Toward Reliable Lipoprotein Particle Predictions from NMR Spectra of Human Blood
An Interlaboratory Ring Test

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Towards reliable lipoprotein particle predictions from NMR spectra of human blood: an interlaboratory ring test

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Abstract: Lipoprotein profiling of human blood by $^1$H Nuclear Magnetic Resonance (NMR) spectroscopy is a rapid and promising approach to monitor health and disease states in medicine and nutrition. However, lack of standardization of measurement protocols has prevented the use of NMR-based lipoprotein profiling in meta-studies.

In this study, a standardized NMR measurement protocol was applied in a ring test performed across three different laboratories in Europe on plasma and serum samples from 28 individuals. Data was evaluated in terms of (i) spectral differences, (ii) differences in LPD predictions obtained using an existing prediction model and (iii) agreement of predictions with cholesterol concentrations in high and low density lipoproteins (HDL and LDL) particles measured by standardized clinical assays.

ANOVA-simultaneous component analysis (ASCA) of the ring test spectral ensemble that contains methylene and methyl peaks (1.4-0.6 ppm) showed that 97.99% of the variance in the data is related to subject, 1.62% to sample type (serum or plasma) and 0.39% to laboratory. This interlaboratory variation is in fact smaller than the maximum acceptable intralaboratory variation on quality control samples. It is also shown that the reproducibility between laboratories is good enough for the LPD predictions to be exchangeable when the standardized NMR measurement protocol is followed.

With the successful implementation of this protocol, which results in reproducible prediction of lipoprotein distributions across laboratories, a step is taken towards bringing NMR more into scope of prognostic and diagnostic biomarkers, reducing the need for less efficient methods.
Supplementary Materials and Methods

Figure S1. Experimental design of the ring test study. NMR spectra from plasma and serum samples of 28 individuals were obtained at the same time in three different locations (Bruker, University of Copenhagen and Unilever). Clinical measurements (HDL-C and LDL-C) for plasma and serum samples separately were obtained for model performance assessment.

Table S1. Spectrometer and probe used in the ring test at each of the three laboratories.

|          | BR                  | KU                  | UL                  |
|----------|---------------------|---------------------|---------------------|
| Magnet   | ASCEND              | UltraShield Plus    | UltraShield Plus    |
| Shim System | BOSS III            | BOSS II             | BOSS II             |
| Console  | AVANCE III HD       | AVANCE III HD       | AVANCE III          |
| Probe    | inverse RT (BBI)    | inverse RT (BBI)    | Inverse Cryo (TCI)  |

Figure S2. Mihaleva mean spectra, plasma ring test mean spectra and serum ring test mean spectra (a) before and (b) after scaling of the ring test data with the scaling factor $c$.

(Equation S1)

$$ r = \frac{SS(A-B)}{\sqrt{SS(A)+SS(B)}} \quad 0 \leq r \leq 1, $$

$$ 0 \leq r = \frac{\text{tr}(A-B)'(A-B)}{\text{tr}(A'A) + \text{tr}(B'B)} = \frac{||A-B||^2}{||A||^2 + ||B||^2} = \frac{SS(A-B)}{SS(A)+SS(B)} $$

for $A = B \rightarrow r = 0$

for $||A||^2 \approx ||B||^2$ (spectral matrices of similar size)
then \( r = 1 \) (for \( \mathbf{A'B} = 0; \mathbf{B'\Lambda} \))

So, in practice \( 0 \leq r \leq 1 \)

\( 0 = \) maximum agreement/similarity

\( 1 = \) maximum disagreement/dissimilarity

**Supplementary Results. Characterization of the ring test population**

**Table S2.** Distribution of the 28 ring test plasma and serum samples and of 189 Mihaleva training samples according to the serum lipid classification of the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (2002). Clinical data for one of the original 190 Mihaleva training samples was not available.

