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Toward Reliable Lipoprotein Particle Predictions from NMR Spectra of Human Blood

An Interlaboratory Ring Test

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DOI

[10.1021/acs.analchem.7b01329](https://doi.org/10.1021/acs.analchem.7b01329)

Publication date

2017

Document Version

Other version

Published in

Analytical Chemistry

[Link to publication](#)

Citation for published version (APA):

Monsonis Centelles, S., Hoefsloot, H. C. J., Khakimov, B., Ebrahimi, P., Lind, M. V., Kristensen, M., de Roo, N., Jacobs, D. M., van Duynhoven, J., Cannet, C., Fang, F., Humpfer, E., Schäfer, H., Spraul, M., Engelsen, S. B., & Smilde, A. K. (2017). Toward Reliable Lipoprotein Particle Predictions from NMR Spectra of Human Blood: An Interlaboratory Ring Test. *Analytical Chemistry*, 89(15), 8004-8012. <https://doi.org/10.1021/acs.analchem.7b01329>

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Supporting Information

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

Sandra Monsonis Centelles^{*†}, Huub C.J. Hoefsloot[†], Bekzod Khakimov[‡], Parvaneh Ebrahimi[‡], Mads V. Lind[§], Mette Kristensen[§], Niels de Roo["], Doris M. Jacobs["], John van Duynhoven^{"[⊥]}, Claire Cannet[#], Fang Fang[#], Eberhard Humpfer[#], Hartmut Schäfer[#], Manfred Spraul[#], Søren B. Engelsen[‡] and Age K. Smilde[†]

[†] Biosystems data analysis, Swammerdam Institute for Life Sciences, Universiteit van Amsterdam, Science Park 904, 1098 XH, Amsterdam, The Netherlands

[‡] Department of Food Science, Chemometrics and Analytical Technology, Faculty of Science, University of Copenhagen, Rolighedsvej 26, DK-1958 Frederiksberg C, Denmark

[§] Department of Nutrition, Exercise and Sports, University of Copenhagen, Rolighedsvej 26, DK-1958 Frederiksberg C, Denmark

["] Unilever R&D, Olivier van Noortlaan 120, 3133 AT, Vlaardingen, The Netherlands

[⊥] Laboratory of Biophysics, Wageningen University, Stippeneng 4, 6708WE, Wageningen, The Netherlands

[#] Bruker BioSpin GmbH, 76287 Rheinstetten, Germany

* E-mail: S.MonsonisCentelles@uva.nl

Abstract: Lipoprotein profiling of human blood by ¹H 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

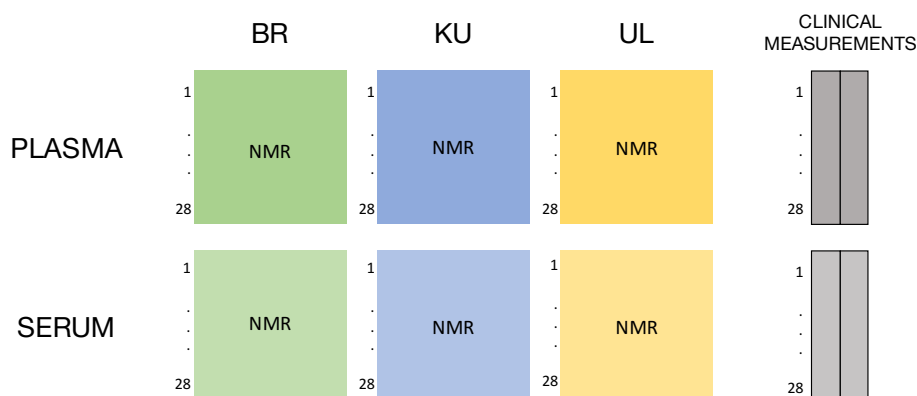


Figure S1. Experimental design of the ring test study. NMR spectra from plasma and serum samples of 28 individuals were obtained at the same time in three different locations (Bruker, University of Copenhagen and Unilever). Clinical measurements (HDL-C and LDL-C) for plasma and serum samples separately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

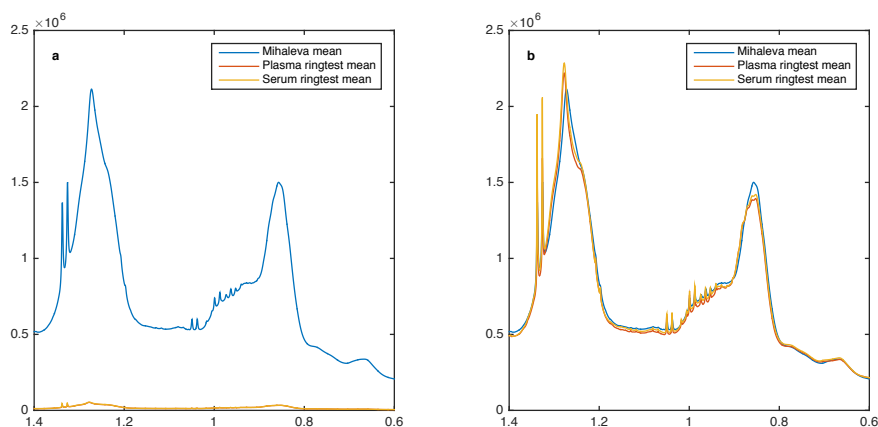


Figure S2. Mihaleva mean spectra, plasma ring test mean spectra and serum ring test mean spectra (a) before and (b) after scaling of the ring test data with the scaling factor c .

$$\text{(Equation S1)} \quad r = \sqrt{\frac{SS(\mathbf{A}-\mathbf{B})}{SS(\mathbf{A})+SS(\mathbf{B})}} \quad 0 \leq r \leq 1,$$

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

$$\text{for } \mathbf{A} = \mathbf{B} \rightarrow r = 0$$

$$\text{for } \|\mathbf{A}\|^2 \approx \|\mathbf{B}\|^2 \text{ (spectral matrices of similar size)}$$

then $\max r = 1$ (for $\mathbf{A}'\mathbf{B} = 0$; $\mathbf{B}\mathbf{L}\mathbf{A}$)

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

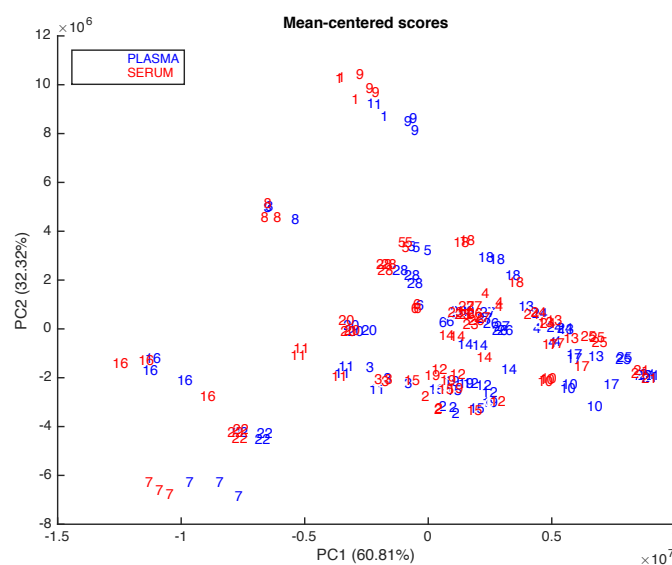


Figure S3. Scores plot of a 2-component PCA model of all spectra (plasma and serum).

