A novel sampling method for analysis of the human gut microbiome

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Introduction

The gut microbiome is the most complex bacterial community in the human body. Alterations in the composition of intestinal microbiota are associated with various disease states including inflammatory bowel disease, obesity and colon cancer.

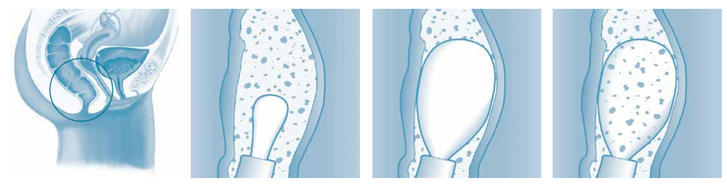
The majority of studies of the human gut microbiome have analysed stool samples although mucosal biopsy specimens have also been used in numerous studies. In this study, we investigated the utility of OriCol™, a novel CE-marked sampling device, for profiling the human gut microbiome.

The OriCol™ Sampling Device

The OriCol™ device, shown in Figure 1, is a simple, convenient method for sampling the rectal mucosa without the need for prior fasting or bowel preparation. Sampling can be performed by a trained healthcare professional in less than 5 minutes.

The device incorporates a nitrile membrane, which after insertion into the rectum via a standard proctoscope, is inflated to make contact with the rectal mucosa. The membrane is then deflated and retracted into the device prior to removal from the patient. Upon retraction the material sampled from the rectal mucosa is retained on the inverted membrane. The device can either be stored frozen or a suitable buffer added to preserve the material for subsequent analysis.

Figure 1. Device, with syringe attached (top) is inserted into the rectum via a standard proctocope. The membrane is inflated and makes contact with the rectal mucosa to collect material from the surface.



Study Overview

This study was undertaken to compare the microbiome profile collected in human stool, rectal swab and rectal mucosa. The purpose of the study was to examine the differences and relationships in gut microbial abundances using the different sampling techniques, and to validate the degree to which the OriCol™ sampling technique can accurately replicate trends in microbial diversity using stool samples as a microbial reference.

Methods

Sample Collection

Samples from the rectal mucosa, obtained using the OriCol™ device, and stool were obtained from 5 healthy volunteers on three separate occasions. A single rectal swab sample was taken from each volunteer immediately before the final OriCol™ sample. The sampling timeline and storage conditions are outlined in Figure 2. Stool samples were initially stored at -20°C in a home freezer followed by -80°C when transferred to the laboratory. OriCol™ samples taken at t1 and t2 were stored without preservation buffer at -20°C for 72h and then transferred to -80°C prior to DNA extraction. The OriCol™ samples taken at t3 were stored at 4°C after the addition of 3 ml of RNASave (Biological Industries, Israel). Fecal Transwab® rectal swabs (MWE, Wiltshire UK) were also stored in RNASave at 4°C rather than the liquid Cary Blair transport medium provided with the swab.

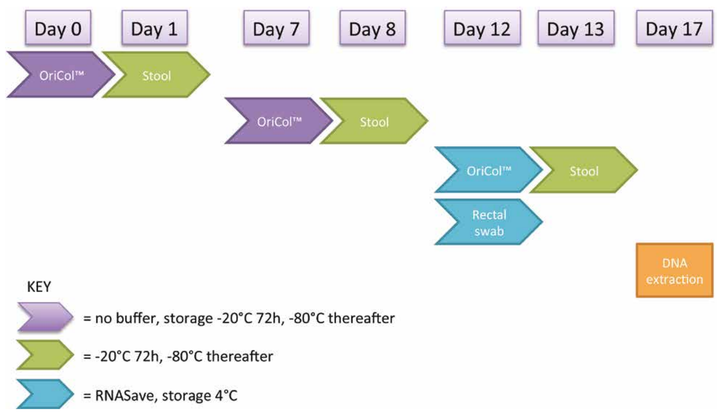


Figure 2. Sample collection and storage workflow.

DNA Extraction

Microbial DNA was extracted from all samples using the PowerFecal® DNA Isolation Kit (MOBIO Laboratories) and frozen at -20°C prior to analysis. Stool samples were processed according to the kit manufacturer's instructions. The material collected using OriCol™ and rectal swab samples was removed, after discarding the RNASave buffer where used, by vortexing in phosphate buffered saline containing 1% Triton X-100 and pelleted prior to DNA extraction.

Microbiome Profiling

The microbial community of samples was profiled using V4 16S rRNA gene sequencing and was carried out as a service by Second Genome, Inc (San Francisco, CA) on a MiSeq instrument (Illumina, San Diego, CA).

