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Commutability Assessment of Candidate Reference Materials for Lipoprotein(a) by Comparison of a MS-based Candidate Reference Measurement Procedure with Immunoassays

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BACKGROUND: Elevated concentrations of lipoprotein(a) [Lp(a)] are directly related to an increased risk of cardiovascular diseases, making it a relevant biomarker for clinical risk assessment. However, the lack of global standardization of current Lp(a) measurement procedures (MPs) leads to inconsistent patient care. The International Federation for Clinical Chemistry and Laboratory Medicine working group on quantitating apolipoproteins by mass spectrometry (MS) aims to develop a next-generation SI (International system of units)-traceable reference measurement system consisting of a MS-based, peptide-calibrated reference measurement procedure (RMP) and secondary serum-based reference materials (RMs) certified for their apolipoprotein(a) [apo(a)] content. To reach measurement standardization through this new measurement system, 2 essential requirements need to be fulfilled: a sufficient correlation among the MPs and appropriate commutability of future serum-based RMs.

METHODS: The correlation among the candidate RMP (cRMP) and immunoassay-based MPs was assessed by measuring a panel of 39 clinical samples (CS). In addition, the commutability of 14 different candidate RMs was investigated.

RESULTS: Results of the immunoassay-based MPs and the cRMPs demonstrated good linear correlations for the CS but some significant sample-specific differences were also observed. The results of the commutability study show that RMs based on unspiked human serum pools can be commutable with CS, whereas human pools spiked with recombinant apo(a) show different behavior compared to CS.

CONCLUSIONS: The results of this study show that unspiked human serum pools are the preferred candidate secondary RMs in the future SI-traceable Lp(a) Reference Measurement System.

Introduction

Lipoprotein(a) is an apolipoprotein B-containing lipoprotein composed of a low-density lipoprotein-like

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particle and a unique glycoprotein called apolipoprotein(a) [apo(a)]. Apo(a) is known for its size polymorphisms caused by a variable number of repetitive domains called kringles, which are highly homologous to kringle 4 (K-IV) of plasminogen (1). In humans, >30 genetically determined apo(a) size isoforms exist, in which the number of K-IV type 2 (K-IV_2) repeats varies from 2 to >40 and the molecular weight ranges from approximately 300 to >800 kD (2). In addition to this remarkable size polymorphism, serum concentrations of Lp(a) are highly variable among individuals ranging from <0.25 nmol/L to >720 nmol/L (<0.1 mg/dL to >300 mg/dL).

Elevated concentrations of Lp(a) are directly related to an increased risk of cardiovascular diseases, making its determination a relevant factor for clinical risk assessment. Lp(a) levels in serum are considered to be mainly genetically determined (3). There is a pronounced inverse correlation between the number of K-IV_2 repeats and the Lp(a) concentration, meaning that individuals with small apo(a) isoforms have higher average serum Lp(a) levels compared to individuals with large isoforms (1). However, there is a wide variability in Lp(a) concentrations within each apo(a) isoform group (4, 5).

Measuring accurate Lp(a) molar concentrations with immunoassay-based measurement procedures (MPs) has proved to be difficult due to the K-IV_2 repeats (6). Most commercial immunoassays use polyclonal antibodies that mainly target the K-IV_2 repeats (7). These antibodies may bind the apo(a) protein more than once and disturb correct measurements in molar terms. Consequently, these methods might have measurement bias leading to an underestimation of the concentration for small isoforms, usually associated with high levels, and an overestimation for large isoforms, usually associated with low levels. Several assay manufacturers try to mitigate this problem of apo(a) isoform–sensitive assays (7) by using multiple independent calibrators with different Lp(a) concentrations (8, 9).

In 2003, a first attempt to harmonize Lp(a) measurements was made by producing the WHO-International Federation for Clinical Chemistry and Laboratory Medicine (IFCC) standard SRM2B, which consisted of a lyophilized pool of human sera with various apo(a) isoforms. This standard was value-assigned with a non-kringle-dependent immunoassay, which is now obsolete (10, 11). In addition, there is insufficient information regarding the commutability of SRM2B and the current stocks are almost exhausted. Despite the availability of this standard, substantial biases (−8 to 22%) have been reported among 6 commercial immunoassays in 2019 (12) and similar results were seen in a Dutch EQA survey in 2018 (6). The harmonization process based on one MP available in only one laboratory was very fragile and is no longer available for worldwide standardization (6, 13).

The IFCC working group on the standardization of apolipoproteins by mass spectrometry (MS) aims to establish a next-generation reference measurement system with SI-traceability, i.e., the highest order of metrological traceability in ISO 17511:2020 (14), for a panel of clinically relevant serum apolipoproteins, including apo(a) (15). The system is being developed along 2 lines: (*a*) the establishment of a multiplex reference measurement procedure (RMP) based on isotope dilution-liquid chromatography-MS (ID-LC-MS) and calibrated with a peptide-based primary RM (16) and (*b*) the development of secondary serum-based reference materials (RMs) certified for their molar concentration of apo(a) with the RMP. The secondary RMs are intended for calibration of the commercial immunoassays and commutability is an essential requirement (17).

