manuscript. Focusing on Lp(a), a next generation ISO 15193 compliant [21], IFCC-endorsed RMP based on quantitative Mass Spectrometry (MS) has been developed. Traceability to SRM2B is unchanged and maintained using a value transfer protocol that assigned values to CLSI-C37 manufactured serum-based secondary RMs. These pooled serum-based RMs are proven commutable and ISO 15194 compliant [22], and currently available in the Leiden Apolipoprotein Reference Laboratory. A network of three harmonized calibration labs is running the apo(a) MS-based RMP, showing its transferability across the globe [23]. Equipped with a *state-of-the-art* calibration hierarchy for Lp(a) standardization, it is now prime time for its global implementation. Details on the stepwise approach and the deliverables of the next-generation Lp(a) RMS are presented in the below paragraph entitled: *From Lp(a) RMS establishment to implementation.*

Metrological traceability as a fundament for sustainable and global Lp(a) test standardization

Every era has its book and in the 21st century the metrological traceability concept was introduced in laboratory medicine. Science evolved and so did metrology, i.e. the science of measurement. The concept of metrological traceability of medical test results was for the first time described in ISO 17511:2003 and intended to make medical test results, reference intervals and clinical decision limits globally exchangeable. After the coming into place of the European IVD Directive (IVDD) 98/79/EC in 1998, metrological traceability was legally required. After all, the IVDD legislation required safe and effective medical test results, although still for a limited number of listed analytes. Its successor, the current EU IVDR 2017/746 is much more stringent and demands extensive evaluation and proof of clinical evidence of all medical tests, the amount and degree depending on the intended use and risk classification of the medical test.

As illustrated in Figure 3, test results produced by CE-marked and/or FDA-approved Lp(a) MPs should nowadays ideally be traceable to internationally recognized materials and methods of higher order, via an unbroken chain of methods and materials, up to the top of the traceability chain [24]. Different stakeholders are involved, each of them with a specific responsibility: at the top of the calibration hierarchy metrology institutes and/or designated reference institutes develop high(est) order primary RMPs and primary/secondary RMs; in the middle part, internationally endorsed calibration labs develop higher order secondary reference measurement procedures. In the lower part IVD-manufacturers develop *easy-to-use* MPs that produce test results traceable to the higher order RMPs/RMs, whereas end-users implement these tests in the medical laboratories and take care that the test performance of the selected MPs is *fit-for-clinical-purpose* in their target groups and setting, and for the envisioned intended use(s). To that end, Lp(a)/apo(a) test results should be traceable, within maximum allowable uncertainty (MAU), to predefined analytical performance specifications (APS) derived from e.g. biological variation data as conceptually described in the Milan consensus hierarchy [25].

The metrological traceability concept, originally described in ISO 17511:2003 and revised in ISO 17511:2020 [24], is nowadays required for establishing internationally endorsed RMSs for enabling global standardization of Lp(a) tests and guaranteed exchangeability of test results in time and space. To make this happen the soft IVDD 98/79/EC has been replaced by the stringent IVDR 2017/746 in all EU-member states. The IVDR came into force per May 2024 and all medical tests marketed in the European Union must currently be certified under the IVDR, which implicitly requires adequate test standardization.

For adoption and implementation of the traceability concept, timely sharing and periodically updating IVD-industrial partners and lab professionals on the progress of IFCC standardization projects is key.

A pilgrim's journey towards the mass spectrometry-based Lp(a) RMS

Under the auspices of the 2nd IFCC Working Group on Apolipoprotein Standardization the conceptual approach and rationale for developing a multiplex apolipoprotein RMSs was first described [26]. A phased approach was agreed upon. Priority was given to the development and evaluation of the candidate RMP (cRMP) for apo(a). To that end, a mass spectrometry (MS)-based cRMP for apo(a) was developed using quantitative bottom-up proteomics targeting 3 proteotypic apo(a) peptides. The method was provisionally validated according to ISO 15193 using a single human serum based calibrator traceable to the former WHO-IFCC RM [11].

