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Transferability and degree of harmonization of an LC-MS based Reference Measurement Procedure for apolipoproteins in a network of calibration laboratories

Introduction

For apolipoprotein standardization, a network of three calibration laboratories has been established for the value assignment of matrix-based reference and EQA materials.
A multiplexed LC-MS based reference measurement procedure (RMP) has been developed for serum apolipoproteins apo(a), apoA-I, apoB, apoC-I, apoC-II, apoC-III and apoE [1].
Transferability and degree of harmonization of the method in a network of three calibration laboratories [2].

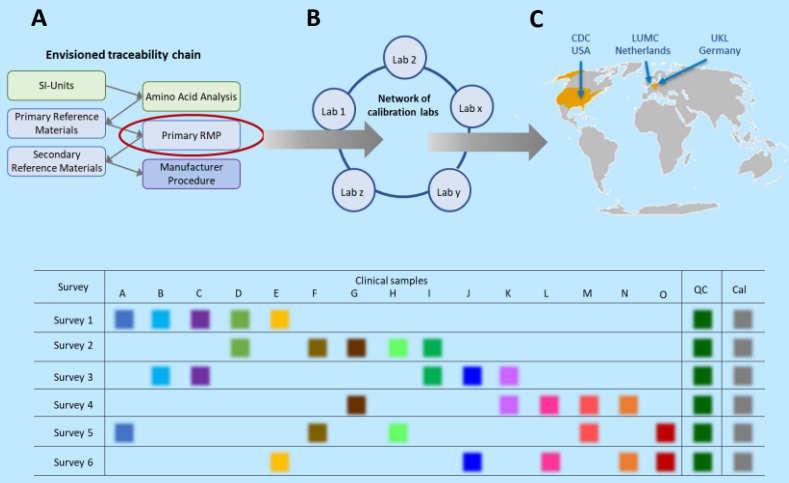

Figure 1. Establishing a network of calibration labs for multiplex apolipoprotein quantitation by mass spectrometry. Traceability of apolipoproteins is envisioned to SI units and a reference measurement procedure using bottom-up proteomics and LC-MS. Within the IFCC WG APO-MS, a reference measurement procedure was developed in accordance with a predefined common accuracy base, agreed at the start of the project **(A)**. A sustainable reference measurement system comprises a network of calibration laboratories, which is one of the terms of reference of the current IFCC Scientific Division **(B)**; for apolipoproteins, a global network of three laboratories in USA, the Netherlands and Germany has been established **(C)**.

Design of ringtrials

Each laboratory received one batch of 72 samples. For six months, 5 human serum samples, 2 QC samples and 5 serum-based calibrators were measured along with a system suitability sample consisting of synthetic peptides, each month (survey). Every clinical sample was included twice in the surveys. A standardized data collection template was used, and data was evaluated using R.

QC

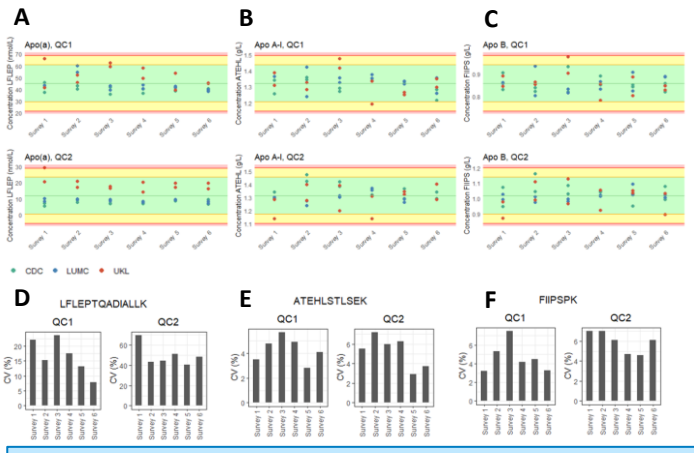

Figure 2. Longitudinal QC performance at the three calibration laboratories over the course of the six survey rounds. Levey Jennings plots showing the QC performance of example peptides from apo(a) (LFLEP), apoA-I (ATEHL) and apoB (FIIPS). Mean, 2SD and 3SD borders are indicated, and different colored dots indicate results from the different laboratories during the different surveys **(A-C).**

Overall precision ((%CV) for all three laboratories combined) per QC sample and per survey indicate an overall slight improvement in precision between survey 1 and survey 6 **(D-F)**.

