

Gut microbiome samples - does stool represent right?

Orly Levitan¹, Dawn B. Burleson¹, Peter McCaffrey², Lanying Ma², Ayin Vala², David A. Johnson³

¹Hygieacare Inc., Norfolk, VA, USA ²VastBiome, San Carlos, CA, USA ³Eastern VA Medical School, Norfolk VA, USA

Introduction

- Most microbiome studies, to date, are based on stool samples. We hypothesized that these samples serve as a poor proxy for understanding the more proximal, inner-colonic microbiome.
- We studied data from metagenomic sequencing of stool samples versus inner-colonic effluent samples collected during bowel preparation (BP) by high-volume water irrigation (Fig. 1).

Methods

- Samples collection:
 - Prospectively, we recruited 20 participants undergoing an FDA-cleared high-volume colon irrigation BP before colonoscopy (Hygieacare[®] Inc., Norfolk VA), in two IRB-approved clinical trials.
 - Inner-colonic samples (n=62) were collected three times during the BP; Representing left (ascending), transverse, and right (descending) colon.
 - Stool samples (n=20) were collected by patients in their homes using a standardized kit.
- Metagenomic sequencing was done using the NovaSeq 6000. Taxonomic classification was performed by Kraken2. Biosynthetic gene clusters (BGC) were identified by the VBx1 analysis pipeline (VastBiome[™], Milbrae CA).

Results

- Patients demographics: average age 65±10 (min-41, max-78), 60% female, 40% male, with BMI 28±6 (min-19, max-43).
- The inner-colonic bacterial communities exhibited a clear biogeography gradient and were significantly different than those of stool samples. The differences were apparent by:
 - Alpha diversity - Shannon index (Fig. 2A)
 - Beta diversity - Principal Coordinates Analysis (PCoA) with Bray-Curtis dissimilarity matrix distances (Fig. 2B)
 - BGC analysis (Fig. 3).
 - Differential abundance of species between the sample types (Fig. 4)

Figure 1 - Study design. Effluent samples were collected over time during the HygiPrep, using visual characteristics of the colon effluent. Stool samples (n=20) were collected at the patients' homes. The different samples represent: Effluent 1- the left (ascending) colon (n=22), Effluent-2 - transverse colon (n=20), and Effluent 3 - right (descending) colon (n=20).

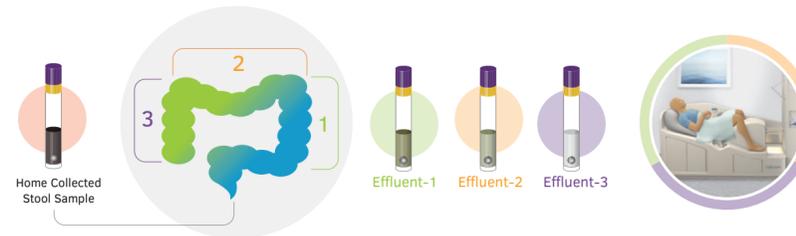


Figure 2 - Phylogenetic diversity. A. Alpha diversity between stool and the three colon effluent samples by the Shannon index. B. Principal Coordinates Analysis (PCoA) using Bray-Curtis dissimilarity between voided stool samples, n=20, Effluent-1, n=22, Effluent-2, n=20, Effluent-3, n=20. Statistical significance- the p value-cut off: ns- p > 0.05; * - p ≤ 0.05; ** - p ≤ 0.01; *** - p ≤ 0.001; **** - p ≤ 0.0001.

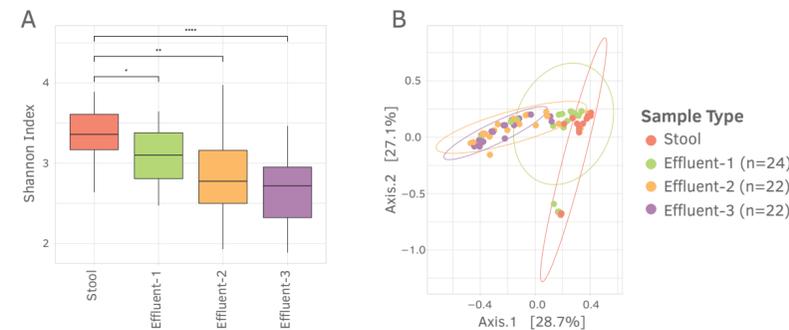


Figure 3 - Biosynthetic gene clusters (BGCs) derived from whole genome sequencing (WGS) of stool and colon effluent samples. Unique BGCs are found in HygiSamples overall and specifically within the different colon effluent representing left (ascending) colon, transverse, and right (descending) colon regions each of which yields unique BGCs and therefore unique functional biodiversity.

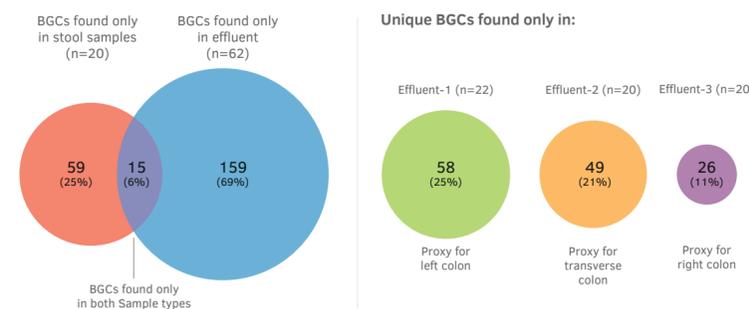
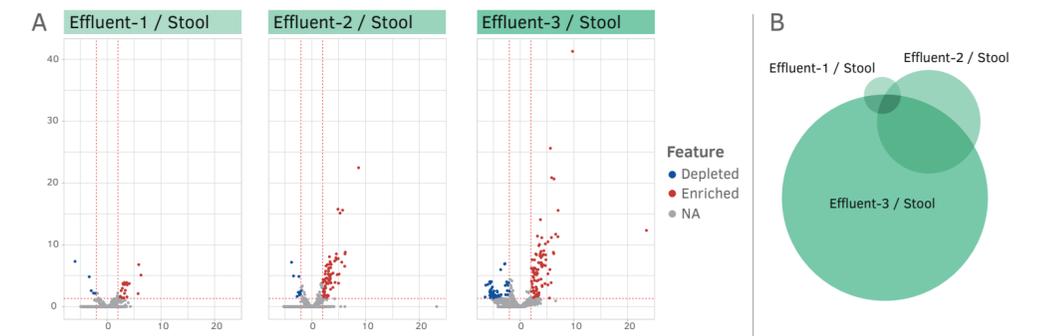


Figure 4 - Differential abundance analysis derived from whole metagenome shotgun (WGS) sequencing. A showing the log₂(FoldChange) between stool samples (n=20) and the different colon effluent samples: left (ascending) colon, transverse colon, and right (descending) colon. B. a schematic Venn diagram showing the proportion of unique bacterial species found in stool versus Effluent samples.



Conclusions

- There are distinctive differences between stool and more proximal, inner-colonic samples microbiome.
- The high percentage of unique inner-colonic BGCs illustrates biosynthetic breadth and diversity found only in those samples.
- The patented collection of microbial samples using high-volume colon irrigation BP is currently the only way to non-invasively obtain microbiome information from within the colon and of doing so without the effects of oral preparatory purgatives.
- Our data suggest that information from inner-colonic microbiome samples contains rich and unique biodiversity key for developing future biomarkers, targeted therapeutics, and personalized medicine.