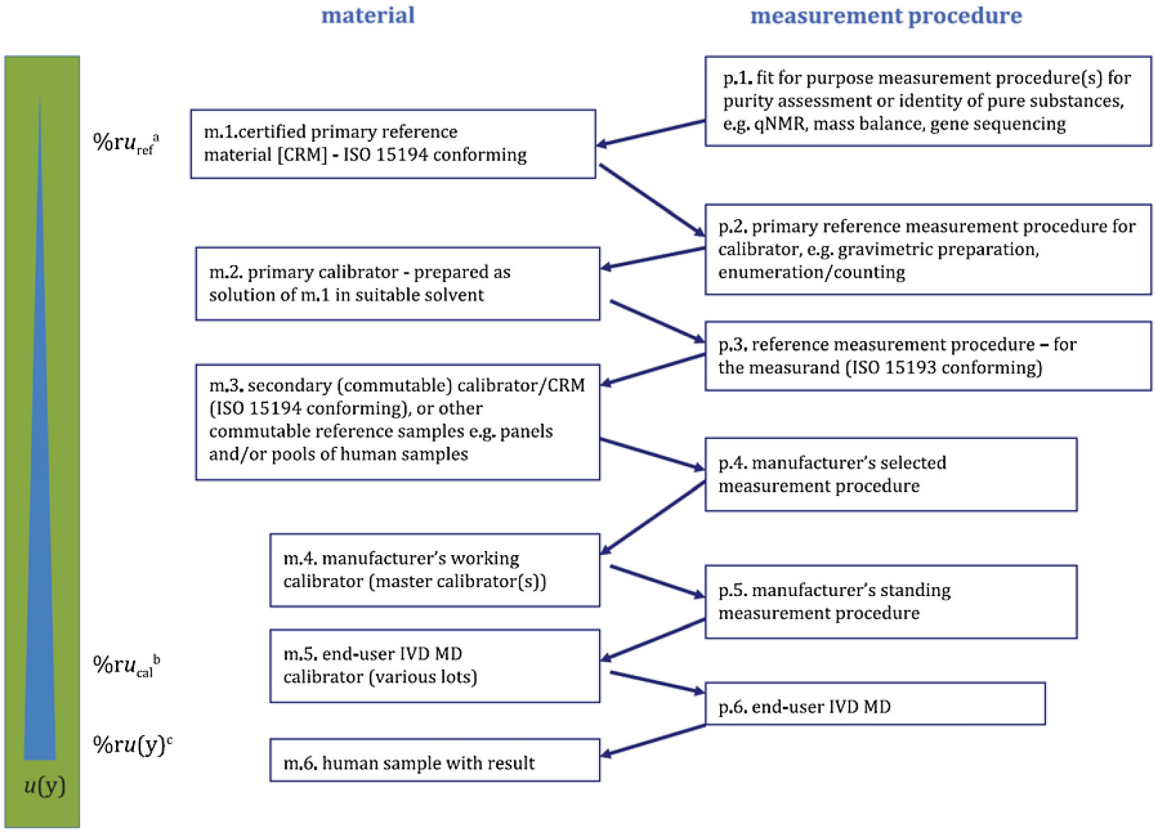


Figure 3: The most ideal and complete metrological traceability chain as described in ISO 17511:2020 [24].

The quantitation of serum apo(a) was by design independent of its size polymorphism, was linear from 3.8 to at least 456 nmol/L, and had a lower limit of quantitation for apo(a) of 3.8 nmol/L using peptide LFLEPTQADIALLK as the quantifying peptide. Interpeptide agreement showed Pearson Rs of 0.987 and 0.984 for peptides GISSTVTGR and TPENYPNAGLTR, and method comparison indicated good correspondence (slopes 0.977, 1.033, and 1.085 for LFLEPTQADIALLK, GISSTVTGR, and TPENYPNAGLTR). Average within-laboratory imprecision of the cRMP was 8.9 %, 11.9 % and 12.8 % for the 3 peptides. This robust, antibody-independent, MS-based cRMP was developed as higher order RMP and is now an essential part of the apo(a) traceability chain and current Lp(a) RMS [27].