In this study, the candidate RMPs (cRMPs) developed by the IFCC working group and 8 immunoassay-based MPs were compared by measuring 39 frozen clinical samples (CS). In addition, a preliminary commutability study was conducted on 14 pilot batches of candidate RMs to identify the most suitable type of material for the future production of a commutable certified RM.

Materials and Methods

MATERIALS: CLINICAL SAMPLES AND CANDIDATE RMS

More details on the selection and preparation of the materials can be found in Part A.1 in the online Data Supplement.

Thirty-nine CS from individual consenting donors were used in this study. The samples were gained, processed, and verified in a way that is ethically and legally compliant for the purposes of diagnostic research and development, production, and quality assurance. Serum samples were prepared according to an updated version of the CLSI protocol C-37 (18), aliquoted in glass vials or polypropylene tubes, and frozen directly after production. Samples were stored at −70°C to −80°C. The effect of one freeze–thaw cycle on the measurement results obtained with immunoassay-based methods was investigated before the start of this study and no significant effect was identified (see Supplementary Data Part A.2). The CS were preselected based on their Lp(a) concentrations (according to an immunoassay-based MP): 18 CS with <50 nmol/L, 7 CS within 50–100 nmol/L, 6 within 100–150 nmol/L, and 8 CS with >150 nmol/L. There was no preselection of the CS based on their apo(a) isoforms or the disease status of the donors.

Fourteen candidate RMs were tested in this commutability study and their specifications are provided in Table 1. Seven candidate RMs (RM1–7) were

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Table 1. Specifications for the 14 candidate RMs measured in the commutability study.

Candidate RM	Provider	Origin	Matrix
RM1	Solomon Park Research laboratories	10 human donors also included in CS	Human serum pool
RM2	Solomon Park Research laboratories	10 human donors also included in CS	Human serum pool
RM3	Solomon Park Research laboratories	10 human donors also included in CS	Human serum pool
RM4	Solomon Park Research laboratories	13 human donors independent from CS	Human serum pool
RM5	Solomon Park Research laboratories	13 human donors independent from CS	Human serum pool
RM6	Solomon Park Research laboratories	10 human donors independent from CS	Human serum pool
RM7	Solomon Park Research laboratories	20 human donors independent from CS	Human serum pool
RM8	JRC EU	Recombinant human apo(a) isoform with 14 K-IV, expressed in pigs	Regular pig serum
RM9	JRC EU	Recombinant human apo(a) isoform with 14 K-IV expressed in pigs, purified	Human serum with native Lp(a) < 3 nmol/L
RM10	JRC EU, INSERM	Recombinant human apo(a) isoform with 13 K-IV expressed in HEK 293	Human serum with native Lp(a) <3 nmol/L
RM11	JRC EU, INSERM	Recombinant human apo(a) isoform with 17 K-IV expressed in HEK 293	Human serum with native Lp(a) <3 nmol/L
RM12	JRC EU, INSERM	Recombinant human apo(a) isoform with 21 K-IV expressed in HEK 293	Human serum with native Lp(a) <3 nmol/L
RM13	JRC EU, INSERM	Recombinant human apo(a) isoform with 29 K-IV expressed in HEK 293	Human serum with native Lp(a) <3 nmol/L
RM14	JRC EU, INSERM	Recombinant human apo(a) isoform with 33 K-IV expressed in HEK 293	Human serum with native Lp(a) <3 nmol/L

Additional information can be found in Supplemental Data Part A.

nonspiked human serum pools. RM1–3 were prepared by pooling 10 serum samples with similar Lp(a) levels and the pooled samples originated from the same donors as the CS. RM4–7 were prepared for the CDC Clinical Standardization Program and the donors were different from those of the CS.

RM8–14 were based on recombinant human apo(a) [r-apo(a)] isoforms with different K-IV_2 repeats spiked in a serum background. Two types of r-apo(a) were included. The first type, used for RM8–9, was r-apo(a) material expressed in transgenic (tg) pigs provided by Kagoshima University, Japan (19). The second type, used for RM10–14, was r-apo(a) isoforms with specific numbers of K-IV_2 repeats provided by INSERM U1140 (Paris, France). The plasmids were obtained by standard cloning methods as described (20) and stably transfected into the human embryonic kidney cell line (HEK) 293 (21). The expressed r-apo(a) isoforms consisted of a specified number of K-IV units (see Table 1), one K-V unit, and the protease domain. The sequence of 10 N-terminal amino acid residues of the purified r-apo(a) was similar to the deduced N-terminal sequence of human apo(a) (22).