Data Analysis

Data analysis was performed by Second Genome using the secondgenomeR package: 0.1.56. Two samples were filtered out of the data set due to insufficient reads; one stool sample (Female B) and one rectal swab sample (Male A).

Results

Sample Diversity

Sample richness was assessed by Operational Taxonomic Unit (OTU) counts and Shannon diversity index in each individual sample (Figure 3). Observations in OriCol™ and stool samples showed consistently similar levels of alpha diversity. Trends between sample triplicates were fairly consistent between the two. This was not observed in the rectal swab samples, three of the four samples included in the analysis had extremely low library sizes and diversity limited to just a few OTUs. This sampling technique appeared to have limited utility either due to insufficient quantity or quality of the DNA collected and was therefore not representative of the true community profiles.

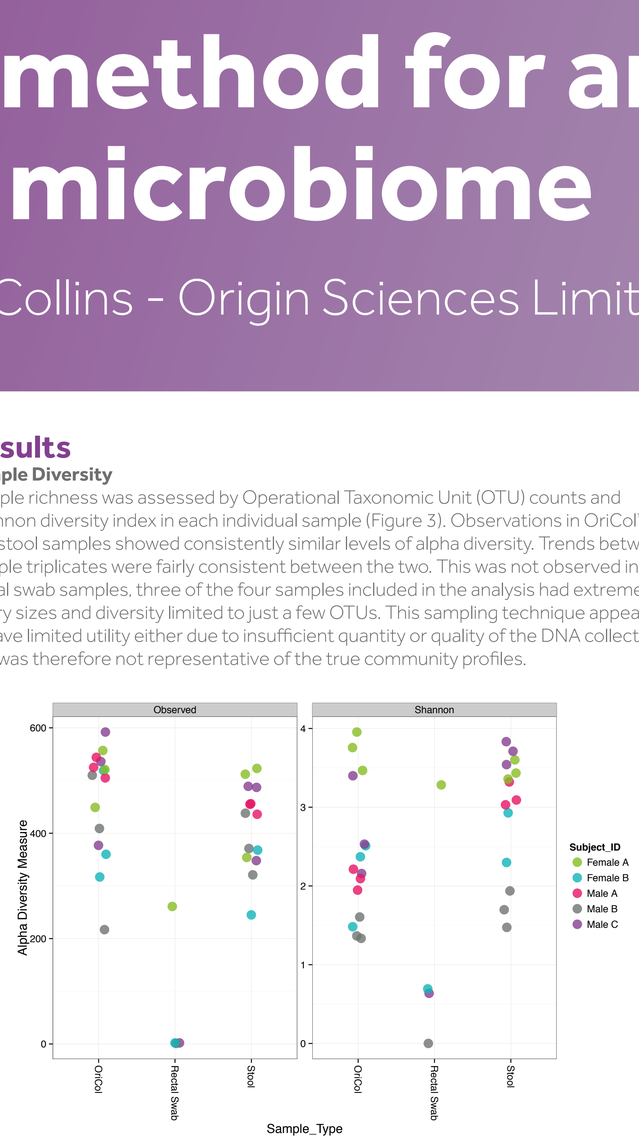


Figure 3. Observed Alpha Diversity and Shannon Diversity Index in all samples.

Sample Composition by Phylum

The relative abundance of the dominant four Phyla in each sample is shown in Figure 4 (the seven other Phyla had abundances of less than 0.1). A high proportion of Bacteriodetes, Firmicutes, Proteobacteria and Actinobacteria is observed in all OriCol™ and stool samples. As expected, the rectal swab samples did not reflect the same phylogenetic diversity due to the low library sizes.

The Bacteriodetes : Firmicutes ratio differed between subjects but was similar in OriCol™ and stool samples. For example, Male B had a higher Bacteriodetes : Firmicutes ratio than the rest of the subjects in both OriCol™ and stool samples. Significantly higher levels of Proteobacteria were observed in OriCol™ over stool for four of the subjects (Female B and Males A, B and C) whilst the opposite was true for Actinobacteria for Female B and Males A and B.