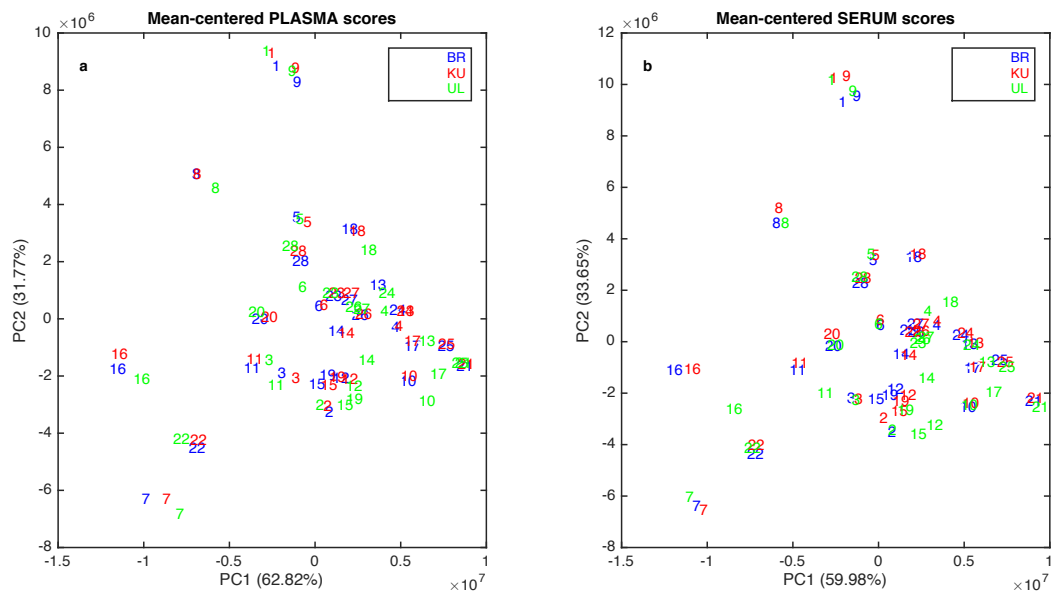


Figure S4. Scores plot of a 2-component PCA model of the (a) plasma and (b) serum spectra.