Apo(a) phenotyping was performed on all 39 CS and the candidate RM4–7 by western blot as described in (23). Technical details and results are shown the Supplemental Data Part A.3.

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LP(A) CONCENTRATION MEASUREMENTS

All CS and the 14 different RMs were measured with 8 immunoassay-based MPs and the cRMP to determine Lp(a) concentration.

The cRMP (developed by 3 calibration laboratories: LUMC, Leiden, The Netherlands; Leipzig, Germany and CDC in Atlanta, GA, USA), is an ID-LC-MS method in which apolipoproteins are subject to proteolytic digestion into peptides and then signature peptides are quantified as surrogates of the protein. In the case of apo(a), 3 peptides (LFLEPTQADIALLK, GISSTTVTGR, and TPENYPNAGLTR) were selected for the K-IV$_2$-independent quantitation of apo(a) (16). The peptides are located outside the K-IV$_2$ repeats and therefore present only once in all apo(a) isoforms. The presence of potential endemic genetic variants was also assessed. In the case of the peptide TPENYPNAGLTR, a genetic variant (rs62621433, ENSP00000395608.2:p.Thr346Ser in Ensembl.org release 100) was observed in several African populations of the 1000 Genomes project with a maximum minor allele frequency of 6% (24). During this study, a single point calibrator was used to estimate apo(a) molar concentration using a transfer calibrator with an assigned value traceable to WHO-IFCC SRM2B.

Eight immunoassay-based MPs frequently used in clinical laboratories were selected for this study (Table 2). The 7 turbidimetric methods used a multipoint calibration curve with 5 different calibrator solutions. The calibration of the nephelometric method was based on one standard that is automatically diluted by the analyzer providing 5 calibration solutions. Three MPs provided results in nmol/L and their results are traceable to SRM2B. The other 5 MPs had results in mg/dL and these values were traceable to the manufacturer's own standards. The Roche Lp(a) method was performed by 2 laboratories using different reagent lots and analyzers but the same lot of calibrator solutions. In the case of the Diasys Lp(a) method and the Sentinel Lp(a) Ultra method, samples were measured by 2 laboratories using different reagents lots and analyzers and the same calibrator lots; however, one reported result in nmol/L and the other in mg/dL.

The measurement ranges of the 8 immunoassay-based MPs do not cover the whole range of Lp(a) concentrations present in CS. Laboratories were therefore instructed to pre-dilute 3 CS with high Lp(a) concentrations in the human serum pool with a negligible Lp(a) level (<3 nmol/L) instead of using the option of automated dilution with a buffer (e.g., NaCl, see Supplementary Data Part A).

EXPERIMENTAL DESIGN

Ten sets of test materials (each containing 39 CS and 14 candidate RMs) were shipped on dry ice to the participating laboratories. All laboratories followed the same study protocol clearly describing the sample handling and the measurement order.

For each set, 243 measurements (3 replicates for CS and 9 replicates for candidate RMs) were performed. The measurements were completed in one day or spread over 3 days. The CS were measured in 3 sequential adjacent replicates and the measurement order of the CS was randomly assigned regarding the Lp(a) concentration. Each candidate RM was measured in 3 groups of 3 sequential adjacent replicates and these 3 groups were evenly spread over the measurement series.

DATA ANALYSIS

The study organizer (Joint Research Center, JRC) performed the statistical data analysis using Microsoft Excel.

Initial evaluation of results: measurement range and precision. Samples with measurement results outside the measurement range of a specific MP were excluded from further evaluations. The 9 MPs have different measurement ranges so the number of samples retained was variable (ranging from 24–39 for the CS and from 8–14 for the candidate RM, Supplemental Table B.1). Precision plots were made to detect potential outliers possibly caused by technical errors and to evaluate the relationship between standard deviation (SD) and concentration. No outliers were detected and there was a proportional relationship between the absolute SD and concentration for all methods, while the relative SD seemed to be constant over the measurement range.

Method comparison. Due to the size polymorphism of apo(a), results expressed in mg/dL cannot be correctly converted into nmol/L; the 2 method groups were therefore kept separate. Data from the 3 immunoassay-based MPs measuring in nmol/L were directly compared to the results obtained with the cRMP using each of the 3 apo(a) peptides. In addition, the remaining 5 methods measuring in mg/dL or g/L were compared to each other.

For each pairwise method comparison, the best fitting linear regression was obtained with the nonparametric method of Passing and Bablok and the Pearson correlation coefficient R was calculated. The presence of sample-specific differences was evaluated using the statistical analysis described in the recommendations from the IFCC WG on Commutability in Metrological Traceability (25) (see Supplemental Data Part A.4). The SD caused by sample-specific differences (i.e., s_{ssd}) was calculated as an indication of the width of the scatter among the CS excluding the effect of the method repeatability. The possible contribution of apo(a) size

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Table 2. The cRMP and immunoassay-based MPs used in the commutability study for the measurement of serum samples and candidate RMs.