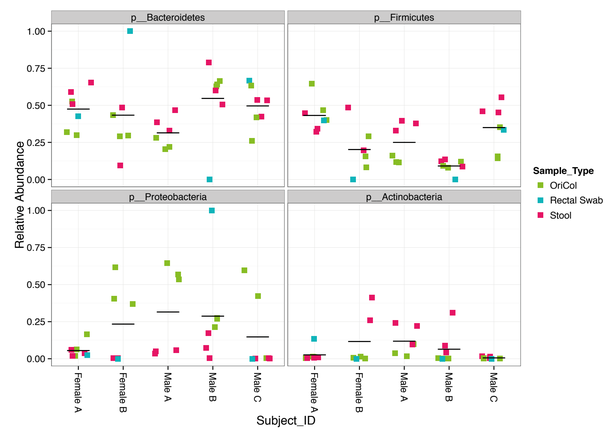


Figure 4. Relative abundance of the dominant four Phyla in each sample.

Sample Composition by Family

The most abundant 9 families are shown in Figure 5. Similar trends in abundances of the Bacteroidaceae are observed in OriCol™ and stool samples. OriCol™ samples showed a consistently higher abundance of Enterobacteriaceae than stool, while the Actinobacteria Bifidobacteriaceae and Bacteriodetes Rikenellaceae had higher mean abundances in stool samples. The other top family abundances were fairly consistent between the OriCol™ and stool samples.

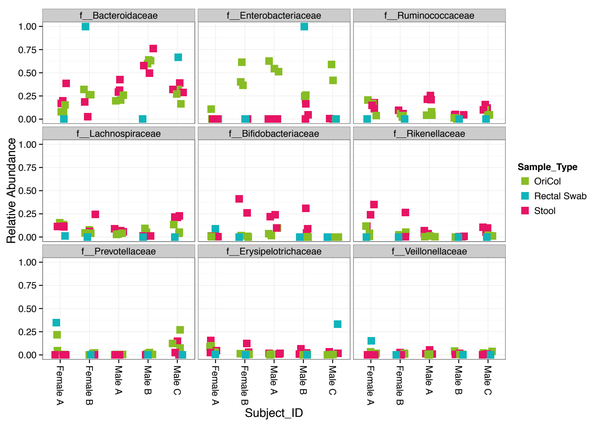


Figure 5. Most abundant nine Families.

The phylogenetic trees for OriCol™ and stool samples are shown in Figure 6. The number of samples containing each family is indicated by the height of the bar. The trees are very similar for both samples types indicating that the difference between OriCol™ and stool is due to a shift in abundance rather than the presence or absence of bacteria.

Family-level tree

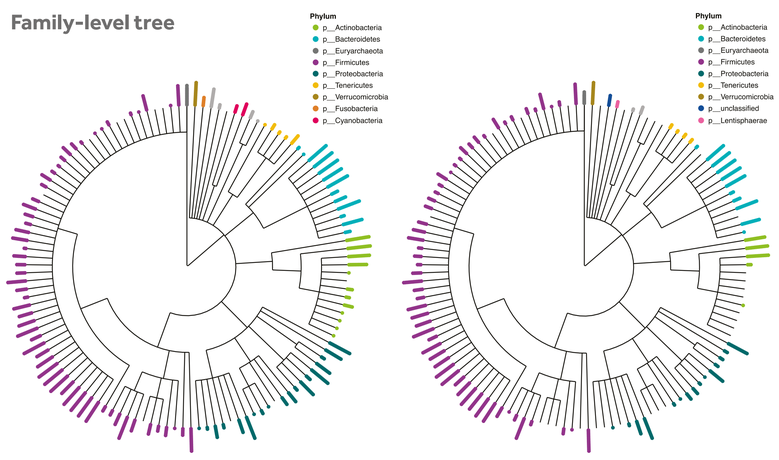


Figure 6. Phylogenetic tree at the Family rank for OriCol™ (top) and stool (bottom).

Whole Microbiome Overview

Comparison of the whole microbiome abundance profiles (beta diversity) between samples showed statistically significant differences in microbial composition between OriCol™ and stool samples when grouped by subject ($p<0.001$) and sample type ($p<0.001$). There were no significant differences over the three time series intervals. Further comparison of the two different OriCol™ storage methods (t1+t2 stored at -80°C versus t3 stored in RNASave) showed no statistically significant difference in microbial composition ($p = 0.896$).

Specific analysis of OriCol™ versus stool samples, paired by subject and time point reveals that the Bacteriodetes family Prevotellaceae was most enriched in OriCol™ samples (Figure 7). A large number of the Firmicutes families were also enriched in OriCol™ over stool as wells as theProteobacteria Camplylobateraceae, Enterobacteriaceae and Oxalobacteraceae. The Actinobacteria familes Coriobacteriaceae and Bifidobacteriaceae, and the Bacteriodetes familes Rikenellaceae and Bacteroidacae were found to be under represented in OriCol™ when compared to stool.