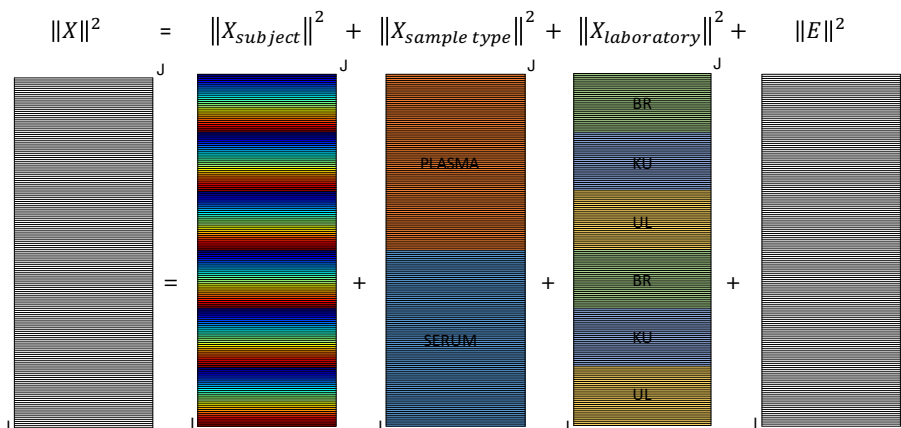
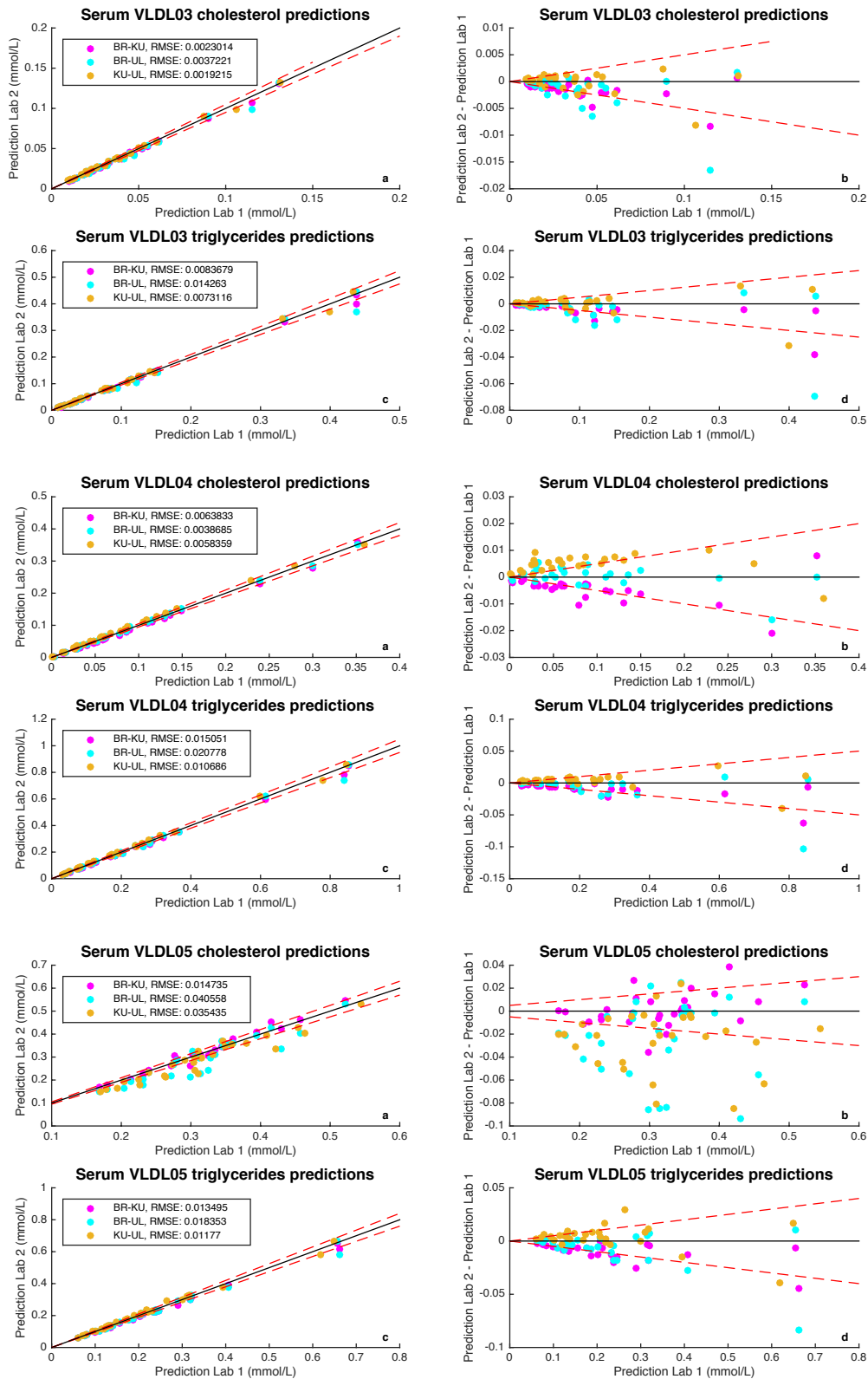
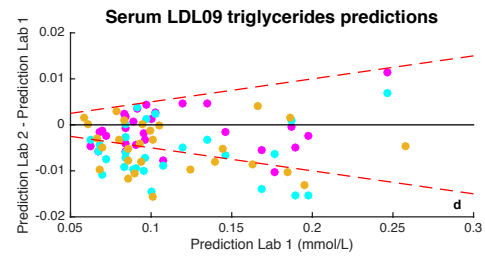
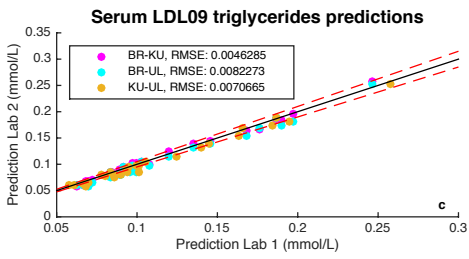
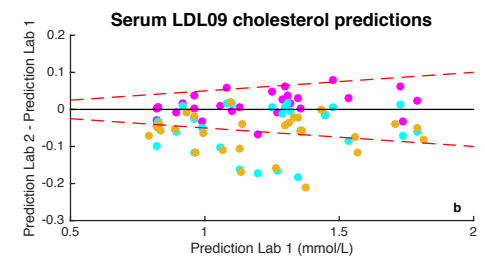
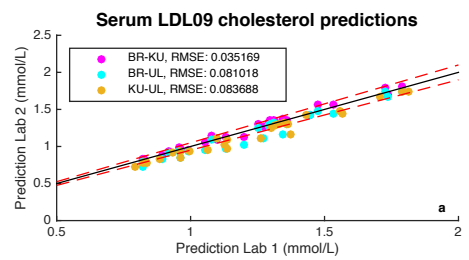
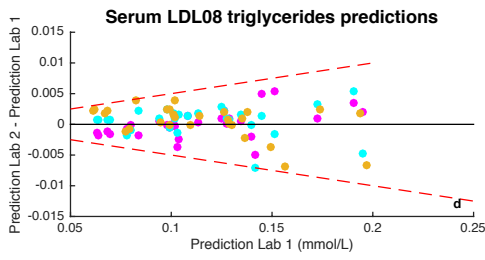
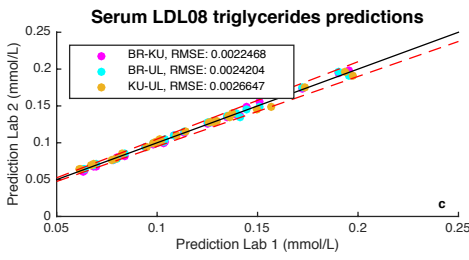
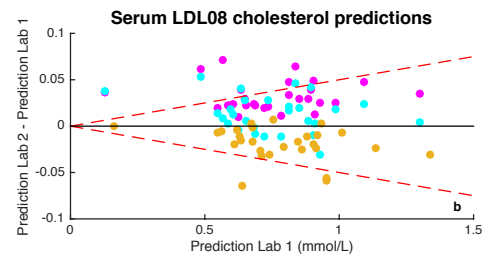
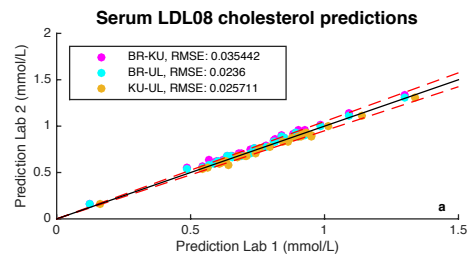
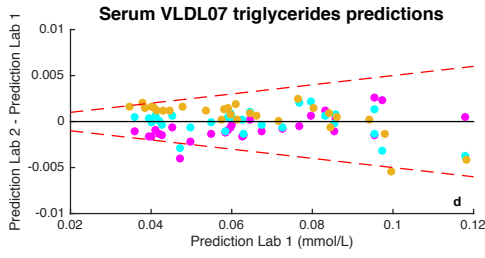
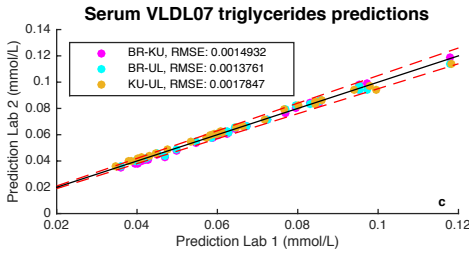
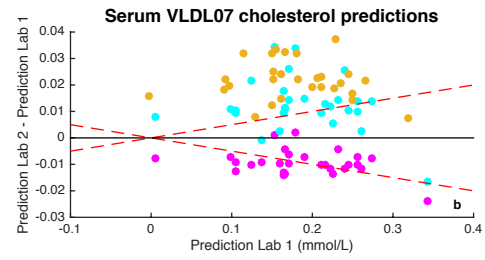
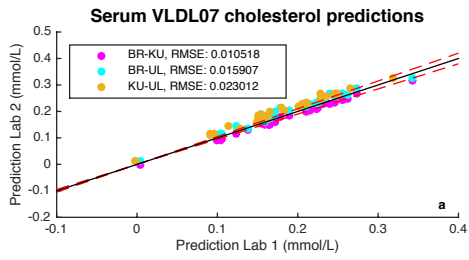
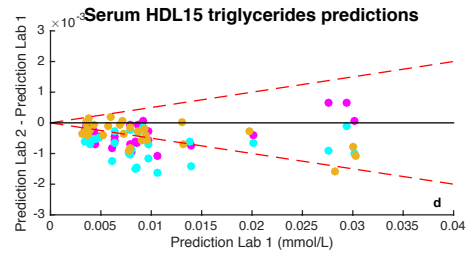
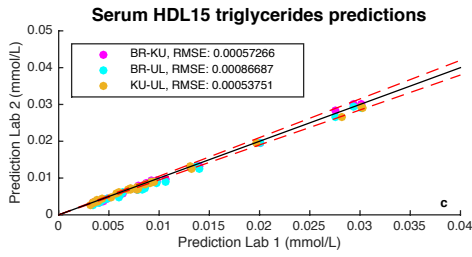
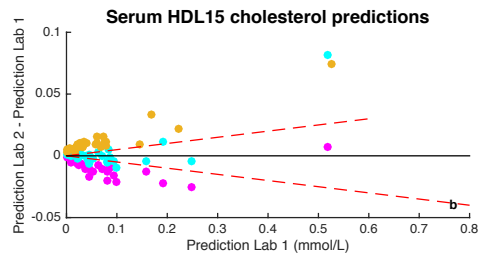
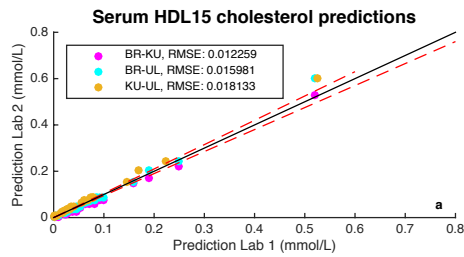
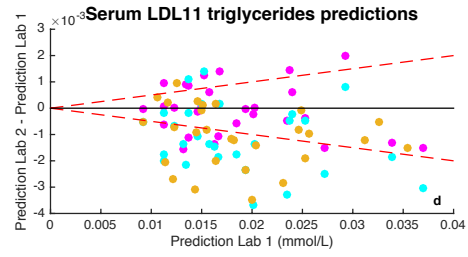
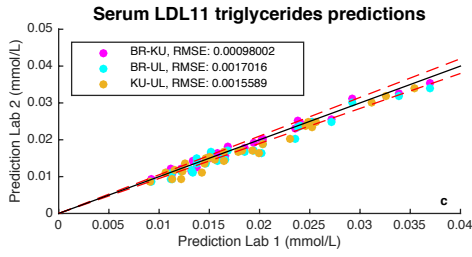
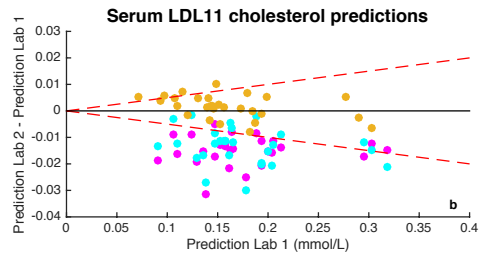
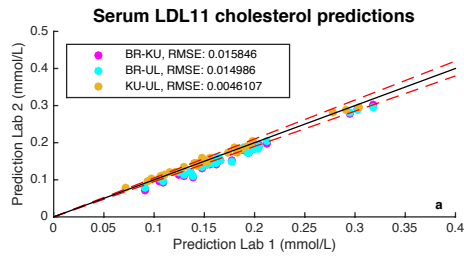
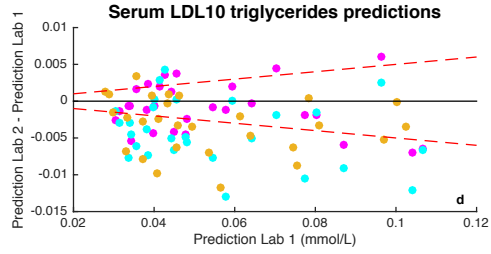
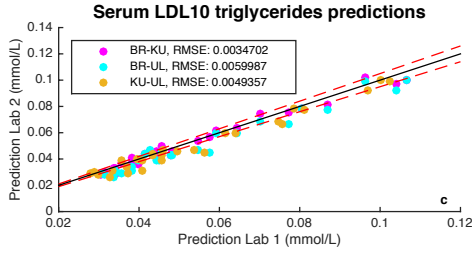
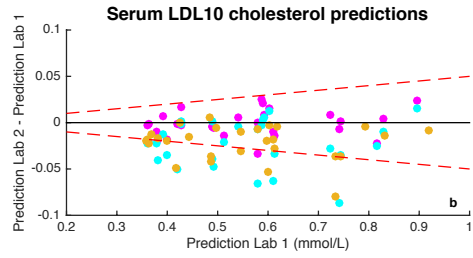
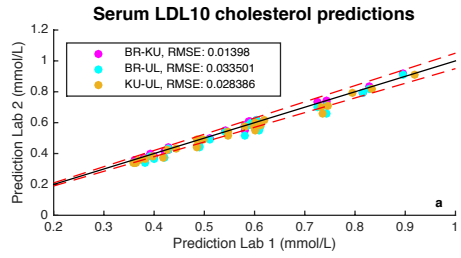


