OMNImet®•GUT: An at-home collection and ambient temperature transport device for fecal metabolomics

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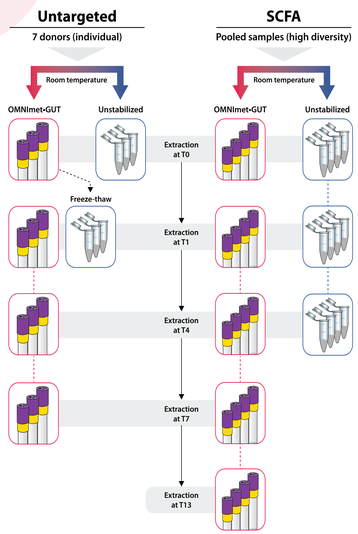
Introduction

Metabolomic analysis of feces is a key methodology for understanding the functional roles of microbial organisms and their interactions within the gut. To date, practical challenges surrounding the collection and stabilization of fecal samples from human volunteers have hampered large-scale metabolomic analyses. In response to the need for a collection and stabilization solution for fecal metabolites, DNA Genotek® has developed the OMNImet®•GUT at-home collection kit. This kit includes a device featuring a volumetrically validated sample input, a built-in mixing ball for rapid homogenization of the collected sample and a metabolite-stabilizing solution.

Methodology

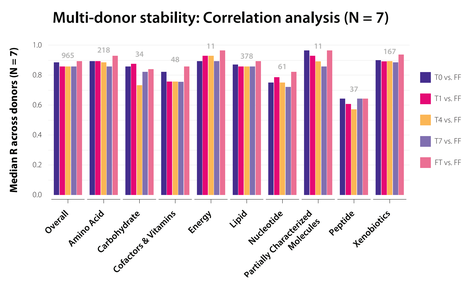
Experimental design for untargeted metabolomics and for targeted SCFA analysis is depicted on the right. For the freeze-thaw (FT) condition, an aliquot from each OMNImet•GUT (ME-200) tube was held at -20°C for 7 days, followed by 4 hours at 30°C. After the indicated storage condition, all samples were held at -80°C until analysis. For untargeted metabolomics, samples were subjected to drying followed by automated biochemical extraction and analysis by liquid chromatography and high resolution tandem mass spectrometry (LC-MS/MS) on Metabolon's Global Platform.[1,2] For SCFA analysis, feces pooled from 7 donors was stored at room temperature for varying lengths of time in OMNImet•GUT and in an unstabilized condition, followed by targeted, quantitative analysis of eight SCFAs. Each tube was centrifuged and an aliquot of the supernatant was derivatized with 2,4-difluorophenyl hydrazine in presence of internal standards. Diluted derivatization reaction mixtures were injected onto an Agilent 1290/AB Sciex QTRAP® 5500 LC-MS/MS system equipped with a C18 reversed phase UHPLC column. The mass spectrometer was operated in negative mode using electrospray ionization (ESI).



Results

Accurate measurement of individual metabolites and metabolic pathways after collection of fecal samples in OMNImet•GUT

Assessment of the fidelity and stability of metabolomic profiles from OMNImet•GUT samples was performed by calculating Spearman correlations for all detected metabolites between flash-frozen (FF) samples and matched OMNImet•GUT samples held for 0, 1, 4 and 7 days at room temperature from 7 donors.

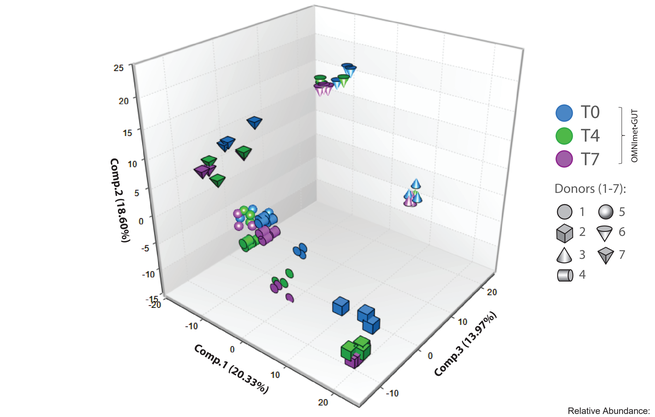


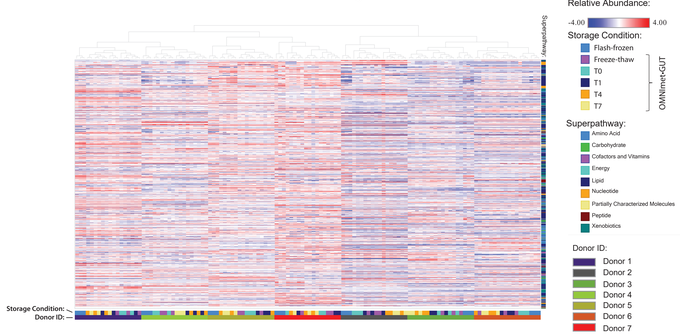
Results (continued)

- A high degree of fidelity (Spearman correlation 0.89) was observed between flash-frozen and OMNImet•GUT collected samples, which were immediately frozen (T0).
- Spearman correlations to flash-frozen raw feces remained in the 0.86-0.88 range after 1, 4 and 7 days of room temperature storage in OMNImet•GUT, indicating sustained metabolite stabilization within the device.
- Amino acid and xenobiotic superpathways, which contain most of the known bioactive microbial metabolites, displayed median Spearman coefficients in the 0.9 range at all time points (T0 to T7) in OMNImet•GUT relative to matched flash-frozen samples.