The apo(a) cRMP described in 2023 was provisionally calibrated and end results were made traceable to SRM2B. Ideally, apo(a) primary, peptide-based calibrators (m.2. in Figure 3) should have been developed to establish the most complete calibration hierarchy for Lp(a) test standardization (Figure 3). Intense research was undertaken to find suitable primary, peptide-based apo(a) reference materials, but revealed suboptimal recoveries of spiked peptides, and was in the end considered as an unfeasible approach. Preliminary data with recombinant apo(a) from HEK392 cell line as primary RM is now considered as a good alternative but this approach will demand additional thorough research and resources. As the momentum of Lp(a) testing for refined CVRM and for prevention in all individuals is now, the IFCC WG on Apo standardization has decided to choose for preserving traceability to SRM2B in the coming years. To that end, value assignment of the serum-based secondary reference materials via a value transfer protocol from the leftover SRM2B RM stock is considered to be the most feasible solution during the coming years (Figure 4). Moreover, one of the calibration labs in the IFCC Working Group on Apolipoprotein Standardization established a more precise semi-automated version of the MS-based apo(a) RMP for envisioned Lp(a) test standardization [28].

In Figure 5 an overview of the major accomplishments of the 2nd IFCC WG on Apo Standardization is shown, including commutability [29] as well as the amendments made compared to the predefined steps in the apo(a) cRMP manuscript [27].