| LIPID              | ATPIII group               | Ring test (plasma) | Ring test (serum) | Mihaleva training |
|--------------------|----------------------------|--------------------|-------------------|-------------------|
| Total cholesterol  | Desirable (<5.18 mM)       | 24                 | 21                | 19                |
|                    | Borderline high (5.18-6.18 mM) | 3                  | 6                 | 61                |
|                    | High (≥ 6.20 mM)            | 1                  | 1                 | 109               |
| HDL-C              | Low (<1 mM)                | 2                  | 2                 | 4                 |
|                    | Average (1-1.55 mM)        | 16                 | 15                | 75                |
|                    | High (>1.55 mM)            | 10                 | 11                | 110               |
| LDL-C              | Optimal (<2.59 mM)         | 12                 | 10                | 12                |
|                    | Near/above optimal (2.59-3.34 mM) | 14               | 14                | 46                |
|                    | Borderline high (3.35-4.12 mM) | 2                 | 4                 | 69                |
|                    | High (4.15-4.90 mM)        | 0                  | 0                 | 46                |
|                    | Very high (>4.90 mM)       | 0                  | 0                 | 16                |
| Total triglycerides| Desirable (<1.70 mM)       | 25                 | 25                | 173               |
|                    | Borderline high (1.70-2.20 mM) | 1                 | 1                 | 16                |
|                    | High (2.30-5.60 mM)        | 2                  | 2                 | 0                 |
|                    | Very high (>5.60 mM)       | 0                  | 0                 | 0                 |

**Supplementary Results. Level 1**

![Figure S3. Scores plot of a 2-component PCA model of all spectra (plasma and serum).](image-url)
Figure S4. Scores plot of a 2-component PCA model of the (a) plasma and (b) serum spectra.

\[
\|X\|_J^2 = \|X_{\text{subject}}\|_J^2 + \|X_{\text{sample \ type}}\|_J^2 + \|X_{\text{laboratory}}\|_J^2 + \|E\|_J^2
\]

Figure S5. Partitioning of the NMR spectral data (mean centered) in the ASCA analysis. The contribution of each of the factors to the explained variance can be determined as the sum of squares of each of the matrices $IxJ$ ($I=168, J=1746$).
Supplementary Results. Level 2. Serum Prediction Lab 1 (mmol/L)

Serum VLDL03 cholesterol predictions

BR-KU, RMSE: 0.0023014  
BR-UL, RMSE: 0.0037221  
KU-UL, RMSE: 0.0019215

Serum VLDL03 triglycerides predictions

BR-KU, RMSE: 0.0083679  
BR-UL, RMSE: 0.014263  
KU-UL, RMSE: 0.0073116

Serum VLDL04 cholesterol predictions

BR-KU, RMSE: 0.0063833  
BR-UL, RMSE: 0.0038685  
KU-UL, RMSE: 0.0058359

Serum VLDL04 triglycerides predictions

BR-KU, RMSE: 0.015051  
BR-UL, RMSE: 0.020778  
KU-UL, RMSE: 0.010686

Serum VLDL05 cholesterol predictions

BR-KU, RMSE: 0.014735  
BR-UL, RMSE: 0.040558  
KU-UL, RMSE: 0.035435

Serum VLDL05 triglycerides predictions

BR-KU, RMSE: 0.013495  
BR-UL, RMSE: 0.018353  
KU-UL, RMSE: 0.01177
Serum VLDL07 cholesterol predictions

Prediction Lab 1 (mmol/L) -0.1 0 0.1 0.2 0.3 0.4
Prediction Lab 2 (mmol/L) -0.2 -0.1 0 0.1 0.2 0.3 0.4

BR-KU, RMSE: 0.010518
BR-UL, RMSE: 0.015907
KU-UL, RMSE: 0.023012

Serum VLDL07 triglycerides predictions

Prediction Lab 1 (mmol/L) 0 0.02 0.04 0.06 0.08 0.1 0.12
Prediction Lab 2 - Prediction Lab 1 -0.03 -0.02 -0.01 0 0.01 0.02 0.03 0.04

BR-KU, RMSE: 0.0014932
BR-UL, RMSE: 0.0013761
KU-UL, RMSE: 0.0017847

Serum LDL08 cholesterol predictions

Prediction Lab 1 (mmol/L) 0 0.5 1 1.5
Prediction Lab 2 - Prediction Lab 1 -0.1 -0.05 0 0.05 0.1

BR-KU, RMSE: 0.035442
BR-UL, RMSE: 0.0236
KU-UL, RMSE: 0.025711

Serum LDL08 triglycerides predictions

Prediction Lab 1 (mmol/L) 0.05 0.1 0.15 0.2 0.25 0.3
Prediction Lab 2 - Prediction Lab 1 -0.015 -0.01 -0.005 0 0.005 0.01 0.015