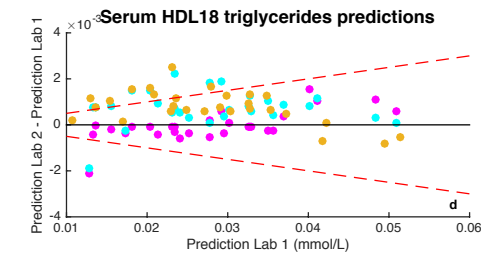
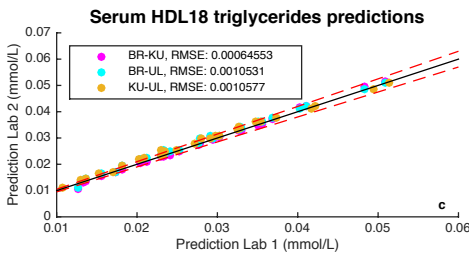
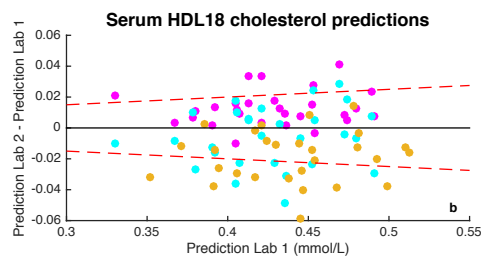
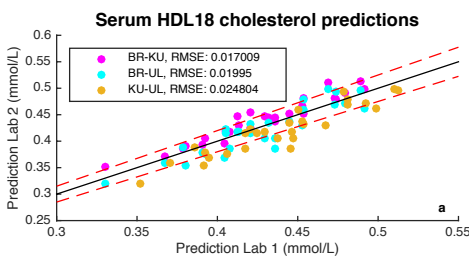
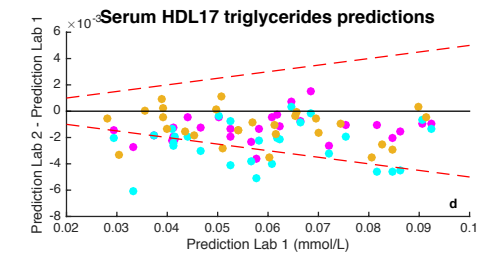
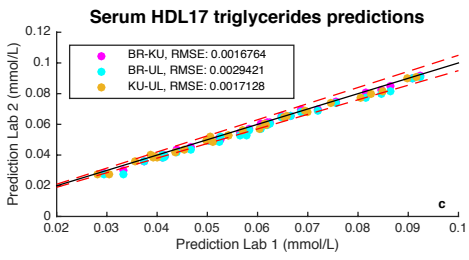
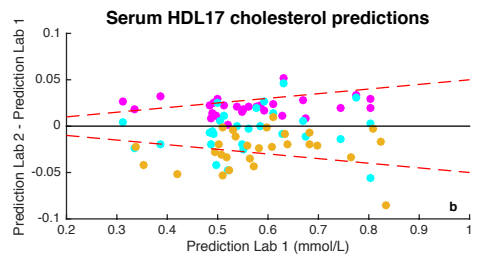
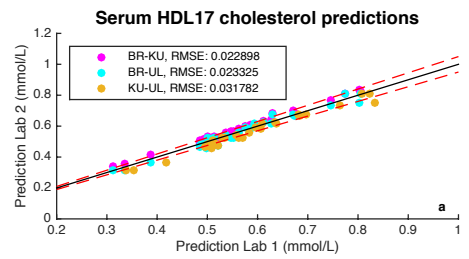
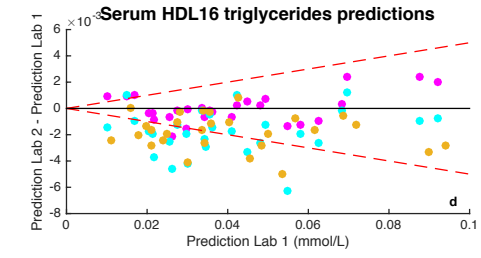
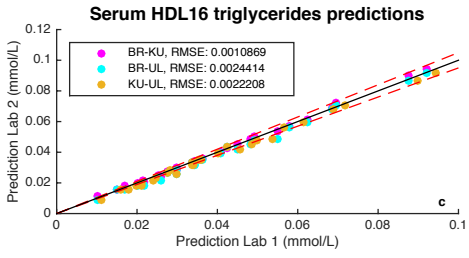
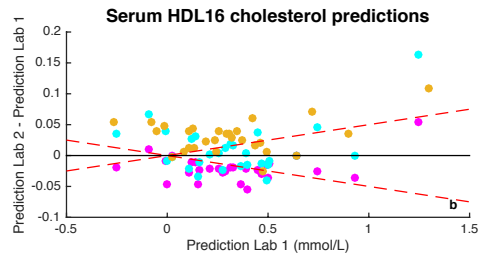
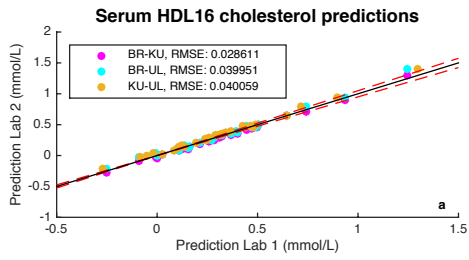
Figure S5. Partitioning of the NMR spectral data (mean centered) in the ASCA analysis. The contribution of each of the factors to the explained variance can be determined as the sum of squares of each of the matrices $I \times J$ ($I=168, J=1746$).