Reporting units	Assay name	Reagent kit and calibrators used	Calibration traceability	Instrument/platform	Method	Measurement range[a]	Laboratory
nmol/L	cRMP	Detailed description in (16)	SRM2B[b]	Agilent 1290 Infinity LC, 6495 triple quadrupole	ID-LC-MS with MRM	1–400	1
	Roche Lp(a) Tina-Quant	Kit: Tina-quant Lipoprotein (a) Gen.2 Calibrators: Preciset Lp(a) Gen.2	SRM2B	Cobas C 501 Cobas C 502	turbidimetry	7–240	1, 2
	Sentinel Lp(a) Ultra	Kit: Lp(a) Ultra (11504D) Calibrators: Lp(a) Cal Set (11522D) (nmol/L values)	SRM2B	Beckman Olympus AU680	turbidimetry	7–240	3
	Diasys Lp(a) 21FS	Kit: Lp(a) 21 FS Calibrators: TruCal Lp(a) 21 (nmol/L values)	SRM2B	Beckman Olympus AU680	turbidimetry	7–240	3
mg/dL	Diasys Lp(a) 21FS	Kit: Lp(a) 21 FS Calibrators: TruCal Lp(a) 21 (mg/dL values)	Manufacturer's internal standard	BioMajesty JCA-BM6010/C	turbidimetry	>3	4
	Abbott Alinity c Lp(a)	Kit: Abbott Alinity c Lp(a) Calibrators: Alinity c Lp(a) STD CAL (01R1401)	Manufacturer's internal standard	Alinity (FA Abbott)	turbidimetry	>3–90	5
	Sentinel Lp(a) Ultra	Kit: Lp(a) Ultra (11504D) Calibrators: Lp(a) Cal Set (11522D) (mg/dL values)	Manufacturer's internal standard	Dimension Vista DV330397	turbidimetry	6–90	6
	Siemens Healthineers Atellica CH	Kit: Atellica CH Lipoprotein(a) [Lp(a)] Calibrators: Lipoprotein(a) Calibrator [Lp(a) CAL]	Manufacturer's internal standard	Atellica CH-930 PP-21	turbidimetry	10–85	6
	Siemens Healthineers N Latex Lp(a)[c]	Kit: N Latex Lp(a) Reagent Calibrator: N Lp(a) Standard SY	Manufacturer's internal standard	BN II 174064	Nephelometry	10–100	6

MRM: multiple reaction monitoring.
[a]The measurement ranges are without pre-dilutions; several methods have the option of automatic dilutions (e.g., with NaCl) and therefore measurement range can be extended to higher levels when applied in clinical laboratories.
[b]Single point calibrator with an assigned value traceable to WHO/IFCC SRM2B.
[c]Results were reported in g/L and recalculated to mg/dL by applying a conversion factor of 100.

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Table 3. Correlation between immunoassay-based MPs reported in nmol/L and candidate RMPs.

Pearson *R* Equation Passing–Bablok fit Sample-specific effects (confidence level) s_{ssd}	Roche Tina-Quant	Sentinel Lp(a) ltra	Diasys Lp(a) 21FS
cRMP (LFLEPTQADIALLK)	0.991	0.993	0.993
	$y = 1.15x - 1.90$[a]	**$y = 1.16x - 2.97$**	**$y = 1.13x - 1.56$**
	Yes (99)[b]	*Yes (99)*	*Yes (99)*
	0.08	*0.09*	*0.08*
cRMP (GISSTTVTGR)	0.991	0.993	0.995
	$y = 1.07x - 0.68$	**$y = 1.06x - 0.90$**	**$y = 1.02x - 0.09$**
	Yes (99)	*Yes (99)*	*Yes (99)*
	0.07	*0.07*	*0.05*
cRMP (TPENYPNAGLTR)	0.968	0.972	0.973
	$y = 1.10x + 0.46$	**$y = 1.11x - 0.12$**	**$y = 1.23x + 1.08$**
	Yes (99)	*Yes (99)*	*Yes (99)*
	0.30	*0.35*	*0.34*

This evaluation was based on the results of 33 CS measured in 3 replicates with each measurement procedure. For acceptable correlation, the correlation coefficient should be >0.975.
The text in regular format corresponds to the correlation coefficient Pearson *R*.
[a]The equation of the linear regression line according the Passing–Bablok analysis is written in bold text.
[b]The text in italics indicates whether significant by sample-specific differences between the CS were detected and s_{ssd} is the SD of the sample-specific differences.

polymorphism to the observed sample-specific differences was also investigated (Supplemental Data Part B.4).