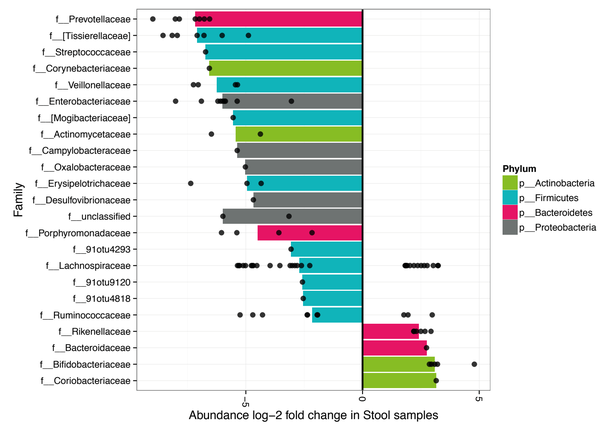


Figure 7. Graphic Summary of Feature (OTU) Selection. The 100 most significant features are shown out of a total of 181 significantly different features selected. Note families 91otu4293, 91otu9120 and 91otu94818 are placeholders for OTUs previously observed in at least 3 other datasets analysed by Second Genome and can be considered as biomarker sequences for as yet unclassified/uncultured taxa.

Subject Comparisons

OriCol™ and stool samples from different subjects were compared to determine whether both sampling methods reported similar trends between subjects. According to pairwise donor comparisons the differential taxa found in OriCol™ and stool samples share substantial overlap. OriCol™ samples tend to show similar over- and under-abundances for the same differential taxa when compared to stool samples, especially for the most significantly different features. There are also differential taxa unique to both OriCol™ and stool samples in the same comparisons. As an example, the comparison Male A versus Female B (shown in Figure 8) showed 276 significantly different features in stool samples and 241 in OriCol™ samples. Of these features, 142 were shared between the two samples types, 134 were unique to stool and 99 unique to OriCol™.

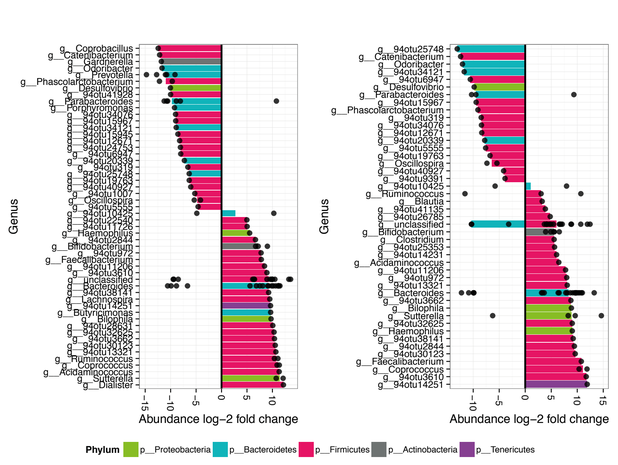


Figure 8. Graphic Summary Feature (OTU) Selection for OriCol™ (left) and Stool (right) samples from Male A versus Female A. The 100 most significant features are shown.

Summary

- The range and trends in alpha diversity between subjects were mirrored in OriCol™ and stool samples.
- Rectal swabs samples show lower diversity and this method of sampling had limited DNA collection and thus, in this study, did not represent the true community profiles.
- Significantly higher levels of Proteobacteria were observed in OriCol™ over stool.
- Whole microbiome abundance profiles are significantly different between OriCol™ and stool samples when grouped by subject or sample type.
- The OriCol™ device is able to capture differences between donors, but the microbial community is different to that from stool samples.

Conclusion

The OriCol™ device offers a novel, simple and convenient method for physician-led sampling of the gut microbiome represented in the rectal mucosa. Samples are collected in a reproducible manner and can be stored at either -80°C or, with the addition of a suitable buffer, at 4°C prior to DNA extraction and analysis.

Future Work

Further exploration of the differences in the communities captured in OriCol™ and stool samples could be achieved using whole metagenome sequencing, which would also provide information on archeal, fungal and viral communities. Comparsion of the microbiome represented in mucosal biopsy samples with that in OriCol™ samples is also planned.

The sample device is being marketed as OriCol™ by Origin Sciences. OriCol™ is CE-marked in accordance with the requirements of the Medical Device Directive 93/42/EEC.

For further information please contact Dr Booth at **jodie.booth@originsciences.com**