Interpeptide comparison

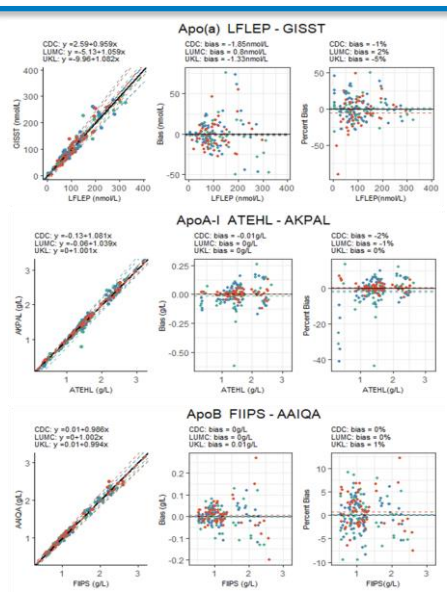

Figure 3. Interpeptide comparison indicating intra-lab analytical selectivity of the harmonized RMP. Scatterplots indicate good concordance between two proteotypic peptides representing the same protein, with Deming regression equations shown for each laboratory (Apo(a)- top; apoA-I middle; apoB bottom) (left). Bias and percent bias, with average bias and average percent bias plotted for each laboratory, are shown in the middle and right plots, respectively.

Level of harmonization

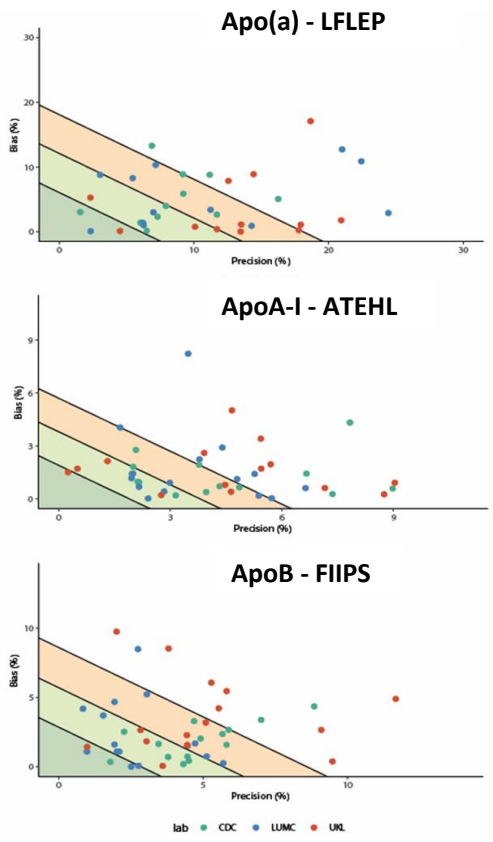

Figure 4. Level of harmonization of the RMP for apolipoproteins at the three calibration labs. Imprecision (%CV) against Bias (%) relative to the all-lab total mean is plotted for each of the individual samples measured. Allowable measurement uncertainty is indicated by lines and colored background: darker green within optimal TEa, lighter green within desirable TEa, orange within minimal TEa, and white background outside minimal TEa. Dots indicate sample results colored per laboratory.

Results: Intra-laboratory precision generally fulfilled predefined performance, which was defined as CVa < 50% of minimal Total Allowable Error. Additional inter-laboratory variation was observed with median interlaboratory variation for the quantifying peptides of **12.0%, 4.9%, 5.1%, 9.9%, 10.0%, 6.9% and 6.8%** for apo(a), apoA-I, apoB, apoC-I, apoC-II, apoC-III and apoE, respectively. For apo(a) specifically, the average interlab CV% for four samples around the cut-off value of 90 nmol/L was 13.7%. In QC samples, the average imprecision for all apos decreased from **6.0% and 18.1%** for QC1 and QC2, respectively, **to 5.2% and 9.5%** over the course of six months, indicating improvement of analytical performance of the network of calibration labs over time.

Protein	peptide	All (%)	CDC (%)	LUMC (%)	UKL (%)
Apo(a)	LFLEP	12.0	7.9	7.0	13.5
ApoA-I	ATEHL	4.9	4.0	3.5	4.7
ApoB	FIIPS	5.1	4.7	2.5	4.4

Table1. Summary of the harmonization potential, as expressed by the median percentage imprecision overall (All) and within the three laboratories (CDC, LUMC, UKL).

Conclusions

This study shows the feasibility of transferring an LC-MS based reference measurement procedure between laboratories. Most Clinical samples fulfilled predefined performance specifications; Ongoing round-robin studies will ensure stable performance of the network of three apolipoprotein calibration labs required to maintain an accurate value-base for apolipoprotein certification of commercial reagents. Further information can be found at https://ifcc.org/ifcc-scientific-division/sd-working-groups/wg-apo-ms/.

Reference [1]. L.R. Ruhaak et al. Clin. Chem. 69(3) (2023) 251-261.
[2]. L.R. Ruhaak et al. submission