Apo-calypse now? Apolipoprotein profiling to reduce residual cardiovascular disease

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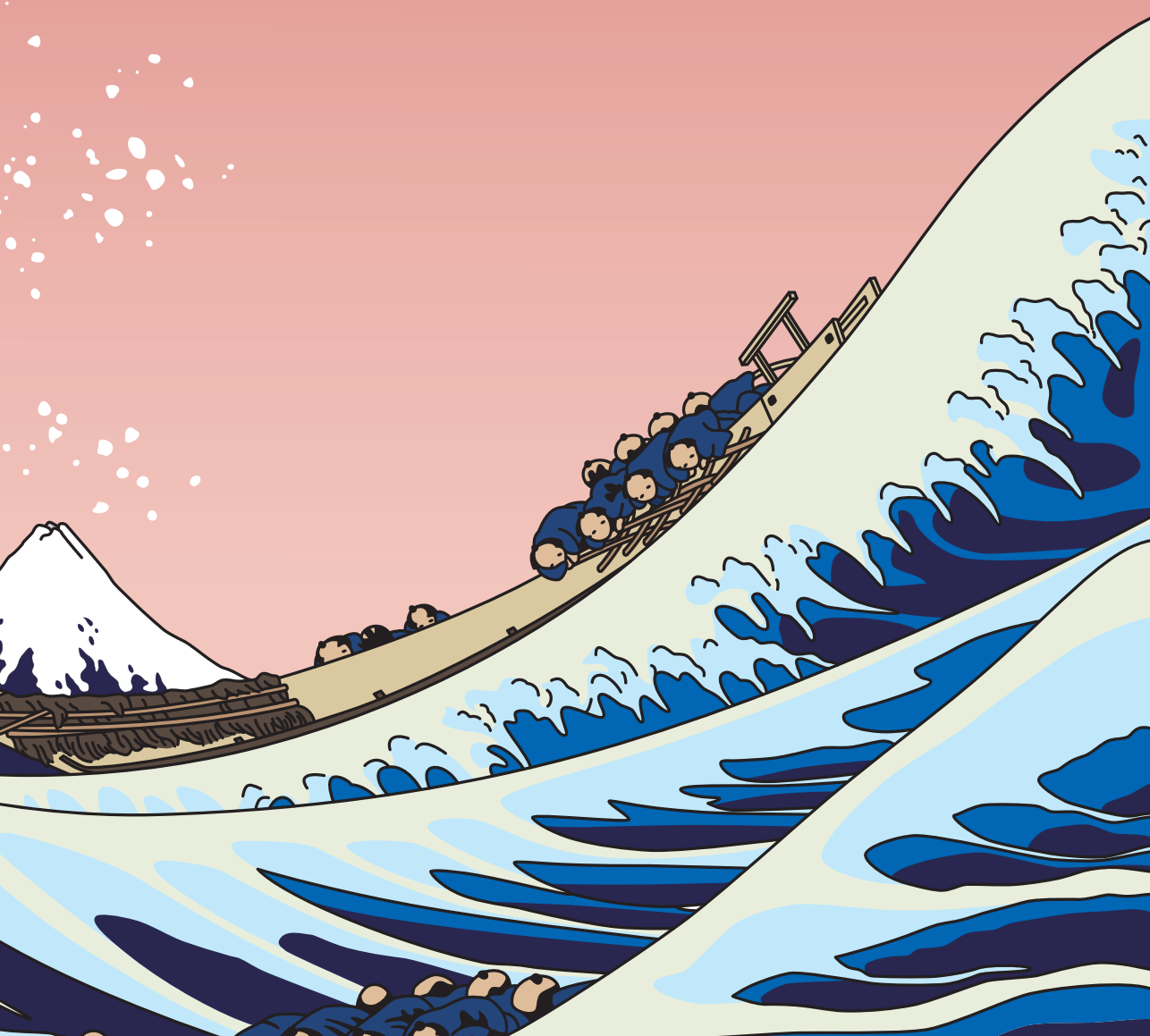
CHAPTER 3



Multiplex Apolipoprotein Test with Mass Spectrometry

MULTIPLEX APOLIPOPROTEIN TEST WITH MASS SPECTROMETRY

The analytical method applied in this thesis was not part of the original research trajectory; however, for clarity, a dedicated brief chapter describing the method has been included.