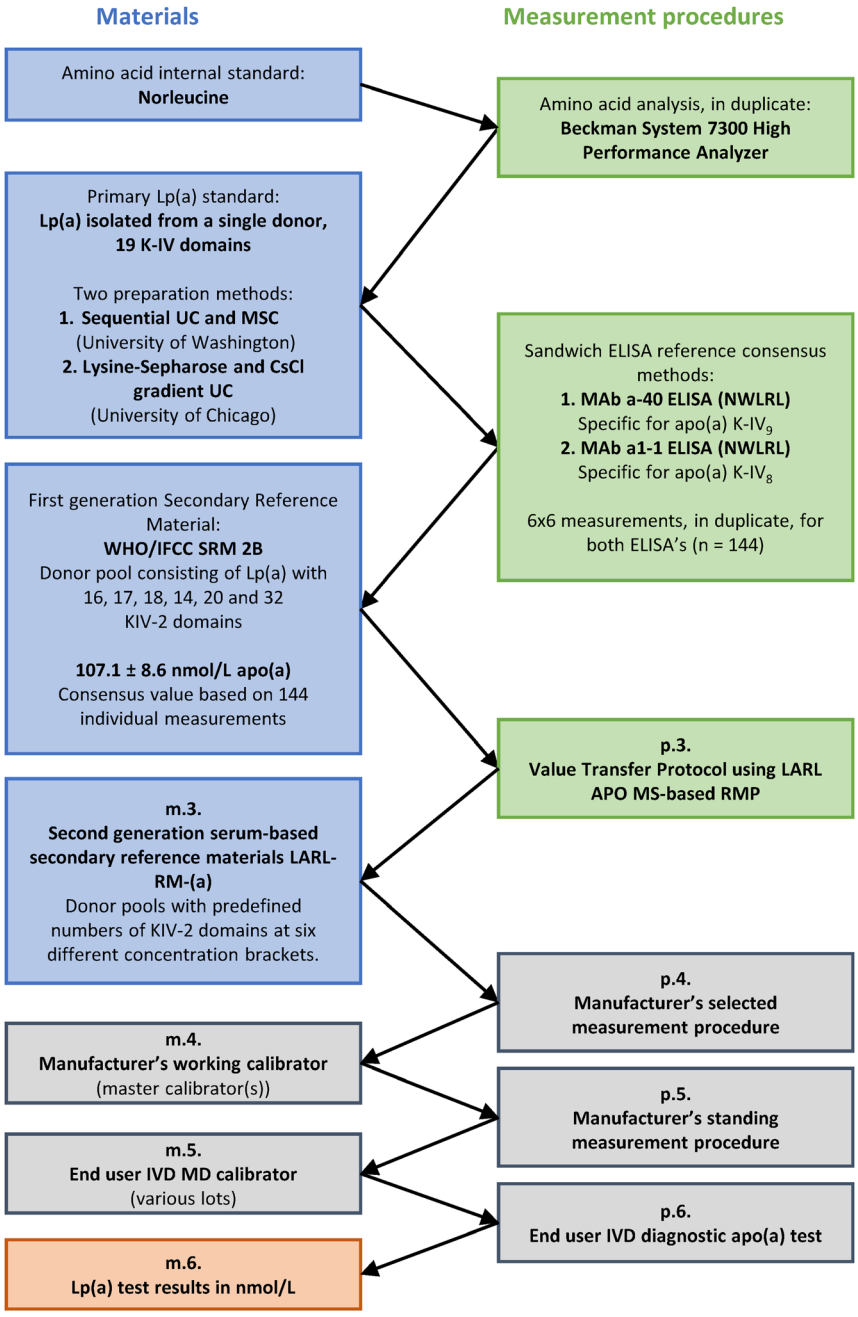


Figure 4: Metrological traceability chain of apo(a)/Lp(a) tests ensuring traceability to the former WHO-IFCC SRM2B reference material with similar absolute Lp(a) test values. The top of the traceability chain is deduced from the Lp(a) standardization publications of Marcovina and colleagues, whereas the mid part is established by the 2nd IFCC Working Group on Apo standardization who developed m.3. and p.3. With this calibration hierarchy Lp(a) test results from IVD-manufacturers (mid part) and end-users (bottom of the traceability chain) will remain SRM2B traceable. Using this approach alignment with the historical database and continuity of absolute values originating from the 1st standardization attempts of NWLRL will be preserved [35].

From Lp(a) RMS establishment to implementation

Conceptual approach to global Apo(a)/Lp(a) standardization in a changing regulatory landscape

Firstly, all stakeholders of the biomarker-to-medical test pipeline should realize that Lp(a)-guided health- and sick care occurs in a global world where data-exchange and comparability of test results have become essential and required by legislations such as the IVDR, the Artificial Intelligence Act and the European Health Data Space in the European Union (EU). Because of current legal requirements, medical test standardization should become inclusive, ideally at or shortly after test introduction on the EU-market. Consequently, a more ethical, holistic and visionary approach of test development and test standardization is needed as there is ongoing evolution in biomarker science, metrology, IVD-related legislation and therapeutics. Metrology has since decades a crucial rule in other sectors

