Impact of preservation conditions on the fecal metabolome and lipidome

De Spiegeleer Margot[1], Huysman Steve[1], Van Meulebroek Lieven[1], Vanhaecke Lynn[1,2]

[1] Department of Veterinary Public Health and Food Safety, Ghent University, Ghent, Belgium
[2] Institute for Global Food Security, School of Biological Sciences, Queen's University, Belfast, United Kingdom
E-mail: Margot.DeSpiegeleer@UGent.be and Lynn.Vanhaecke@UGent.be

Eminent attention is emerging towards fecal fingerprinting, in that it unravels the symbiotic interplay between the host, diet and intestinal ecosystem enabling characteristic signatures of responsive metabolic discrepancies regarding gut (patho)physiology and beyond. It is instrumental, however, to capture and conserve a realistic snapshot of the dynamic metabolome covered upon (outpatient) sampling to circumvent erroneous interpretations and subsequent deceptive associations regarding the disease and parameter(s) under observation. Hence, this study tackles the present-day lack of efficient preservation strategies regarding the stability of the fecal polar metabolome and lipidome, using in-house developed and validated extraction and UHPLC-HR-Q-Orbitrap-MS methods (*Vanden Bussche et al., 2015* & *Van Meulebroek et al., 2017, Anal. Chem.*). By means of targeted profiling (n>400) and untargeted fingerprinting in concert with univariate and multivariate data analysis, the effect(s) of freeze-thawing (up to 2 cycles) and storage duration (up to 25 weeks), integrated with storage temperature (-20°C and -80°C) and (an)aerobicity were assessed. The impact of aerobicity was found to be negligible, which could be ascribed to the implementation of lyophilization of the fecal samples. Conversely, the freeze-thawing was pointed out predominant, suggesting that reactivated metabolic and/or microbial mechanisms may have evoked the elevated concentration levels observed in amino and fatty acids amongst others. Monitoring the long-term stability, particularly the low-molecular-weight metabolites (<400 Da) exhibited significant alterations during the timecourse of storage. To sum up, our recommendation sounds to aliquot intact fecal samples which may be stored no longer than 4 weeks at -20°C and 8 weeks at -80°C.