Commutability assessment of the candidate RM. For the commutability assessment of the candidate RM, only the results of the cRMP peptides LFLEPTQADIALLK and GISSTTVTGR were used, while TPENYPNAGLTR results were not used as the correlation with the immunoassay-based MP was less good. The statistical analysis was based on the difference in bias approach (25) with ln-transformed concentrations to obtain a constant bias over the whole measurement range. The commutability criterion was set at 15%, which corresponds to approximately twice the largest expanded uncertainty associated with the difference in bias in this study.

Results

CORRELATION BETWEEN CRMP AND IMMUNOASSAY-BASED MP REPORTED IN NMOL/L

Lp(a) concentrations measured in the CS using the 3 peptides in the cRMP were compared to the results obtained with the 3 immunoassay-based MPs reported in nmol/L units (in total 9 pairwise comparisons). The correlation between the results obtained with the cRMP and the immunoassay-based MP was very good for the peptides LFLEPTQADIALLK and GISSTTVTGR (Pearson *R*: 0.991–0.995) (Table 3). Significant (at 99% confidence) sample-specific differences were observed for each method comparison and the SD associated with the sample-specific differences (indicated as s_{ssd}) ranged from 0.05 to 0.09. Comparison of the results obtained with the peptide TPENYPNAGLTR and the 3 immunoassay-based MPs showed weaker correlations (Pearson *R*: 0.968–0.973) and larger sample-specific differences (s_{ssd}: 0.30–0.34). As peptide TPENYPNAGLTR is prone to genetic variation in the African population, it is possible that this deviation is caused by this or similar mutations (16). Omission of one CS with clearly outlying results improved the Pearson *R* (>0.986) and reduced s_{ssd} (<0.18).

The Passing–Bablok linear regression analysis of the results of the immunoassay-based MP and the cRMP using the peptides LFLEPTQADIALLK and GISSTTVTGR, showed slopes that ranged from 1.02 to 1.16 (Table 3 and Fig. 1). For 5 of the 6 comparisons, these slopes were significantly (95% confidence) different from one indicating the presence of a small relative bias.

As mentioned before, the results of the cRMP are sensitive to the number of K-IV_2 repeats in the apo(a) isoforms, while the immunoassay-based MP could be apo(a) isoform–sensitive. The sample-specific differences observed in the method comparison between the cRMP and the immunoassay-based MP could therefore

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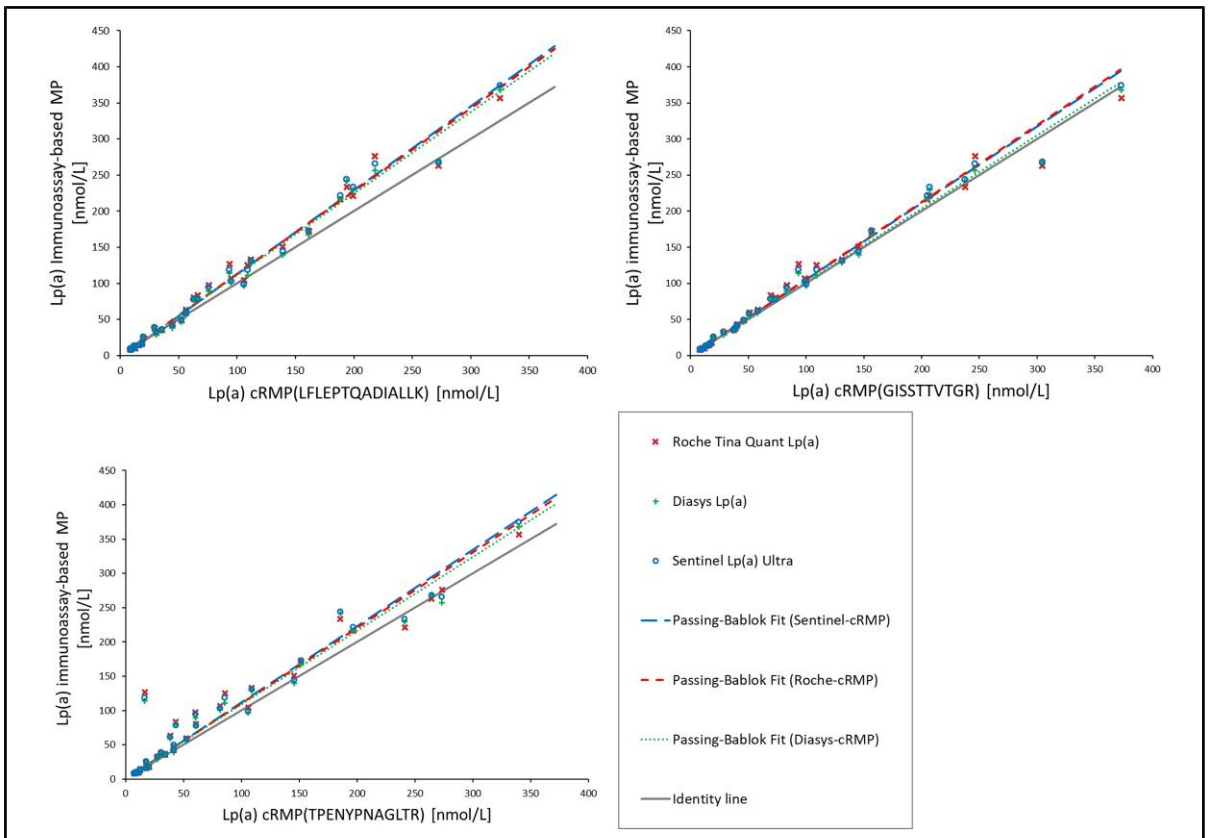


