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iSWAB-MB: The collection device with minimal bias on microbiome compositional profile.

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Introduction:

Unlike well-established blood collection methods, the preservation of fecal samples for microbiome studies requires substantial considerations and development. The ideal transportation and storage temperature for fecal samples should be maintained between −20 °C and −80 °C, which serves as the gold standard to preserve the biological representativeness of the associated microbial community. Several commercially available stabilization buffers have been designed to preserve the microbiome profile at room temperature (RT). This advancement significantly enhanced convenience and reduced the costs associated with transportation and storage. However, the potential bias that could be introduced to the microbial community is a major concern when using stabilization buffers. In this study, fecal samples collected in Mawi buffer were compared with frozen fecal samples to assess the efficiency of Mawi buffer in preserving the microbial community profiles.

Method:

Fecal samples were collected using three lots of iSWAB-MB devices from three donors and stored at RT after collection. Frozen fecal samples were collected from the same three donors using 2mL microcentrifuge tubes and stored in dry ice immediately after collection. Triplicates of samples were processed for DNA and RNA extractions using QIAamp

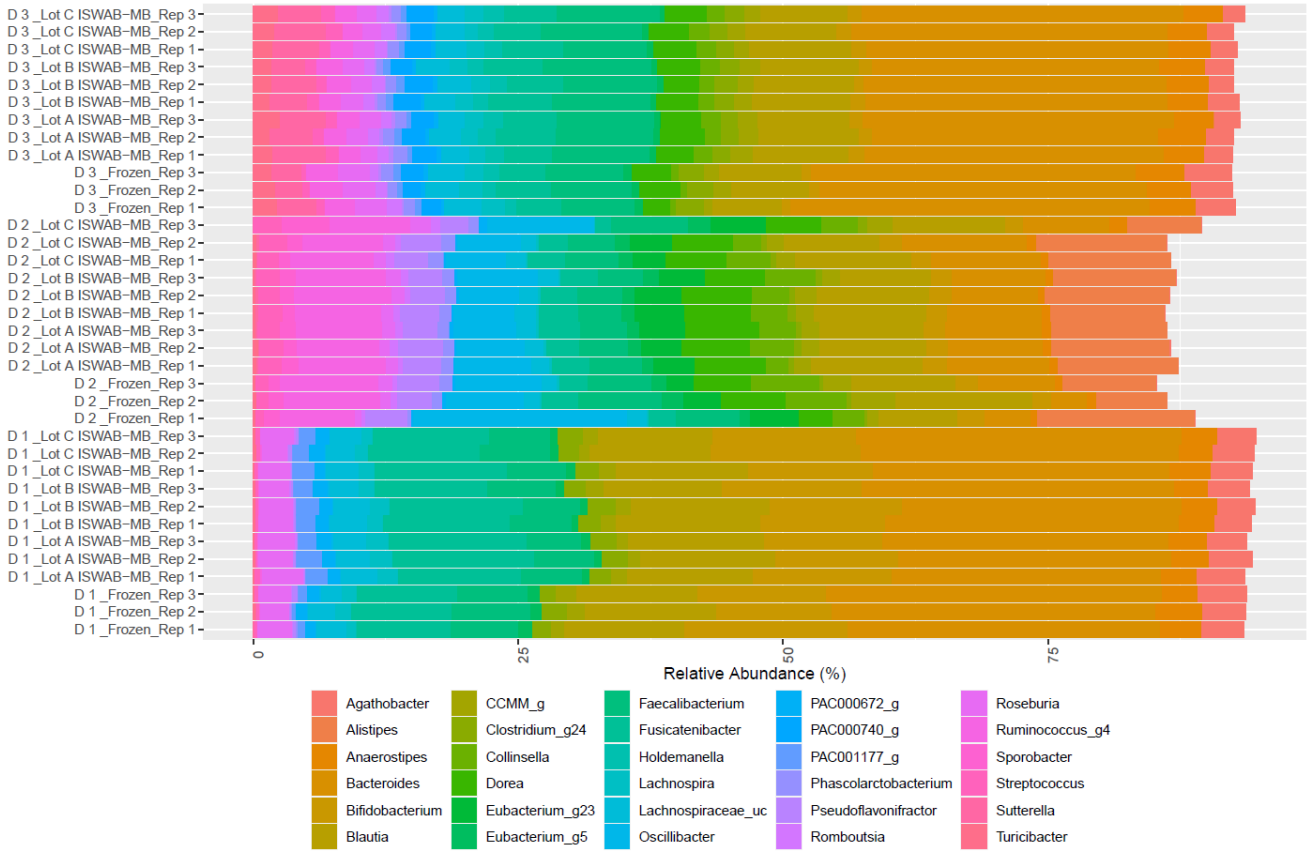

Figure 1 Relative abundance (%) of top 30 genera of bacteria in frozen fecal samples and samples collected using iSWAB-MB based on 16S rRNA gene V3-4 region sequencing result. Colors represented genera of bacteria. Samples from donor 1 to donor 3 were shown from bottom to top of the figure.

PowerFecal Pro DNA kit and RNeasy PowerFecal Pro kit, respectively. Amplicon (16S V3-V4) and metatranscriptomic sequencing and bioinformatic analysis were performed by EzBiome (MD, USA) to reveal the associated microbial community on the DNA and RNA samples and compare the efficiency in preserving the biological representativeness of microbial community in these samples.

