# Optimization of sample preparation for NMR-based metabolomics of infant stool

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## 1. Introduction

Research of the faecal metabolome is becoming increasingly popular since it contributes to our understanding of the complex interplay between host, diet and gut microbiota[1]. Although efforts have been made to optimize and standardize human faecal metabolomics[2], the accurate characterization of the infant faecal metabolome still lags behind. The collection of infant stool is non-invasive and easy which makes gut microbiome and metabolome studies – especially in birth cohorts – very attractive. Unfortunately, there is still no optimized sample handling protocol for proton nuclear magnetic resonance ($^1$H NMR) spectroscopy of infant stool. The aim of this study is to examine the influence of different sample preparation conditions on the $^1$H NMR metabolite profile of infant stool and to determine the optimal protocol for metabolomics analysis.

## 2. Approach

Five infants (aged between 4 and 9 months) were enrolled in this study. For each infant, ten stool samples were collected at one time point and immediately stored at -20°C. In total, 40 aliquots were obtained per infant and subjected to different sample preparation conditions. The standard/reference protocol was developed based upon previous literature[3,4]. Next, the impact of (a) type of extraction solvent; (b) dilution ratio; (c) homogenization method; (d) filtration; (e) duration of centrifugation, on the faecal $^1$H NMR metabolite profile was examined.

## 3. Results

Figure 1 illustrates $^1$H NMR spectra derived from faecal samples of the studied infants.

Preliminary results show that the signal-to-noise ratio of faecal $^1$H NMR spectra is largely affected by the dilution factor, i.e. a higher dilution shows a lower signal-to-noise ratio. Homogenization of the samples by bead beating results in higher relative concentrations of SCFA as compared with homogenization by a vortex shaker. Elongation of the centrifugation duration shows more removal of particulate matter, resulting in higher-quality spectra.

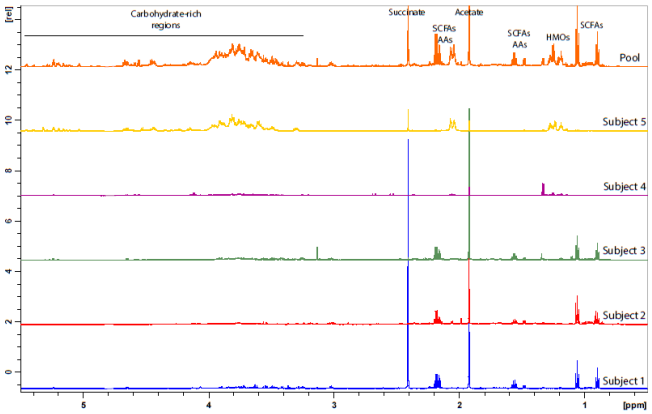


**Figure 1.** Overview of $^1$H NMR spectra (region 0.5-5.5 ppm) derived from faecal samples of infants (subject 1 to 5) and one pooled faecal sample processed according to the standard protocol. AA: amino acids; HMO: human milk oligosaccharides; SCFA: short chain fatty acids.

## 4. Discussion

In order to obtain the best signal-to-noise ratio and highest-quality $^1$H NMR spectra of infant faecal samples, we provisionally recommend to: (i) use a dilution ratio of 1:5 faeces-to-water (ii) homogenize the sample using bead beating followed by (iii) 30 min. centrifugation. $^1$H-NMR spectroscopy measurements are on-going to investigate the impact of other dilution solvents (e.g. acetonitrile) and filtration on the quality of the infant faecal metabolome. Ultimately, our findings will contribute to the development of an optimized sample preparation protocol for NMR-based metabolomics of infant stool, and its subsequent application in gut microbiome and metabolome research.

## References

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