Fig. 1. Correlation plots between candidate RMPs (cRMPs) and immunoassay-based MPs reported in nmol/L. Correlation is based on the results for 33 CS (average of 3 replicate measurements). The results of the 3 immunoassay-based MPs are plotted on the *y*-axis while the results obtained with the cRMP for one of the non-kringle specific peptides is plotted on the *x*-axis.

be due to the presence of different apo(a) isoforms in the CS. This hypothesis was investigated by dividing the CS into 3 subgroups depending on their apo(a) isoforms, but no clear link between the apo(a) isoforms and the sample-specific differences was observed (Supplemental Data Part B.4).

METHOD CORRELATION BETWEEN IMMUNOASSAY-BASED MPS

Comparison of the 3 immunoassay-based MPs reported in nmol/L among each other showed a very good linear correlation (Pearson R >0.997) and none or very small sample-specific differences. The Passing–Bablok linear regressions demonstrated slopes ranging from 0.95 to 1.03, which were not significantly different from 1, indicating no significant bias between these 3 methods (Supplemental Fig. B2 and Supplemental Table B2).

The comparison of the 5 immunoassay-based MPs reported in mg/dL showed a very good correlation among the 4 turbidimetric MPs (Pearson R >0.997) and none or very small sample-specific differences (s_{ssd}: 0.02–0.04). The slopes of the Passing–Bablok linear regressions ranged from 0.89 to 1.10 and there was a small but significant (at 95% confidence) relative bias for 4 of the 6 method comparisons. The correlation between the one nephelometric MP and the 4 turbidimetric MPs was weaker (Pearson R 0.987–0.994) and the sample-specific differences were also larger (s_{ssd}: 0.06–0.08). The slopes of the Passing–Bablok linear regressions ranged from 0.69 to 0.77, clearly indicating the presence of a significant (at 99% confidence) bias (Supplemental Fig. B3 and Supplemental Table B3). This significant bias could be caused by the fact that the nephelometric method is calibrated with one master calibrator, which is automatically diluted by the analyzer to 5 solutions containing the same apo(a) isoforms, making the assay more sensitive to apo(a) size polymorphism (9, 12). The current assigned value for the master calibrator might result in an additional bias.

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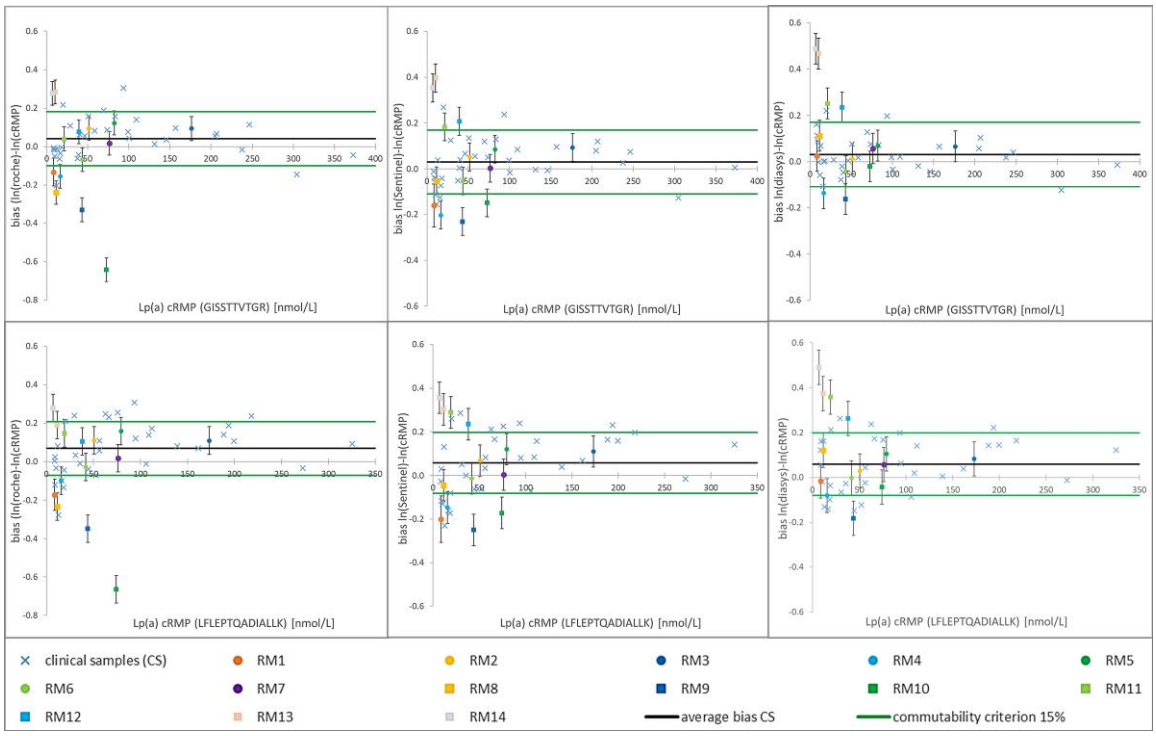


