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The importance of sample collection and controlling other sources of variability for high quality data in microbiome research

The human microbiome

The field of microbiome research has generated overwhelming enthusiasm in recent years. Advances in culture-independent methods to study the microbial communities in and on the human body have made it clear that these microorganisms are intricately linked to health and disease, opening the door to a new era of microbiome-based diagnostics and therapeutics.

So, what can we learn from the microbiome?

The gut microbiome in particular plays an integral role in our health and well-being, and we now know that microbial imbalances can influence our risk of developing cardiovascular disease[1], nervous system disorders[2], metabolic disorders[3] and inflammatory diseases[4], among others. The gut microbiome can even influence how a patient will respond to a particular therapy. Because of this, researchers are turning to microbiome analysis to identify key biomarkers of health and disease and to develop diagnostic classifiers and new treatment strategies for a range of health conditions. To realize the full potential of microbiome-based healthcare innovations, accurate characterization of these complex microbial communities is essential.

Controlling sources of variability in microbiome research

There are many variables in microbiome research that need to be considered and suitably controlled to generate robust results and avoid misleading signals. This article will primarily focus on controlling variability during sample collection, however, it's important to note that variation in any one of the sources listed below can result in large changes to the original microbial community:

Sample collection and preservation: Microbial communities change once removed from their natural environment, so sample collection, transportation logistics and storage are critical factors in ensuring that what is measured is an accurate reflection of the source material. We will explore this more below.

Laboratory processing: Once the sample is ready for processing, there are other major sources of potential variability that need to be considered to get the best possible data. Consistency in sample handling, DNA extraction methods, sample processing controls, reagent batch management and cohort batching are all critical.

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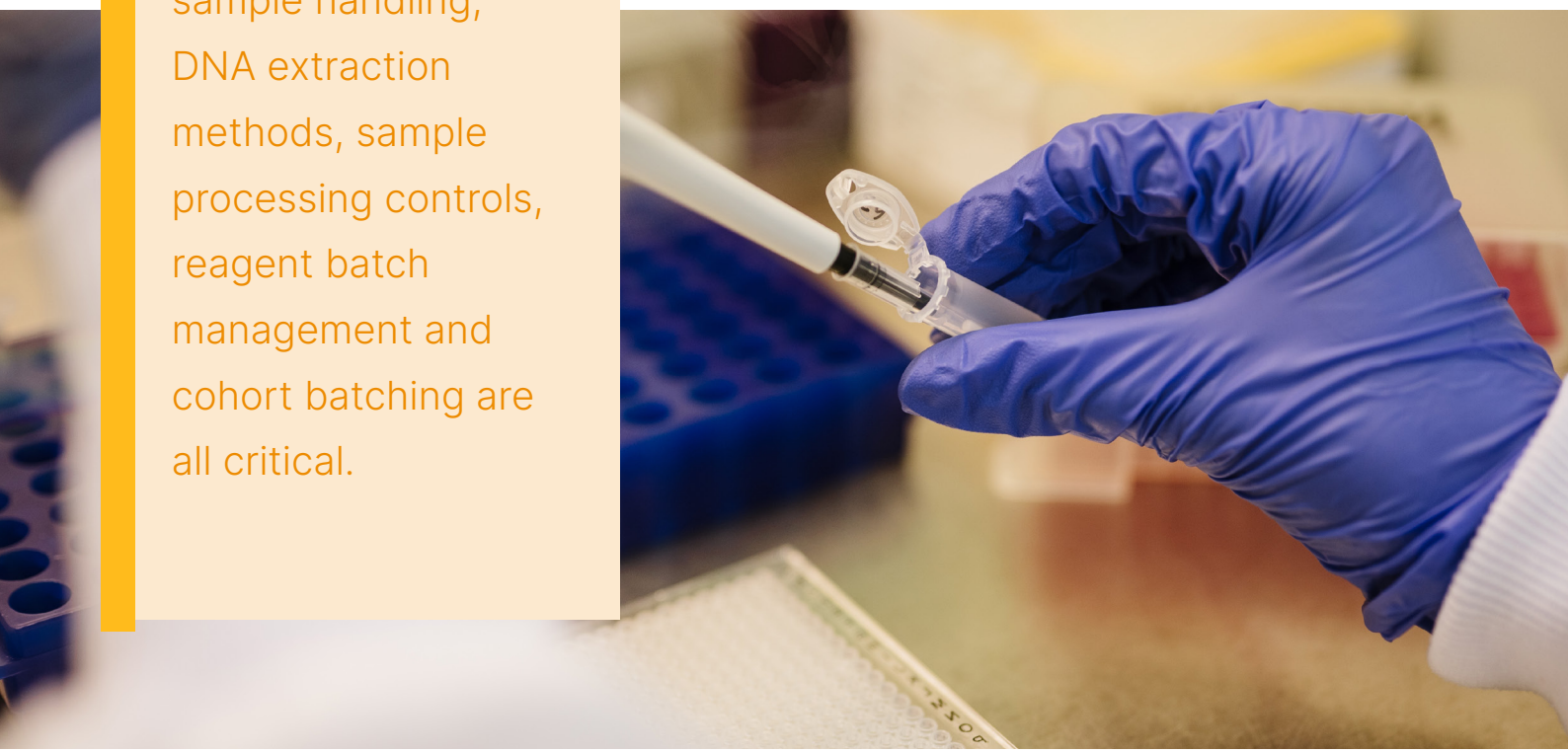