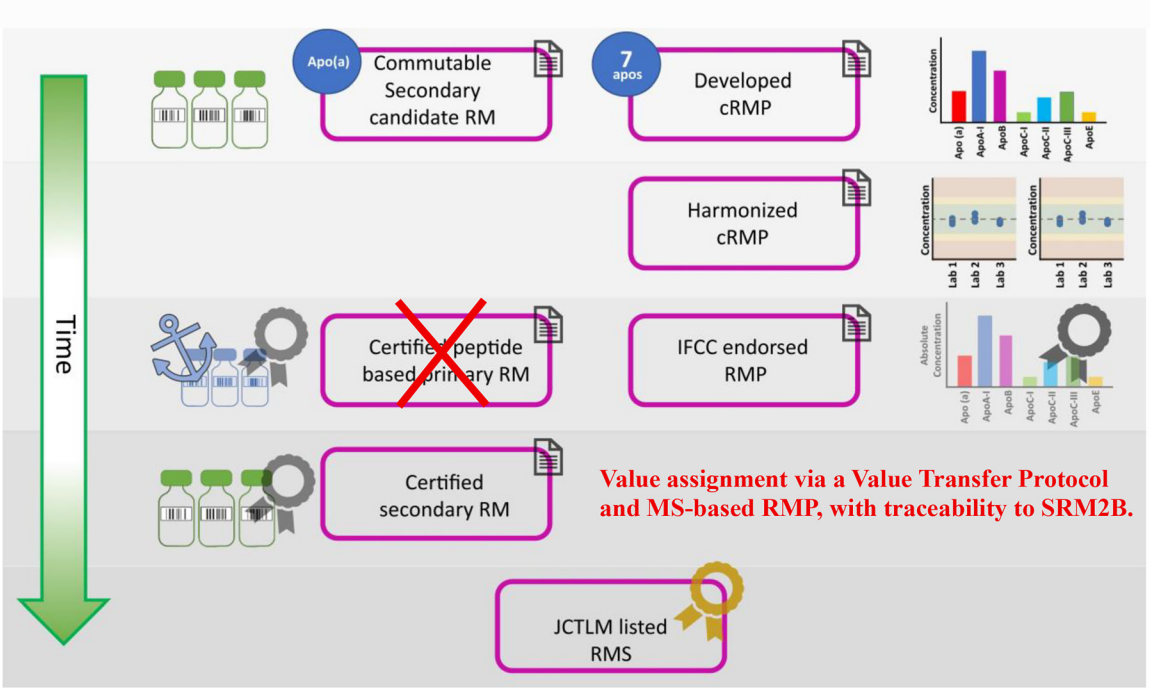


Figure 5: Overview of the original (purple rectangles) and alternative steps (marked in red) towards development of a RMS for apo(a)/Lp(a) standardization. Originally, the development of a candidate RMP (cRMP) for apo(a)/Lp(a) was described in a conceptual paper [26], whereas its validation was described by Ruhaak et al. [27]. Transferability of the cRMP, and a sufficient degree of harmonization among three calibration laboratories of the IFCC WG Apolipoproteins-MS, has been accomplished and the publication is under revision [23]. Secondary, matrix-based reference materials have been produced in compliance with ISO 15194. After intense research, establishing peptide-based primary RMs was considered not to be a feasible approach. The IFCC WG on Apo standardization decided to opt for value transfer from the remaining SRM2B RMs to the pooled serum-based secondary reference materials via a value transfer protocol. Commutability of the selected secondary serum-based reference materials was proven by Dikaios et al. [29]. An additional publication on the improved semi-automated apo(a) RMP has been submitted [28]. Compliance to ISO 15193; ISO 15194; ISO 15195 and ISO 17025 has been documented. Accreditation by the Dutch Board for Accreditation for global apo(a) standardization activities is underway. Document icons indicate accepted and submitted publications.

(such as the food, film, automotive, flower, chip, (bio)sensor and mobile phone industries, …) as it shapes modern societies. Nevertheless, its strategic relevance is only very recently appreciated in the healthcare sector, and since long put aside as minor or irrelevant even by key opinion leaders [30]. It also does not help that education on metrology is not yet embedded in curricula of medical and lab diagnostic professionals [31]. The former “false” negative outcome studies are showcases how clinical associations with myocardial infarction, stroke and peripheral arterial disease were masked due to flawed Lp(a) tests. It should be noted that correct implementation of higher order Lp(a) RMSs has been instrumental to disclose Lp(a)’s atherogenicity and thrombogenicity. It is the example from which lessons in metrology should be taken! Additionally, for realizing personalized precision diagnostics and tailored CVRM [32, 33] the required alignments between biomarker science, metrology, technology and IVD-related legislation are demonstrated in Figure 6. In this era of precision medicine and in order to keep EU-citizens longer healthy and sick care affordable and sustainable, IVD-manufacturers should adopt and implement the key principles of the metrological traceability concept early during test development.