Supplementary Results. Level 2. Serum









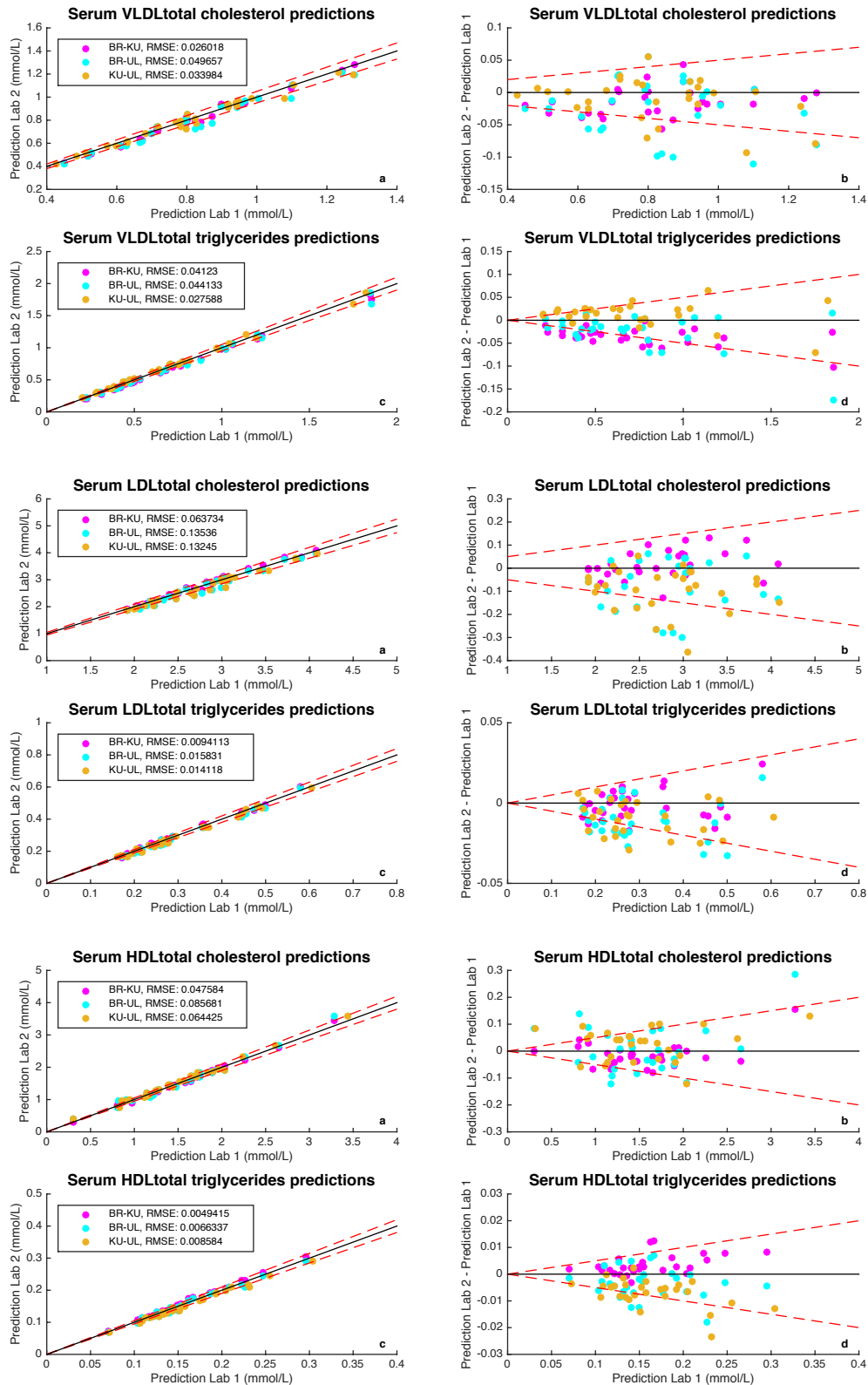
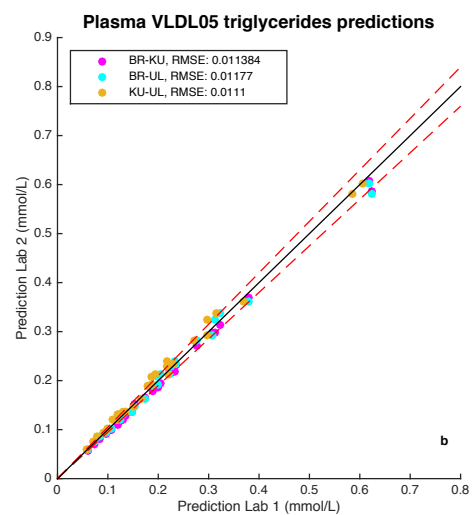
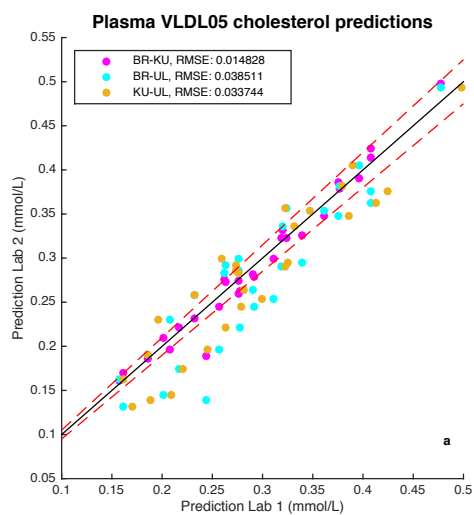
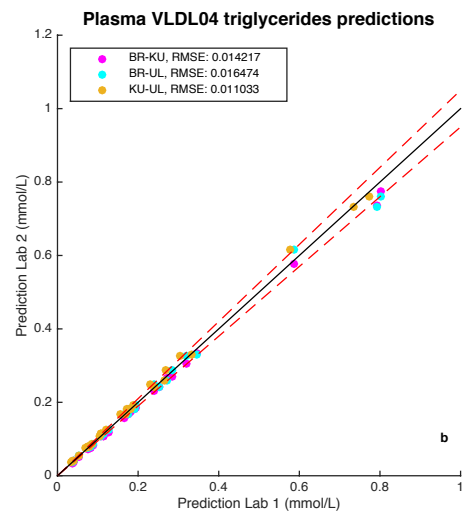
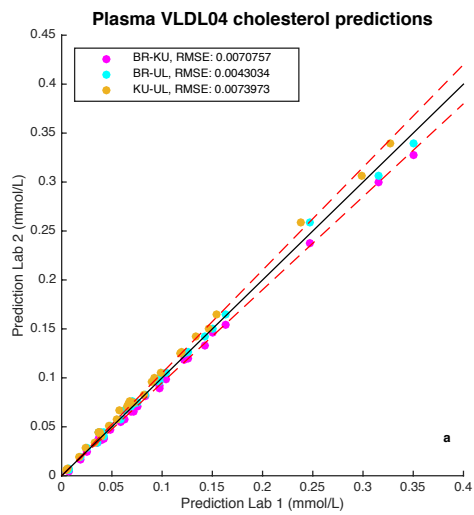
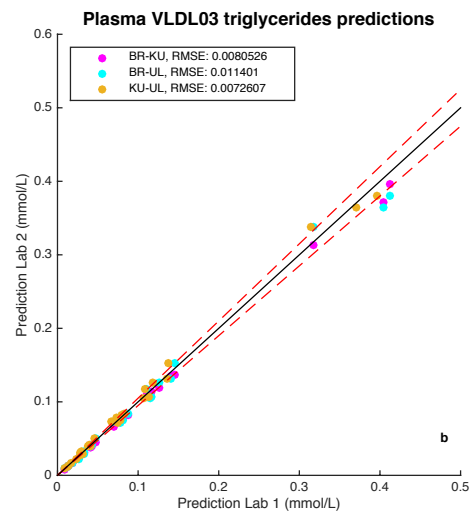
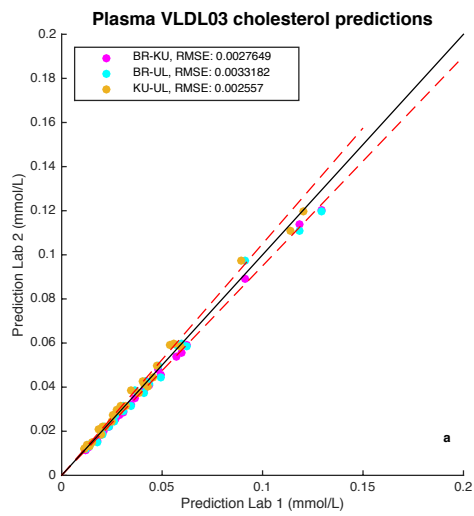
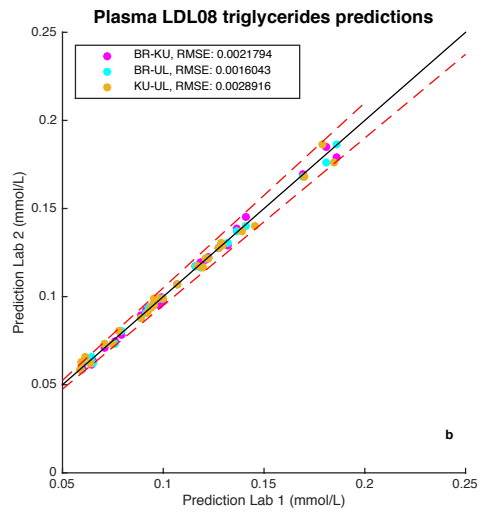
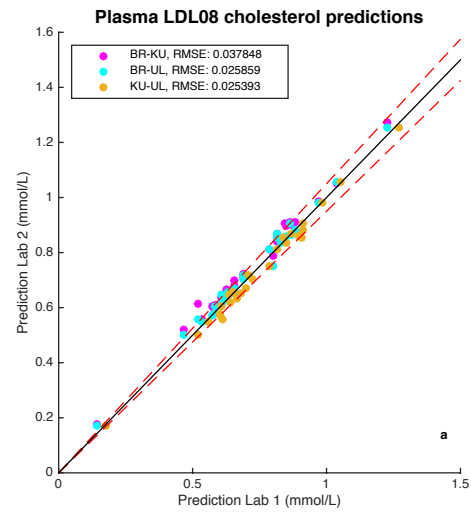
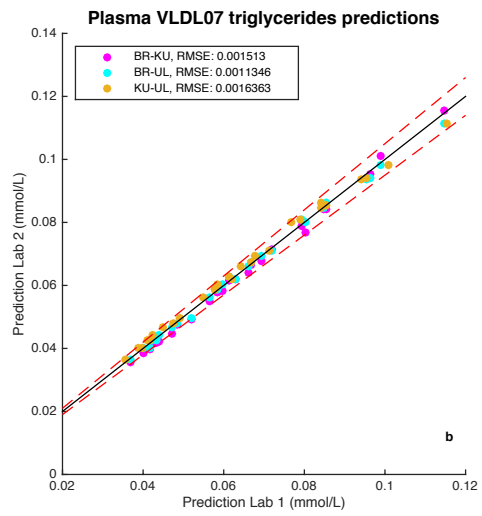
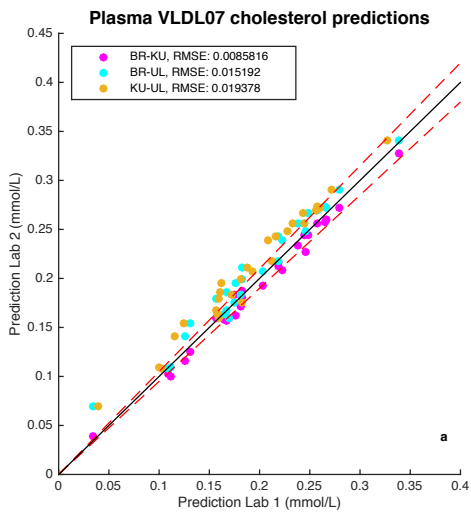
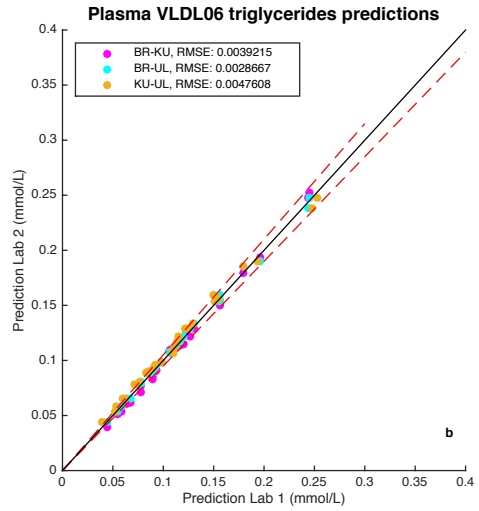
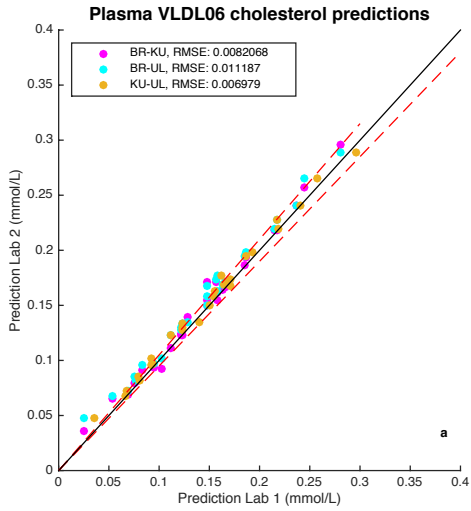
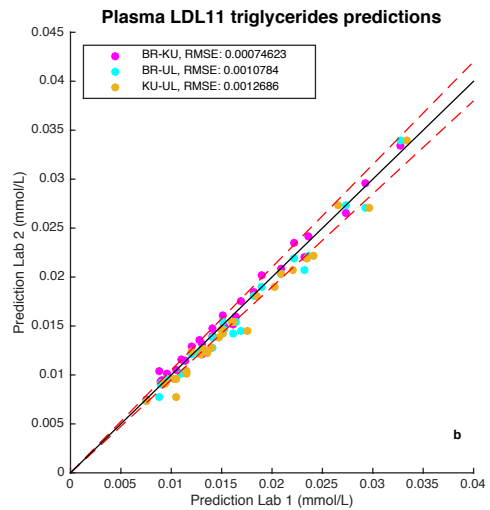
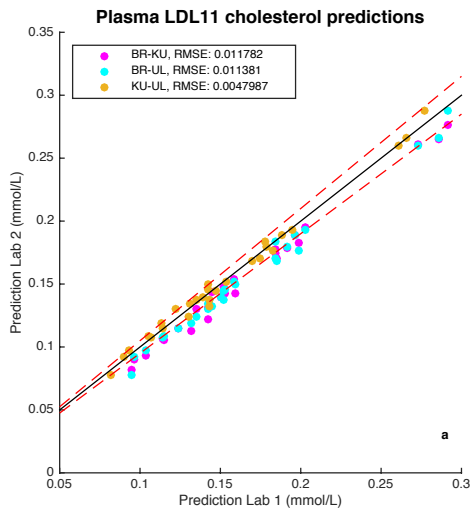
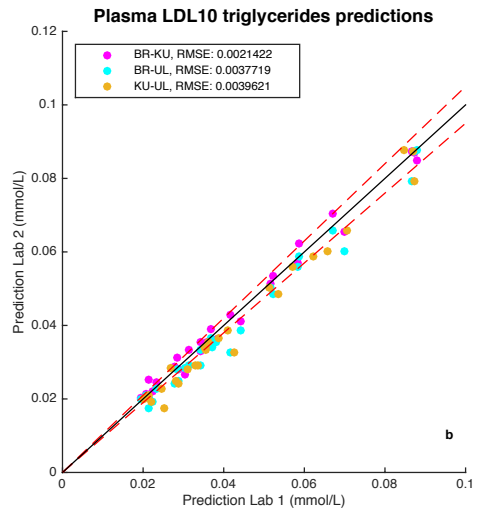
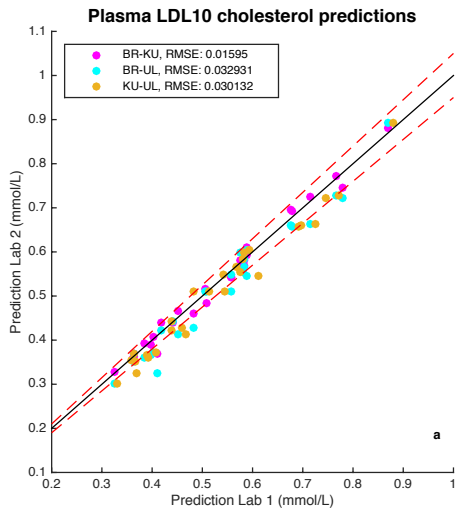
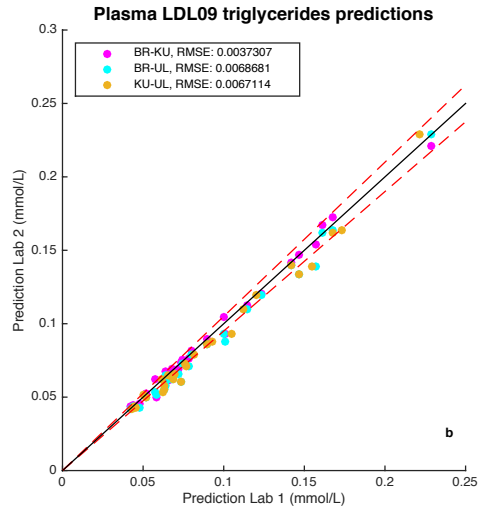
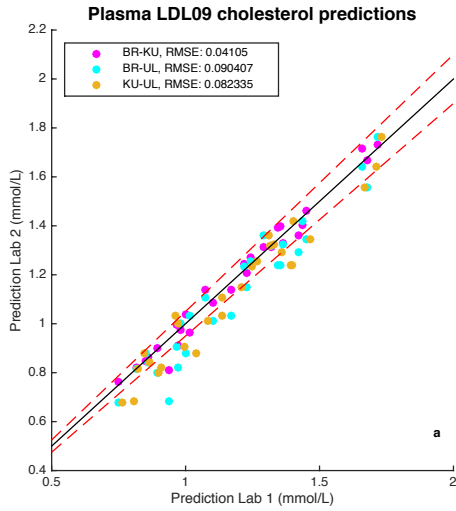