Results and Discussions:

For each donor, the taxonomic profiles at the genus level were found to be similar between iSWAB-MB samples and frozen fecal samples, as indicated by both metagenomic (Figure 1) and metatranscriptomic (Figure 2) analysis. The taxonomic profiles exhibited no significant differences (as determined by the PERMANOVA test on Bray Curtis distance) between frozen fecal samples and iSWAB-MB samples in both DNA (p=0.985) and RNA (p = 0.107) profiles. iSWAB-MB demonstrated excellent reproducibility between aliquots and buffer lots, whereas frozen fecal samples exhibited greater variance between replicates, particularly with RNA samples. This variability in frozen fecal samples may be attributed to the challenge of achieving homogeneity in solid samples potentially leading to bias among aliquots with different sampling positions, whereas samples preserved with buffer are more easily homogenized.

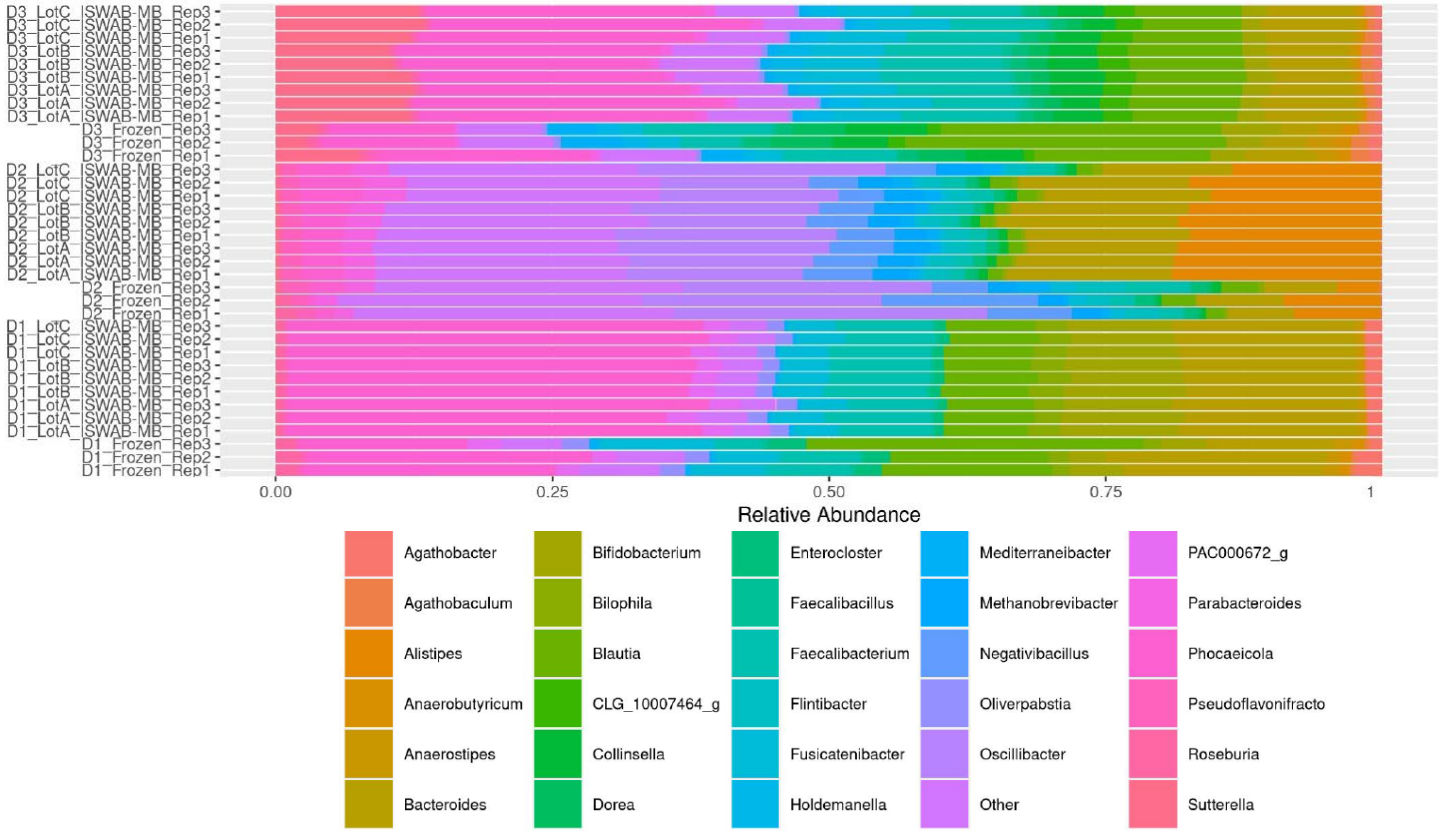

Figure 2 Relative abundance (%) of top 30 genera of bacteria in frozen fecal samples and samples collected using iSWAB-MB based on metatranscriptomic sequencing result. Colors represented genera of bacteria. Samples from donor 1 to donor 3 were shown from bottom to top of the figure.

Conclusion:

The utilization of iSWAB-MB devices presents an effective and groundbreaking solution for the collection, transportation, and storage of fecal samples at room temperature with minimal bias in the microbial community. This advancement holds immense potential for revolutionizing both research and clinical testing, enabling a universal approach to sample collection and facilitating comparisons across studies conducted by various research groups and laboratories. Furthermore, the preservation of samples with iSWAB-MB devices ensures exceptional reproducibility, allowing for reliable retesting in the future. This aspect opens intriguing possibilities for reconfirmation or reevaluation when future generations of technologies emerge, thereby propelling scientific progress to new frontiers.