

Effects of sample processing on the metabolome and proteome

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Aims

Metabolic and proteomic analysis can be affected by sample processing and storage conditions. This part of the work package was designed to investigate sample processing conditions and downstream analyses with the aim to inform strategies for the use of cohort samples.

Effects of sample processing on sample integrity

In order to investigate whether processing influences sample integrity a biological resource was established as outlined in Figure 1. Ethical approval was obtained from the NRES Committee South West-Frenchay with an end date of March 2024. Non-fasting EDTA and serum blood samples were collected from 50 healthy volunteers and treated to 6 different storage conditions (Figure 2) before processing to mimic clinical and non-clinical settings. Condition 1 is the 'gold standard', conditions 2 to 6 mimic conditions common for sample collection in cohort studies. Samples were processed

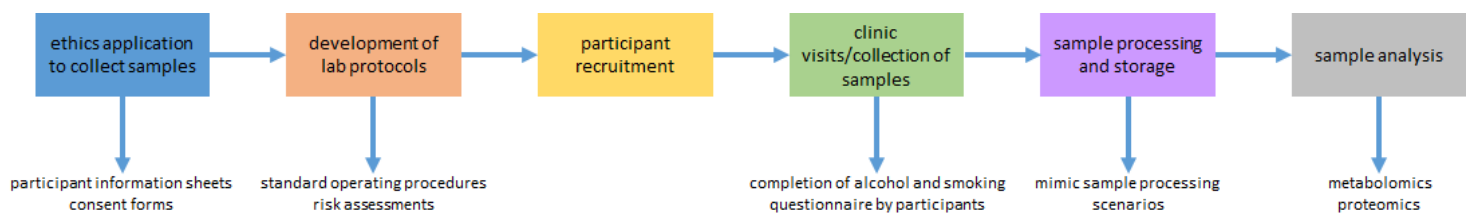


Figure 1: Establishing a resource to analyse the effects of sample processing on sample integrity

	Conditions					
	1	2	3	4	5	6
Temperature stored before processing	4°C	4°C	RT	4°C	RT	unknown
Time processed after collection	<90 min	24hr	24hr	48hr	48hr	post

Figure 2: Conditions blood tubes were maintained before processing. Key: RT-room temperature; post-samples were posted back to the lab by Royal Mail next day delivery service before being processed

using standard laboratory protocols and the aliquots generated frozen at -80°C. At the clinic visit participants were invited to answer a questionnaire on alcohol intake and smoking habits. The number of participants, the type of samples collected, and how they were processed is provided in Table 1.

Samples collected	No of participants
EDTA plasma collected using conditions 1-5	26
EDTA plasma collected using conditions 1-6	12
Serum collected using conditions 1-5	38
Individuals with EDTA plasma and serum collected using conditions 1-5	26

Table 1: Number of sample types collected with which processing conditions.

The effect of sample processing on the metabolomic profile

Using the EDTA plasma and serum samples that had been collected, as per Table 1, over 230 quantified metabolomics measures were obtained per sample using a 1D proton NMR spectroscopy-based platform (^1H)¹²³. The analysis covered three 'molecular windows' to characterise lipoproteins, low molecular weight metabolites and lipids. This work is currently being drafted for publication. In summary, time and temperature mostly impacted the concentration of low molecular weight metabolites. In particular those associated with glycolysis and a number of amino acids. Differences were observed in the degradation patterns between EDTA plasma and serum samples. Sample processing conditions had minimal effect on lipoprotein and lipid levels. When comparing results from sample collections across the CLOSER cohorts this work may be useful for correcting potential biases in small molecule measures resulting from variation of collection and processing procedures.

The effect of sample processing on the proteomic profile

To assess whether sample processing conditions effect the proteomic profile EDTA plasma samples from 3 of the volunteers, conditions 1-5, were analysed. Less samples were analysed compared to the metabolomics work due to the substantial difference in cost between the 2 analysis methods. Analysis was undertaken using tandem mass tagging followed by nano LC mass spectrometry. This work is being drafted for publication alongside the metabolomics data. To identify differentially expressed proteins conditions 2-5 were separately compared to condition 1 (the gold standard). Only E5RK69 (annexin) was found to be differentially expressed, to significance, when condition 5 was compared against condition 1. Therefore, there was no substantial evidence to suggest that the proteomic profile of a sample greatly alters due to processing conditions. Although this may be due to the limited sample size of the pilot study.

Outcomes

A biological resource has been established so that analyse platforms can be piloted to see if sample processing could introduce bias to results. Following the metabolomic and proteomic work samples from all 6 processing conditions are still available for analysis and ethical approval has been obtained for their use until 2024.

The metabolomic and proteomic work is being written up for publication. Due to the high cost of the proteomic analysis, which limited the number of samples to be analysed, it is difficult to draw a conclusion about whether processing conditions affect the proteomic profile. The cost of tandem mass tagging followed by nano LC mass spectrometry makes it infeasible for cohort studies to use this method for proteomic analysis.

Cohort studies may be restricted in the setting in which they are able to collect samples and may not have the available resources to process samples to the 'gold standard'. Pilot work may therefore need to be undertaken by cohort studies to determine if their analyte of interest is stable under different processing conditions. The biological resource generated for this work package could be used for such pilot studies.

References

- ¹ Soininen P, Kangas AJ, Würtz P, Tukiainen T, Tynkkynen T, Laatikainen R, Järvelin MR, Kähönen M, Lehtimäki T, Viikari J, Raitakari OT, Savolainen MJ, Ala-Korpela, M. High-throughput serum NMR metabolomics for cost-effective holistic studies on systemic metabolism. *Analyst*. 2009;134;1781-1785
- ² Inouye M, Kettunen J, Soininen P, Silander K, Ripatti S, Kumpula LS, Hämäläinen E, Jousilahti P, Kangas AJ, Männistö S, Savolainen MJ, Jula A, Leiviskä J, Palotie A, Salomaa V, Perola M, Ala-Korpela M, Peltonen L. Metabonomic, transcriptomic, and genomic variation of a population cohort. *Mol. Sys. Biol.* 2010;6;441

³ Soininen P, Kangas AJ, Würtz P, Suna T, Ala-Korpela M. Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. *Circ. Cardiovasc. Genet.* 2015;8;192-206