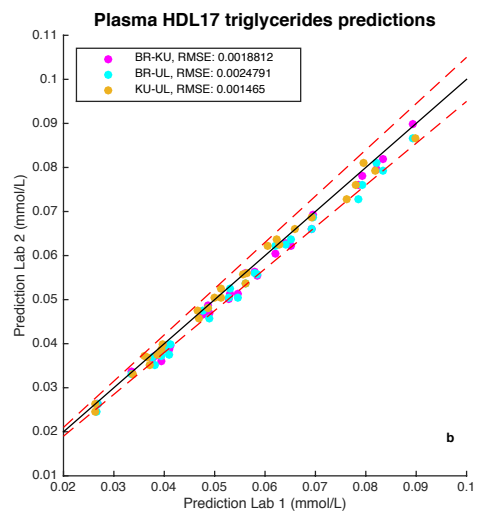
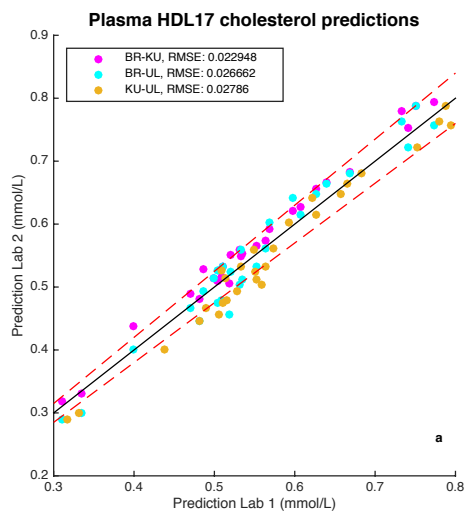
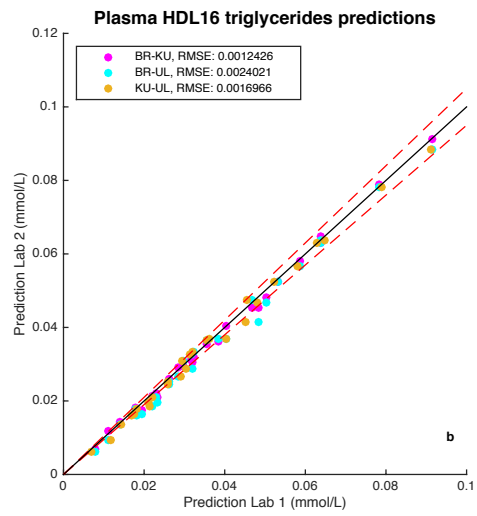
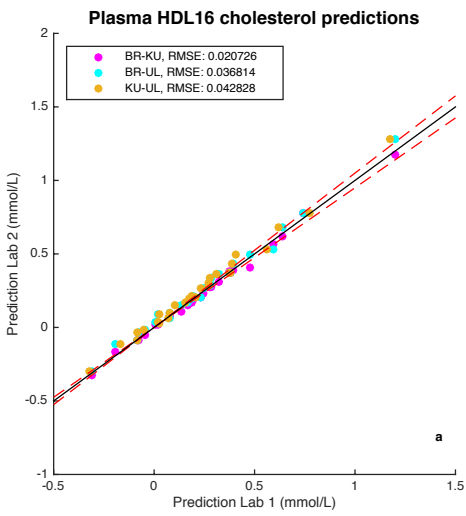
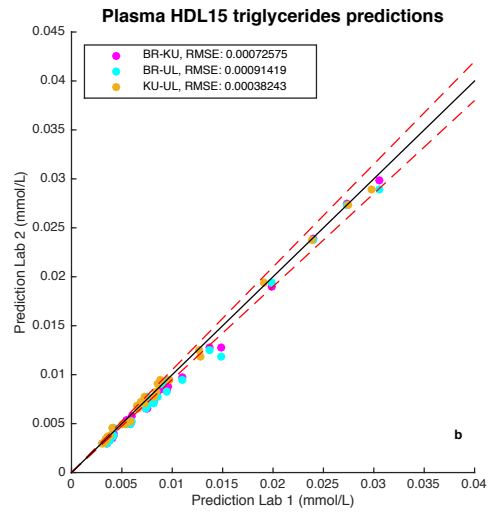
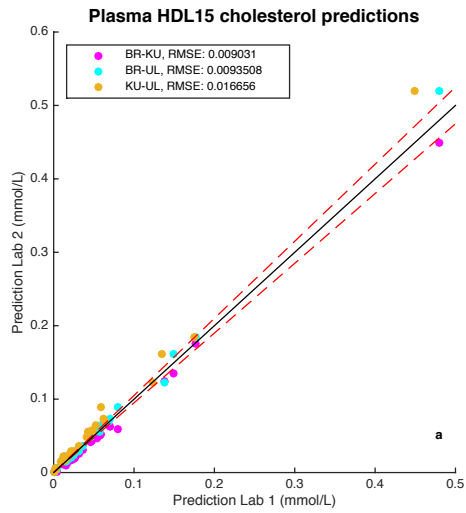
Figure S6. Between-lab differences in serum cholesterol and triglycerides predictions for all subclasses (except VLDL06, see main text) and main fractions obtained using the Mihaleva-derived PLS model. BR versus KU, BR versus UL and UL versus KU (a and b) cholesterol and (c and d) triglycerides serum 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%.