Secondly, effective implementation of Lp(a) clinical guidelines demands multi-disciplinary collaboration across biomarker researchers, clinicians, IVD-industries, laboratory specialists, metrologists and methodologists. Also healthcare policy makers currently aim to reform the unsustainable and costly curative healthcare systems for non-communicable diseases in Western Societies –such as CVD– as well as the marginal improvements in absolute cardiovascular risk reduction without solving the substantial residual cardiovascular risk ([34]; dfb60cde-21a5-426d-8616-e394a326abc2_en). It is prime time for Personalized Precision Medicine (PPM) and Preventive, Predictive, Personalized, Participatory healthcare systems, that are patient centric and clinically and cost-effective in all EU-citizens. This can only be accomplished if we break down silos between stakeholders involved in biomarker discovery, and medical test and drug development and if we

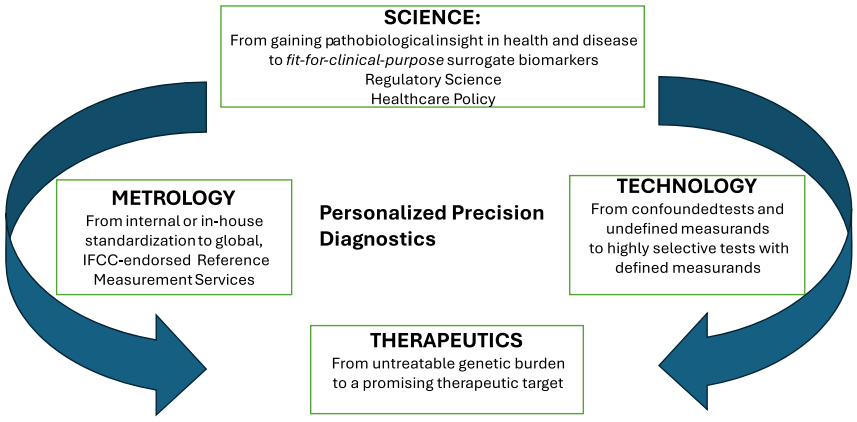


Figure 6: Parallel evolution in science, metrology, technology and therapeutics is revolutionizing Lp(a) management. Multidisciplinary collaborations between Lp(a) biomarker scientists, technology developers, healthcare policy officers, IVD-legislators and pharma-industries are key enablers for realizing the full potential of personalized precision cardiovascular diagnostics, including Lp(a) management.

accomplish global standardization and exchangeability of test results, to realize the full potential of evolving biomarker science, metrology, technology and therapeutics. By changing our thoughts and focusing on strategic standardization management, the lab diagnostic sector will transition more successfully to personalized risk stratifications, diagnoses, treatments of sick patients and to preventive public health management.

Practical 2-step approach

The potential of a serum panel, value assigned with the 2nd generation IFCC-endorsed mass spectrometry-based RMP, to harmonize Lp(a) measurements from different immunoassays has recently been demonstrated by Miida and colleagues [35]. The successful Japanese Lp(a) harmonization study is considered to be an important first milestone, demonstrating the feasibility and potential of Lp(a) test kit standardization across IVD-manufacturers, notwithstanding the existence of sample specific scatter.

Consequently, trueness verifiers and a certification protocol are established by the Leiden Apolipoprotein Reference Laboratory (LARL), to give guidance to IVD-companies by evaluating their Lp(a) test accuracy. To accomplish this, certification sets holding 40 single donor, CLSI C37-A prepared serum samples that cover the measuring range of commercial immunoassays, were value assigned and made traceable to SRM2B. Equivalence of Lp(a) data in the measuring range between commercial immunoassays and the MS-based RMP can be evaluated against predefined analytical performance criteria described in the certification protocol (Table 1). IVD-companies applying for Lp(a) certification services will get insight into their current accuracy. Before the start of the comparison study at the manufacturer's site, the IVD-company gets information on the certification process. Moreover, the IVD-company needs to ensure platform/application readiness and has to demonstrate ahead preparational requirements as outlined below. I.e., before the start of the certification study, IVD-companies must document that their analytical system meets at minimum the following requirements:

- The instrument must be capable of producing discrete number values.
- Precision testing should have been performed at relevant concentration levels.
- Maintenance must have passed required preventive maintenance procedures and must be in optimal condition to perform the trueness verification analyses.
- Reportable range should be known and reported.