The semi-automated LC-MS/MS assay was developed for the simultaneous quantification of nine serum apolipoproteins (ApoA-I, ApoA-II, ApoA-IV, ApoB, ApoC-I, ApoC-II, ApoC-III, and ApoE) as well as qualitative phenotyping of ApoE isoforms. [1,2] The assay employs five value-assigned human serum samples for external calibration, each traceable to internationally recognized WHO-IFCC reference materials: SRM-2B for Apo(a), SP1-01 for ApoA-I, and SP3-08 for ApoB. [1,2] Serum samples are diluted 20-fold in 96-well plates, and stable isotope-labeled (SIL) peptides are added as internal standards. After tryptic digestion, the reaction is quenched, peptides are concentrated via solid phase extraction, and then analyzed using a 6495 triple quadrupole MS in multiple reaction monitoring mode (Figure 1). Sample preparation is semi-automated using a 96-channel BRAVO automated liquid handling platform, ensuring precision and throughput.

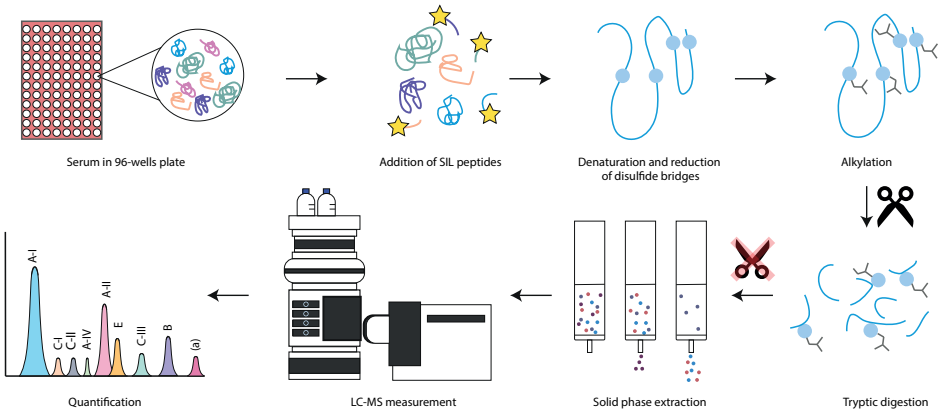


Figure 1: Workflow illustrating the processing steps of serum proteins, including the addition of internal standard peptides, denaturation, reduction, alkylation, and enzymatic digestion with trypsin, followed by solid phase extraction, LC-MS/MS measurement, and subsequent quantification for proteomic analysis.

Two signature peptides per apolipoprotein were selected, one for quantification and at least one for confirmation, to ensure accuracy and detect potential peptide interferences, genetic variants or isoforms (Table 1). For Apo(a), two peptides were selected outside the KIV2 domain, making the quantification independent of the number of repeats. Additional peptides allow specific quantification of ApoB-100 versus total ApoB and discrimination of ApoE isoforms (ApoE2, ApoE3, ApoE4) for phenotyping.

Table 1: Proteotypic peptides used for apolipoprotein quantification and ApoE phenotyping.

Protein	Quantifying Peptides	Qualifying Peptides
Apo(a)	LFLEPTQADIALLK	GISSTTVTGR, GTYSTTVTGR
ApoA-I	AKPALEDLR	VQPYLDDFQK
ApoA-II	SPELQAEAK	EQLTPLIK
ApoA-IV	LEPYADQLR	SLAPYAQDTQEK, LTPYADEFK
ApoB	TGISPLALIK	TEVIPPLIENR, FPEVDVLTK (ApoB-100)
ApoC-I	EFGNTLEDK	TPDVSSALDK
ApoC-II	ESLSSYWESAK	TYLPAVDEK
ApoC-III	GWVTDGFSSLK	DALSSVQESQVAQQAR
ApoE	SELEEQLTPVAEETR	LGPLVEQGR, LAVYQAGAR
ApoE Phenotype	**Phenotypic peptides**	
ApoE2	C[CM]LAVYQAGAR	
Non-ApoE4	LGADMEDVC[CM]GR	
ApoE4	LGADMEDVR	

A system suitability sample containing synthetic peptides reflecting both endogenous and SIL peptides is used to monitor the performance of the LC-MS/MS. Bilevel internal quality control (IQC) native serum samples are measured in triplicate per batch. This standardized procedure ensures robust and high quality data generation.[3] The assay performance is demonstrated in **Chapter 4** of this thesis, acquiring high precision and robustness over time across relevant clinical concentration ranges.

REFERENCES

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3. Smit NPM, Romijn FPHTM, van Ham VJJ, Reijnders E, Cobbaert CM, Ruhaak LR. Quantitative protein mass-spectrometry requires a standardized pre-analytical phase [published correction appears in Clinical Chemistry and Laboratory Medicine (CCLM)]. 2023;61:55-66. doi: doi:10.1515/cclm-2022-0735