Individual donor metabolomic profiles are maintained after collection in OMNImet•GUT

Principal component analysis (PCA, top) and hierarchical clustering analysis (HCA, bottom) on matched samples from 7 donors that had been flash-frozen or stored at room temperature in OMNImet•GUT for varying lengths of time were performed to assess the impact of storing samples in OMNImet•GUT on the ability to derive biological insights from human subject data.

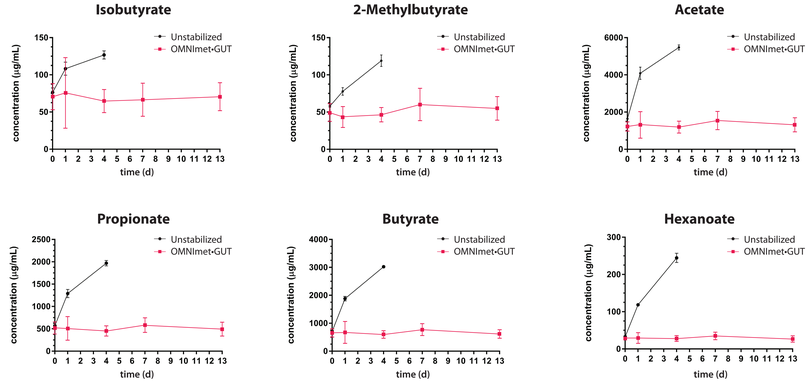




- In both the PCA and the HCA, samples clustered clearly by donor, while differences among storage conditions and time points were minor relative to inter-donor differences.
- No time-dependent trends were consistently observed in either the PCA or the HCA, indicating that storage in OMNImet•GUT for up to 7 days at room temperature preserves the unique metabolomic fingerprint of human fecal samples.

Short-chain fatty acids (SCFAs) are stable in OMNImet•GUT for at least 7 days at room temperature

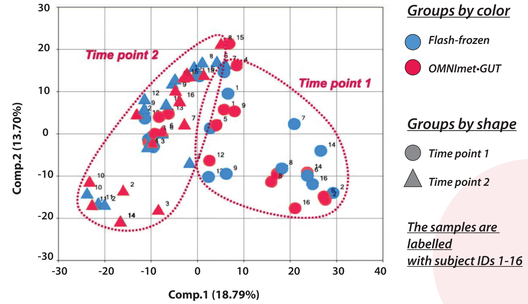
SCFAs are key mediators of gut microbial activity and play important roles in regulating the immune system, maintaining the gut epithelial barrier and preventing disorders such as metabolic syndrome, inflammatory bowel disease and certain types of cancer.[3]



While concentrations of SCFAs in raw samples increased approximately twofold to tenfold after 4 days at room temperature, concentrations of SCFAs in OMNImet•GUT samples remained unchanged between 0 and 13 days at room temperature. Concentrations of isovalerate and valerate over the same period of time displayed a similar stability pattern (data not shown). These results indicate that OMNImet•GUT quenches bacterial metabolism and that SCFAs are stable in the device for at least 13 days at room temperature.

Stabilization of metabolites of infant stool collected in OMNImet•GUT

A hospital-based field test was conducted where matched samples (n = 16 infant donors, each donor sampled at 2 time points; mean time between samples = 23 days, range 5-44 days) were both flash-frozen and stored in OMNImet•GUT tubes for 3-4 days separately.[4] In the PCA of the 1,064 metabolites detected in both flash-frozen and OMNImet•GUT tubes, samples from the same individuals clustered closely regardless of collection method. For both collection methods there was a partial separation between samples from the 2 time points, which is consistent with both methods being able to uncover time-dependent metabolomic changes that occurred across patients during their gut development.



Principal component analysis (PCA) *of Frozen vs. OMNImet•GUT fecal samples collected at 2 time points. The PCA was generated using 1,064 metabolites detected in both the Frozen and OMNImet•GUT tubes.*

Ambient temperature collection and stabilization of infant stool in the OMNImet•GUT device yielded comparable results to flash-freezing in terms of the distinct metabolomic profiles of subjects and the biochemical signature of microbiome development over time.[4]

Conclusions

The results presented here demonstrate the benefits of OMNImet•GUT:

- **Convenient at-home collection of fecal samples for targeted and untargeted metabolomics**
- **Stabilization of the gut metabolomic profiles for 7 days at room temperature**
- **No cold chain required for shipping, handling or storage**
- **LC-MS/MS compatibility (including Metabolon's Global Platform and SCFAs)**
- **Suitable for the collection and analysis of fecal samples from donors of any age, including infants**

1 Evans, A.M., Bridgewater, B.R., Liu, Q., Mitchell, M.W., Robinson, R.J., et al. (2014). High resolution mass spectrometry improves data quantity and quality as compared to unit mass resolution mass spectrometry in high-throughput profiling metabolomics. *Metabolomics* 4: 132. http://doi.org/10.4172/2153-0769.1000132
2 Ford, L., Kennedy, A.D., Goodman, K.D., Pappan, K.L., Evans, A.M., Miller, L.A.D., Wulff, J.E., Wiggs, B.R., Lennon, J.J., Elsea, S., et al. (2020). Precision of a clinical metabolomics profiling platform for use in the identification of inborn errors of metabolism. *J Appl Lab Med* 5, 342-356. http://doi.org/10.1093/jalm/jfz026
3 Tan, J., McKenzie, C., Potamitis, M., Thorburn, A.N., Mackay, C.R., Macia, L. The role of short-chain fatty acids in health and disease. *Adv Immunol.* 2014;121:91-119. https://doi.org/10.1016/B978-0-12-800100-4.00003-9
4 Ramamoorthy, S., Levy, S., Mohamed, M., et al. An ambient-temperature storage and stabilization device performs comparably to flash-frozen collection for stool metabolomics in infants. *BMC Microbiol* 21, 59 (2021). https://doi.org/10.1186/s12866-021-02104-6

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