Fig. 2. Commutability assessment of 14 candidate RM for Lp(a) according to a difference in bias approach. This assessment was based on the results of 33 CS measured in 3 replicates and 14 candidate RMs measured in 9 replicates. The relative bias was calculated as the difference between the ln-transformed mean concentration measured with the immunoassay-based MPs and the ln-transformed mean concentration measured with the cRMP based on the non-kringle specific apo(a) peptides (GISSTTVTGR) or (LFLEPTQADIALLK). The error bars on the bias of individual candidate RM represent the expanded uncertainty associated with the estimated difference in bias.

COMMUTABILITY ASSESSMENT

The commutability assessment between the immunoassay-based MPs and the cRMPs showed that 5 out of the 7 candidate RMs based on unspiked human serum pools had a good commutability profile: the outcome was commutable or inconclusive with the data points inside the limits of the commutability criterion. The 2 unspiked serum pools with low Lp(a) concentration (<20 nmol/L) were noncommutable for some method combinations but this could be due to imprecision of the immunoassay-based MPs in the lower measurement ranges. In contrast, all 7 candidate RMs based on r-apo(a) isoforms (RM8–14) spiked into serum were noncommutable for at least one of the method comparisons (Figs. 2 and 3).

The 14 RMs were also assessed for their commutability among the immunoassay-based MPs (Supplemental data parts B.5 and B.6). The conclusions of the commutability assessment were similar: the 5 candidate RMs based on unspiked serum pools with a concentration >20 nmoL/L (or >10 mg/dL) had better commutability profiles than the other candidate RMs.

Discussion

Efficient implementation of measurement standardization based on the concept of metrological traceability can be achieved if all conditions and requirements described in the international standards on metrological traceability, RMP, and RMs are fulfilled (14, 26, 27). Problems in past standardization projects have illustrated the importance of 2 requirements: (*a*) a sufficiently close correlation between results of each MP and the RMP in the traceability chain and (*b*) good commutability of the RM(s) intended for use as common calibrator(s) (28, 29). The correlation between the results obtained with the clinical laboratory MP and the cRMP illustrates the degree of equivalence that can be achieved for individual CS after the finalization of the standardization process. A lack of correlation caused by differences in selectivity for the measurand will produce biased results on individual samples (i.e., sample-specific differences), which cannot be removed by calibration, even with perfectly characterized and commutable

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Candidate RM	cRMP(GISSTTVTGR)			cRMP(LFLEPTQADIALLK)		
	Roche Lp(a) Tina-Quant	Sentinel Lp(a) Ultra	Diasys Lp(a) 21FS	Roche Lp(a) Tina-Quant	Sentinel Lp(a) Ultra	Diasys Lp(a) 21FS
RM1	?	?	C	N	N	?
RM2	C	C	C	C	C	C
RM3	C	C	C	C	C	C
RM4	?	N	?	?	?	?
RM5	?	C	C	?	C	C
RM6	?	?	C	?	?	C
RM7	C	C	C	C	C	C
RM8	N	?	?	N	?	?
RM9	N	N	?	N	N	N
RM10	N	?	C	N	N	?
RM11	C	?	N	?	N	N
RM12	C	?	N	C	?	?
RM13	N	N	N	?	N	N
RM14	N	N	N	?	N	N

Fig. 3. Outcome of the commutability assessment of 14 candidate RMs for Lp(a) according to a difference in bias approach. Commutability of candidate RM was assessed according to the difference in bias analysis [as described in (25)] between the cRMP (using the peptides LFLEPTQADIALLK and GISSTTVTGR) and the 3 immunoassay-based MPs reported in nmol/L. Commutability letter code: C, commutable; N, noncommutable; ?, inconclusive.