BR-KU, RMSE: 0.0022468
BR-UL, RMSE: 0.0024204
KU-UL, RMSE: 0.0026647

Serum LDL09 cholesterol predictions

Prediction Lab 1 (mmol/L) 0.5 1 1.5 2
Prediction Lab 2 - Prediction Lab 1 -0.3 -0.2 -0.1 0 0.1 0.2

BR-KU, RMSE: 0.035169
BR-UL, RMSE: 0.081018
KU-UL, RMSE: 0.083688

Serum LDL09 triglycerides predictions

Prediction Lab 1 (mmol/L) 0.05 0.1 0.15 0.2 0.25 0.3
Prediction Lab 2 - Prediction Lab 1 -0.02 -0.01 0 0.01 0.02

BR-KU, RMSE: 0.0046285
BR-UL, RMSE: 0.0082273
KU-UL, RMSE: 0.0070665
Figure S6. Between-lab differences in serum cholesterol and triglycerides predictions for all subclasses (except VLDL06, see main text) and main fractions obtained using the Mihaleva-derived PLS model. BR versus KU, BR versus UL and UL versus KU (a and b) cholesterol and (c and d) triglycerides serum predictions. (a and c) On the x axis, the value of the prediction of the first laboratory; on the y axis, the value of the prediction of the second laboratory. (b and d) Zoom-in of the differences between labs: On the x axis, the value of the prediction of the first laboratory; on the y axis, the difference between the prediction of the second laboratory and the prediction of the first one. The black line is drawn where predictions are equal, the red dashed lines indicate a deviation of 5%.
Supplementary Results. Level 2. Plasma

Plasma VLDL03 cholesterol predictions
- BR-KU, RMSE: 0.0027649
- BR-UL, RMSE: 0.0033182
- KU-UL, RMSE: 0.002557

Plasma VLDL03 triglycerides predictions
- BR-KU, RMSE: 0.0080526
- BR-UL, RMSE: 0.011401
- KU-UL, RMSE: 0.0072607

Plasma VLDL04 cholesterol predictions
- BR-KU, RMSE: 0.0070757
- BR-UL, RMSE: 0.0043034
- KU-UL, RMSE: 0.0073973

Plasma VLDL04 triglycerides predictions
- BR-KU, RMSE: 0.014217
- BR-UL, RMSE: 0.016474
- KU-UL, RMSE: 0.011033

Plasma VLDL05 cholesterol predictions
- BR-KU, RMSE: 0.014828
- BR-UL, RMSE: 0.038511
- KU-UL, RMSE: 0.033744

Plasma VLDL05 triglycerides predictions
- BR-KU, RMSE: 0.011384
- BR-UL, RMSE: 0.01177
- KU-UL, RMSE: 0.0111
Plasma VLDL06 cholesterol predictions

Plasma VLDL06 triglycerides predictions

Plasma VLDL07 cholesterol predictions

Plasma VLDL07 triglycerides predictions

Plasma LDL08 cholesterol predictions

Plasma LDL08 triglycerides predictions
Plasma LDL09 cholesterol predictions
- BR-KU, RMSE: 0.04105
- BR-UL, RMSE: 0.090407
- KU-UL, RMSE: 0.082335

Plasma LDL09 triglycerides predictions
- BR-KU, RMSE: 0.0037307
- BR-UL, RMSE: 0.0068681
- KU-UL, RMSE: 0.0067114

Plasma LDL10 cholesterol predictions
- BR-KU, RMSE: 0.01595
- BR-UL, RMSE: 0.032931
- KU-UL, RMSE: 0.030132

Plasma LDL10 triglycerides predictions
- BR-KU, RMSE: 0.0021422
- BR-UL, RMSE: 0.0037719
- KU-UL, RMSE: 0.0039621

Plasma LDL11 cholesterol predictions
- BR-KU, RMSE: 0.011782
- BR-UL, RMSE: 0.011381
- KU-UL, RMSE: 0.0047987

Plasma LDL11 triglycerides predictions
- BR-KU, RMSE: 0.00074623
- BR-UL, RMSE: 0.0010784
- KU-UL, RMSE: 0.0012686
Prediction Lab 1 (mmol/L) 0 0.1 0.2 0.3 0.4 0.5
Prediction Lab 2 (mmol/L) 0 0.1 0.2 0.3 0.4 0.5
Plasma HDL15 cholesterol predictions
BR-KU, RMSE: 0.009031
BR-UL, RMSE: 0.0093508
KU-UL, RMSE: 0.016656
Plasma HDL15 triglycerides predictions
BR-KU, RMSE: 0.00072575
BR-UL, RMSE: 0.00091419
KU-UL, RMSE: 0.00038243