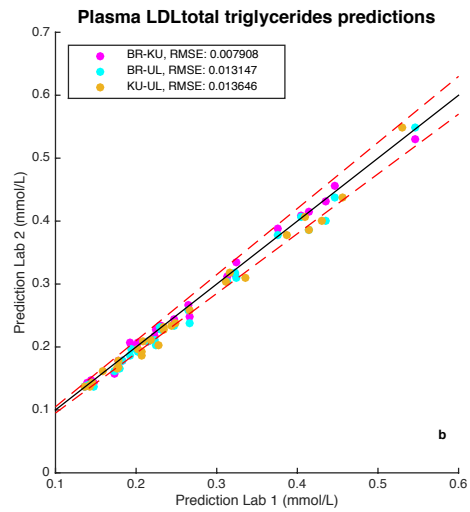
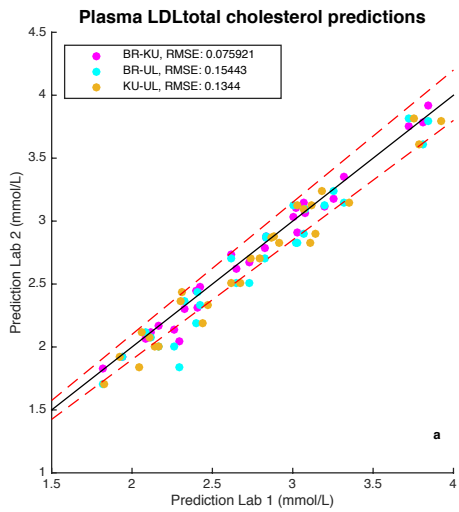
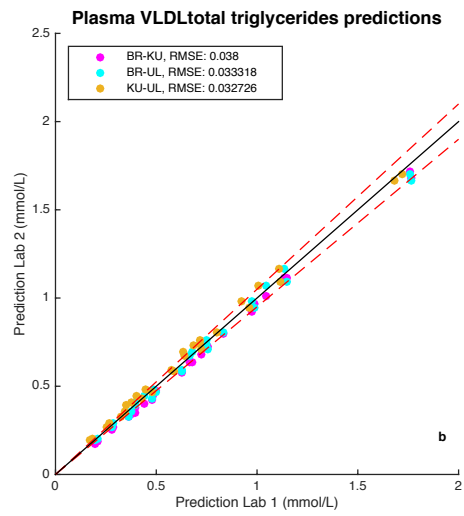
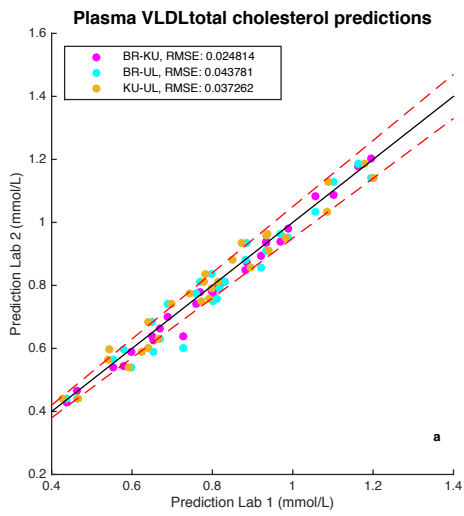
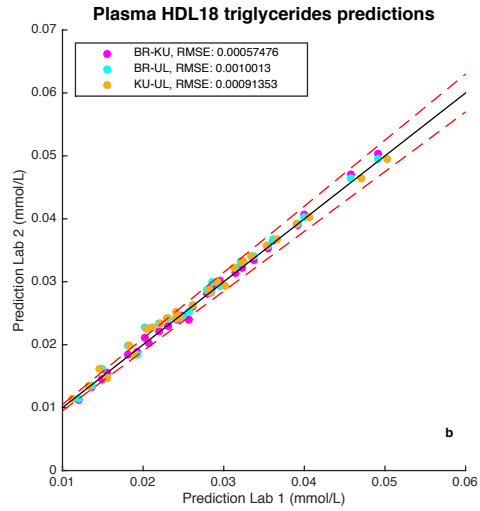
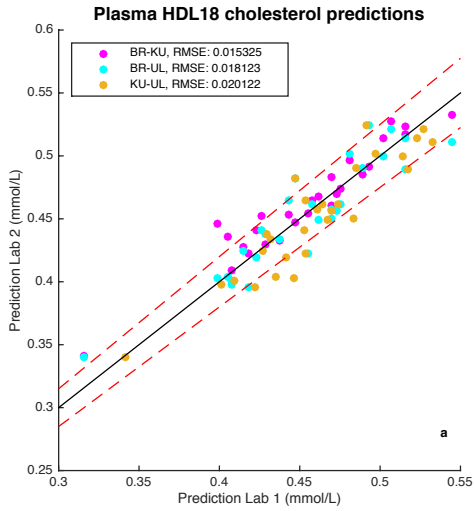
Supplementary Results. Level 2. Plasma