Data generation and analysis: Prioritizing high-resolution sequencing, accurate profiling, and appropriate tertiary analysis methods will also help you get the most robust results from your study. We will explore many of these factors in subsequent articles.

Throughout each of these steps, it's important to keep factors consistent so that non-experimental variables are minimized. It is therefore worth doing significant planning at the beginning of your study to consider each of these sources of variation and choose the methods that will give you the best results.

One of the first sources of variation to consider is sample collection. Other important sources of variation will be explored in subsequent articles in this series.

The need for reliable sample collection

An increasing number of studies are using stool-based gut microbiome analyses for the discovery of disease biomarkers and therapeutic targets. These generally rely on room temperature preservation methods to collect and store samples until they can be frozen or analyzed. However, if samples are not preserved adequately, shifts in the original microbial community can occur which can result in researchers chasing false leads.

Dr Alena Pribyl, Senior Scientist at Microba, explains, "When we were evaluating sample collection methods for Microba, we realized that the field was lacking sufficient benchmarking studies using metagenomic sequencing to evaluate the ability of different methods to preserve microbial communities from the human gut. So, we decided to undertake our own study".

"We were surprised to see considerable variation in diversity, compositional and functional profiles, and technical reproducibility between common sample collection methods", said Dr Pribyl. "The most concerning finding was that some methods resulted in an inconsistent outgrowth of facultative anaerobes such as Escherichia spp. and Citrobacter spp. When assessing the gut microbiome, this can have major consequences for the interpretation of results and can lead researchers to incorrect conclusions."

The team found that the best performing collection method was a swab that was actually designed for forensic DNA sampling and is based on active desiccation of the sample. The swab could accurately stabilize microbial communities at temperatures of –20°C, room temperature and 50°C for a period of 4 weeks. Additional testing (unpublished) extended this time out to 10 weeks at room temperature. Another benefit of the device is that it is liquid-free and easy to use, requiring only a small swab of fecal material from toilet paper, rather than the confronting, and often complex, 'catch and collect' method used by many other collection devices. Based on these findings, Microba now recommends this swab for all stool microbiome studies.

"We also found that in addition to high compliance by participants using this method, their ability to collect a sample accurately was extremely high."

The impact of sample collection on research outcomes

If the method used to collect fecal samples fails to provide an accurate, stable and reproducible representation of gut microbial communities, you lose the potential to gain meaningful results. Even more, having a versatile and easy-to-use method is an important consideration to ensure high participant compliance rates in large studies where samples may be transported over long distances.

The past two decades of research have made it clear that the microbiome holds important insights into human health and disease. But if we can't analyze it accurately, we are missing out on an incredible opportunity to transform human health. This involves controlling sources of variation in microbiome studies, starting with using a reliable sample collection method.

Further reading

Pribyl, AL, Parks, DH, Angel, NZ et al. Critical evaluation of faecal microbiome preservation using metagenomic analysis. ISME Commun. 2021;1(14)

Illumina & Microba: Empowering microbiome research

Microba Life Sciences and Illumina work together to accelerate microbiome research. Combining Microba's high-quality proprietary gut microbiome Analysis Platform with Illumina's revolutionary Next Generation Sequencing tools, researchers have access to world-leading, accurate metagenomic data to drive new discovery from the microbiome.

References

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