Prediction Lab 1 (mmol/L) -0.5 0 0.5 1 1.5
Prediction Lab 2 (mmol/L) -1 -0.5 0 0.5 1 1.5 2
Plasma HDL16 cholesterol predictions
BR-KU, RMSE: 0.020726
BR-UL, RMSE: 0.036814
KU-UL, RMSE: 0.042828
Plasma HDL16 triglycerides predictions
BR-KU, RMSE: 0.0012426
BR-UL, RMSE: 0.0024021
KU-UL, RMSE: 0.0016966

Prediction Lab 1 (mmol/L) 0.3 0.4 0.5 0.6 0.7 0.8
Prediction Lab 2 (mmol/L) 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1
Plasma HDL17 cholesterol predictions
BR-KU, RMSE: 0.033986
BR-UL, RMSE: 0.036862
KU-UL, RMSE: 0.03796
Plasma HDL17 triglycerides predictions
BR-KU, RMSE: 0.0018812
BR-UL, RMSE: 0.0024791
KU-UL, RMSE: 0.001465
Prediction Lab 1 (mmol/L) 0.3 0.35 0.4 0.45 0.5 0.55

Prediction Lab 2 (mmol/L) 0.25 0.3 0.35 0.4 0.45 0.5 0.55 0.6

Plasma HDL18 cholesterol predictions

BR-KU, RMSE: 0.015325
BR-UL, RMSE: 0.018123
KU-UL, RMSE: 0.020122

Plasma HDL18 triglycerides predictions

BR-KU, RMSE: 0.00057476
BR-UL, RMSE: 0.0010013
KU-UL, RMSE: 0.00091353

Prediction Lab 1 (mmol/L) 0.01 0.02 0.03 0.04 0.05 0.06

Prediction Lab 2 (mmol/L) 0 0.01 0.02 0.03 0.04 0.05 0.06 0.07

Plasma VLDLtotal cholesterol predictions

BR-KU, RMSE: 0.024814
BR-UL, RMSE: 0.043781
KU-UL, RMSE: 0.037262

Plasma VLDLtotal triglycerides predictions

BR-KU, RMSE: 0.038
BR-UL, RMSE: 0.033318
KU-UL, RMSE: 0.032726

Prediction Lab 1 (mmol/L) 0.4 0.6 0.8 1 1.2 1.4

Prediction Lab 2 (mmol/L) 0.2 0.4 0.6 0.8 1 1.2 1.4 1.6

Plasma LDLtotal cholesterol predictions

BR-KU, RMSE: 0.075921
BR-UL, RMSE: 0.15443
KU-UL, RMSE: 0.1344

Plasma LDLtotal triglycerides predictions

BR-KU, RMSE: 0.007908
BR-UL, RMSE: 0.013147
KU-UL, RMSE: 0.013646

Prediction Lab 1 (mmol/L) 1.5 2 2.5 3 3.5 4

Prediction Lab 2 (mmol/L) 1 1.5 2 2.5 3 3.5 4 4.5

Plasma LDLtotal cholesterol predictions

BR-KU, RMSE: 0.075921
BR-UL, RMSE: 0.15443
KU-UL, RMSE: 0.1344

Plasma LDLtotal triglycerides predictions

BR-KU, RMSE: 0.007908
BR-UL, RMSE: 0.013147
KU-UL, RMSE: 0.013646

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Figure S7. Between-lab differences in plasma cholesterol and triglycerides predictions for all subclasses and main classes obtained using the Mihaileva-derived PLS model. BR versus KU, BR versus UL and UL versus KU (a) cholesterol and (b) triglycerides serum predictions. On the x axis, the value of the prediction of the first laboratory; on the y axis, the value of the prediction of the second laboratory. The black line is drawn where predictions are equal, the red dashed lines indicate a deviation of 5%.

Supplementary Results. Level 3

Figure S8. Ring test HDL cholesterol residual plot (PLS predictions vs. HDL-C clinical values).
Figure S9. HDL-C predictions of the PLS model on plasma spectra from the three laboratories. The black line is drawn where prediction and measurement are equal, the red dashed lines indicate plus or minus 5%.

Figure S10. LDL-C predictions of the PLS model on (a) serum and (b) plasma spectra from the three laboratories. The black line is drawn where prediction and measurement are equal, the red dashed lines indicate plus or minus 5%.