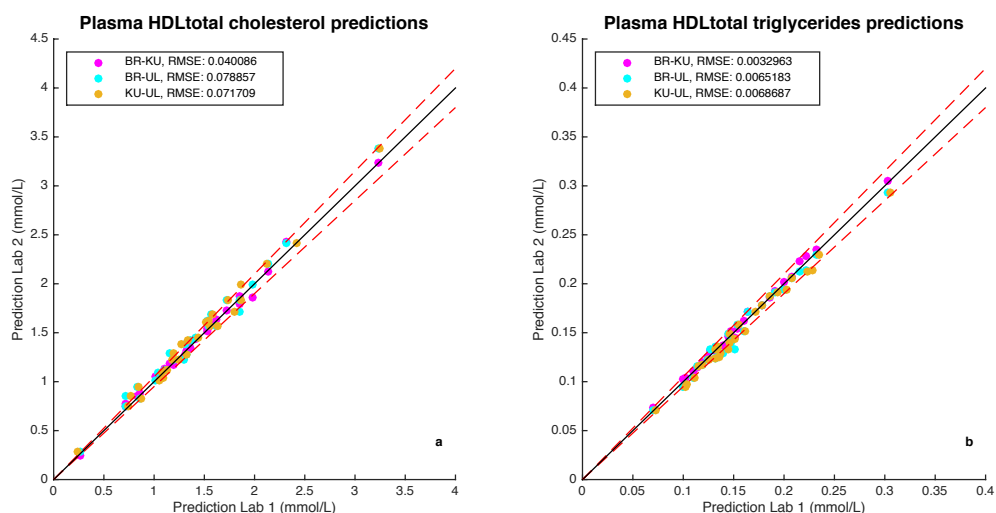


Figure S7. Between-lab differences in plasma cholesterol and triglycerides predictions for all subclasses and main classes obtained using the Mihaleva-derived PLS model. BR versus KU, BR versus UL and UL versus KU (a) cholesterol and (b) triglycerides serum predictions. On the x axis, the value of the 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

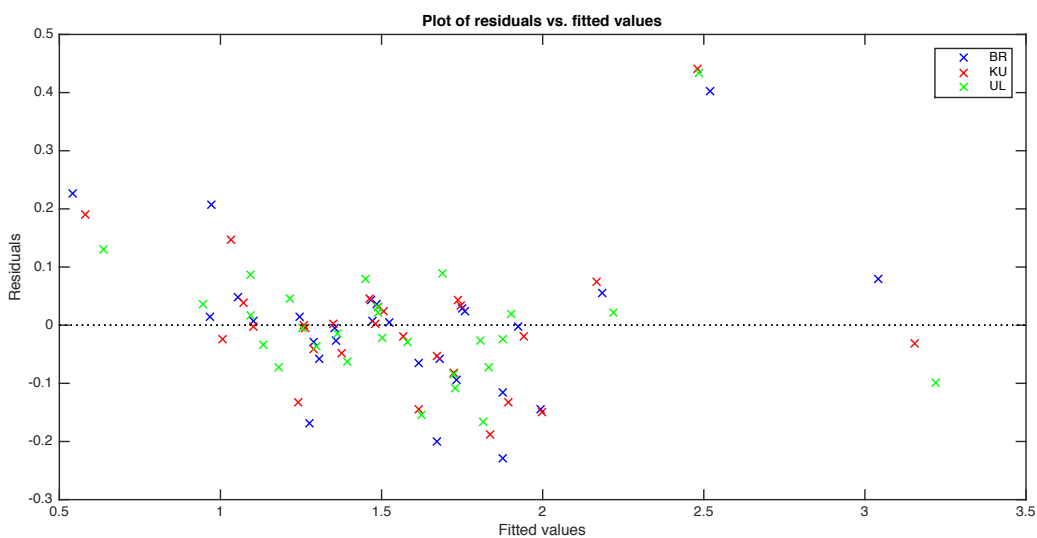


Figure S8. Ring test HDL cholesterol residual plot (PLS predictions vs. HDL-C clinical values).

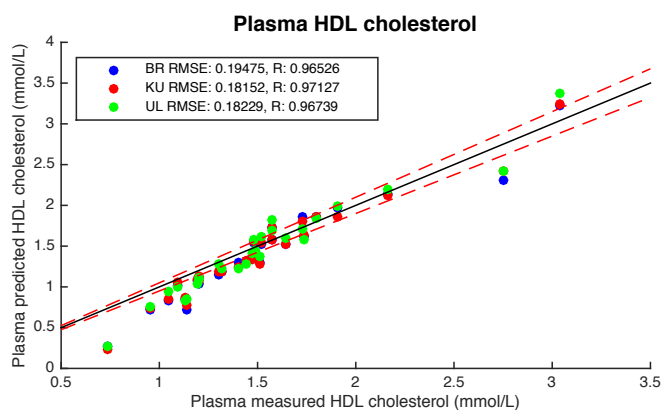


Figure S9. HDL-C predictions of the PLS model on plasma spectra from the three laboratories. The black line is drawn where prediction and measurement are equal, the red dashed lines indicate plus or minus 5%.

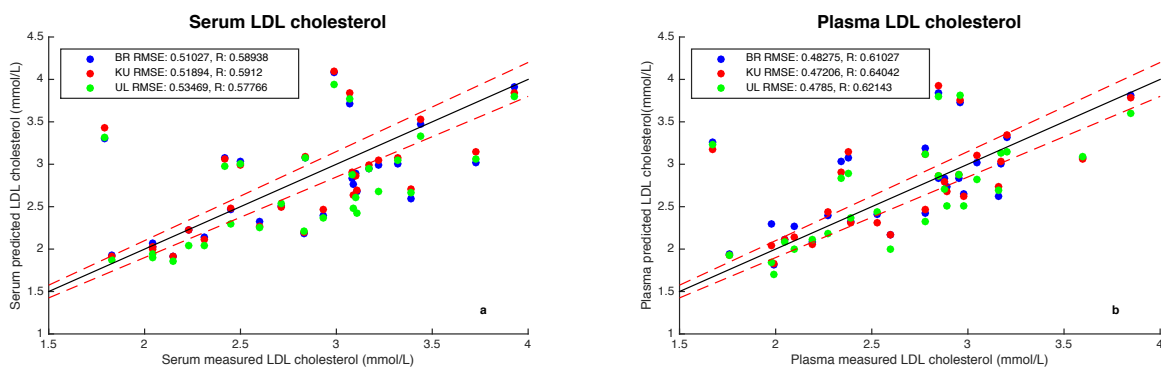


Figure S10. LDL-C predictions of the PLS model on (a) serum and (b) plasma spectra from the three laboratories. The black line is drawn where prediction and measurement are equal, the red dashed lines indicate plus or minus 5%.