A special LARL form has to be completed ahead and describes the preparational requirements needed to ensure adequate system performance. After analyzing the samples according to the instructions in the certification protocol on the manufacturers' platforms with specific CE-marked or (C) FDA-approved applications, the data as well as the IQC- and calibrator data have to be sent to the LARL office. Reporting is accomplished by means of a Document of Comparison. Opportunities are given to discuss the findings virtually or live.

To accomplish (re)standardization of biased commercial Lp(a) tests, sufficient amounts of calibrators have been developed at six Lp(a) levels within and above the measuring range of immunoassays. To that end donor sera from prescreened, selected and cleared donors with Lp(a) levels in predefined bins were pooled to have sufficient amount of matrix-based calibrators for the coming years [36]. The calibrators are value assigned with the IFCC-endorsed MS-based RMP and will become available after accreditation and recognition of the LARL as international calibration lab.

Note that EQA-organizers who provide proven commutable serum samples to their participating medical labs can ask LARL to value assign their EQA-materials.

Table 1: Analytical performance criteria used to demonstrate equivalence between commercial Lp(a) test results and Lp(a) RMS results [28].

Apolipoprotein	Parameter	Criterion	Statistical approach
Apolipoprotein(a)	*r*	>0.975	Pearson's correlation
	Slope	0.95–1.05	Passing-Bablok regression
	Median % bias below dilution cut-off	<5 %	Mathematical median of biases
	Median % CV	Concentration-dependent; based on Van Neer et al. [28] below 30.5 $x^{-0.31}$, where x is the concentration of the sample	CV of QC results
	Within-method outlier	1	Grubb's test for outliers

Certificates will be reported, with assigned values and expanded measurement uncertainties, to be used as target values with MAU in their accuracy-based EQA-surveys.

Future perspectives

The future of Lp(a) testing is bright and the challenges related to its apo(a) size polymorphism are better understood and to a reasonable extent addressed in most immunoassays [4, 13]. However, potential impact of other apo(a) heterogeneity on test accuracy related to genetic variation and the high degree of post-translational modifications (N- and O-glycosylation) are not yet unraveled [2, 3].

A 2nd generation IFCC-endorsed mass spectrometry-based RMS is in place for the roll out of global Lp(a) standardization. The objectives of the IFCC SD working group on Apo standardization are currently shifting from establishing an Lp(a) RMS to adoption and implementation of global Lp(a) standardization. To make this happen, interested (IFCC) corporate members and third parties will be invited to take part in this endeavor. Creating awareness and educating lab professionals, clinicians, and IVD- and pharma-industry on the clinical relevance and challenging metrology aspects of Lp(a) testing is key for ensuring adequate personalized CVRM. It is anticipated that the (re) standardization process will run smoothly because traceability to the former SRM2B is maintained, as well as the recommendation to express Lp(a) particle number in nmol/L. Only the Lp(a) RMP is renewed and has been demonstrated to produce equivalent results with immunoassays within MAU. For IVD-manufacturers who already claim traceability to SRM2B and express Lp(a) levels in molar units in their *Instructions For Use*, the claims on metrological traceability of Lp(a) test results in the product inserts will hardly change. Regulators are advised to consider the change in traceability to the MS-based RMP as natural evolution of higher order methods in specialized calibration labs and should not burden individual IVD-manufacturers by asking evidence that is already generated by IFCC endorsed calibration laboratories. However, IVD-companies who do not yet have traceability of Lp(a) test results to SRM2B and/or do not report in molar units, will likely be confronted with regulators (FDA in the USA, Chinese FDA in China and Notified Bodies in the EU) that consider the (re)standardized molar Lp(a) test as a new test. To conclude, the Leiden Apolipoprotein Reference Laboratory is ready to guide IVD-manufacturers in their Lp(a) (re)standardization process, for ensuring exchangeable and accurate Lp(a) test results with effective implementation of Lp(a) clinical guidelines and personalized cardiovascular precision diagnostics.

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