RMs. This persistent bias could limit the capacity of the clinical laboratory MP to meet the analytical and clinical performance goals (28).

These study results show a good correlation between the current Lp(a) immunoassay-based MPs reported in nmol/L and the cRMP (Pearson $R \geq 0.991$) for the peptides LFLEPTQADIALLK and GISSTTVTGR indicating that these peptides are suitable for the RMP quantification. The average bias between the immunoassay-based MP and the cRMP results is small, but significant sample-specific differences were observed. For the peptide TPENYPNAGLTR, the correlation was unsatisfactory (Pearson R below the predefined 0.975 criterion). For the 5 immunoassay-based MPs reported in mg/dL some method comparisons showed a significant bias. This was excepted because these methods are traceable to different internal standards and the calibration approach is different. The good correlation among the methods (Pearson $R \geq 0.987$) indicates that the availability of a common calibrator for the immunoassay-based MPs will probably lead to more comparable results.

In this study, both the cRMP and the immunoassay-based MP reported in nmol/L produced results traceable to the WHO standard SRM2B. The obtained results are equivalent and the methods can be considered harmonized. However, the SRM2B-based traceability chain was only a provisional approach as this had many limitations and is no longer available for worldwide standardization (6). The final goal for the RMP is an SRM2B independent calibration based on value-assigned peptide calibrators. Once established, this SI-traceable calibration will have the advantage that new batches of certified RMs can be produced independently of the previous batches and there is no accumulation of associated uncertainties. A peptide-based calibration approach requires sufficient proof of the equimolar and complete digestion of the apo(a) protein present in the Lp(a) particles into the target peptides and these experiments will be the next step in the development of the cRMP.

Recently, an alternative cRMP for Lp(a) based on LC–MS/MS has been published, but this method is neither IFCC-endorsed and/or JCTLM listed so far. In addition, there is currently no network of calibration laboratories established to prepare sustainable implementation of standardization for the future. It should also be noted that there are no data available about the comparison of this method with the immunoassay-based MPs that are frequently used in clinical laboratories (30).

The results of this initial commutability study show that RMs based on unspiked human serum pools are good candidates for future certified RMs, whereas human pools spiked with either tg pig r-apo(a) or HEK r-apo(a) show different behavior compared to CS, making them unsuitable as matrix-based certified RMs. The noncommutability of the candidate RM based on r-apo(a) isoforms could be caused by many factors, including the fact that the r-apo(a) are not bound to a Lp(a) particle. Differences in posttranslational modification could also not be excluded; however, both types of r-apo(a) were produced in mammalian cells. Finally, it is also possible that the unbound r-apo(a) was altered by active enzyme systems or bound to proteins like fibrinogen, present in the human serum background.

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The applied commutability criterion (i.e., 15%) was quite large and was based on the uncertainty associated with the commutability assessment in this study. This approach allowed exclusion of candidate RMs that are clearly noncommutable but it does not prove that candidate RMs fulfilling this criterion will be sufficiently commutable to be used as common calibrators for immunoassay-based MPs. A final conclusion on commutability would require an additional study with a larger number of CS (to reduce the impact of sample-specific differences) and a commutability criterion based on medical requirements (17).

The production of new candidate RM batches based on human serum pools will be the next step. Commutable RMs may be obtained by pooling a larger number of individual sample donations within a specific Lp(a) concentration range regardless of their apo(a) isoforms. However, the selection of individual samples and how they are pooled may introduce arbitrariness, and reproducing new RM batches with identical properties would be challenging. An alternative option might be to produce commutable RMs by pooling several individual donations preselected to contain only specific apo(a) isoforms representing the most suitable isoforms in the specific Lp(a) concentration range.

Together with the cRMP, the commutable RMs will form the basis of the future SI-traceable reference measurement system for Lp(a). However, one should keep in mind that the sample-specific differences seen in this study between the cRMP and the immunoassay-based MPs will remain even after calibration with commutable secondary RMs. For each specific immunoassay-based MP, achievement of the clinical performance goals for individual CS should be checked and redesign of the MPs might still be needed.

Supplemental material

Supplemental material is available at *Clinical Chemistry* online.

Nonstandard Abbreviations: Lp(a), lipoprotein(a); MP, measurement procedure; MS, mass spectrometry; RMP, reference measurement procedure; RM, reference material; apo(a), apolipoprotein(a); cRMP, candidate RMP; CS, clinical samples; K-IV$_2$, repetitive kringle subunit 4, type 2 in the apo(a) molecule; IFCC, International Federation for Clinical Chemistry and Laboratory Medicine; ID-LC-MS, isotope dilution-liquid chromatography mass spectrometry; CDC, Centers for Disease Control and Prevention; r-apo(a), recombinant apolipoprotein(a); HEK, human embryonic kidney (cells); JRC, Joint Research Center; SI, international system of units.

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