Whitepaper:

# INVESTIGATING THE HUMAN MICROBIOME USING NEXT-GENERATION SEQUENCING

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## INTRODUCTION

The human microbiome contains microorganisms from the three domains of life, and viruses, that co-habit in different human body sites.[28] Although the human gut microbiome is maybe the most known, since our gastrointestinal tract harbors the highest amount of microorganisms in our body, many other microbiomes have been discovered, such as the oral, skin, vaginal, urinary tract, airway, and even the blood microbiome.[1,4,7,23,25] The term "human microbiota" should be reserved to refer to the presence of the microorganisms themselves, whereas the term "human microbiome" reflects its broader implications to the environment, i.e., the human host.[21] Indeed, the human microbiome can be considered as our 'second genome', being an overlay of our own genetic blueprint, the human genome. Microorganisms provide human bodies with ecosystem services that our own cells have not the capability for, and that play critical roles in human health.[16,25] It is clear now that the human microbiome is a dynamic entity, which is affected by a variety of factors, such as human genetics, birth mode, age, antibiotics usage, diet, lifestyle, and air pollution.[3,14,22,24]

Recent technological advances including next-generation sequencing (NGS) have allowed us to gain an unprecedented look into the human microbiome, identifying who are the community members and what they are doing. Metagenomics combines the usage of NGS technologies to massively sequence isolated DNA from a human microbiome sample and analyze the sequencing data obtained using bioinformatics tools.[27,28] In this way, characterization of the metagenome, being the collection of genomes and genes from the members of the human microbiota, can be performed.[21] Metagenomics allows to interrogate an environmental sample in a culture-independent way, which is useful to discover the presence of microorganisms that resist cultivation. It's important to differentiate between targeted and untargeted metagenomics, the former being specific to the amplification of a marker gene whereas the latter captures the full repertoire of genetic information from a sample.[13]

## THE HUMAN MICROBIOME IN HEALTH AND DISEASE

The variability in the human microbiome is much larger than that of the human genome. While it remains to be defined what a 'healthy microbiome' truly signifies, it has been shown that human microbiome perturbation (dysbiosis) is associated with many diseases, including obesity, type 2 diabetes, hepatic steatosis, inflammatory bowel disease (IBD) and several types of cancer.[7,19,20] In this context, it is challenging to identify microbial biomarkers of a disease since interpersonal diversity of the human microbiota has been found to occur all the way down to the strain-level.[14,32]

Also, microbial-wide association studies (MWAS), inspired by genome-wide association studies (GWAS), are used to find microbial variants that explain the phenotype in case-control studies.[9,15] Thus, integrating human and microbial genomic data sets will likely provide a path to better predict the risk of human disease.[16] Moving from association to causation and finally translational science is a long way. Longitudinal prospective studies complemented by mechanistic

experiments in animal models are required to establish whether a certain microbiome causes disease.[14] However, translational science from animal models come with limitations due to the complexity of species-specific host-microbe interactions.[26]

## CLINICAL DEVELOPMENT

Since the human microbiota has been increasingly recognized as having an important role in the onset and the progression of many diseases, the development of novel therapeutic strategies to manipulate the human microbiota has emerged as an evolving need in medicine.[8] Recently, the potential applications for the microbiome as therapeutic target have been extended to several different therapeutic areas among which infectious diseases, oncology, and central nervous system diseases (referring to the so-called "gut-brain axis"). As biotech and pharma companies are trying to harvest the natural benefits of the microbiome and translate them into microbiome-based therapeutics, they are also increasingly faced with the complexity of the human microbiome.

Well-designed (randomized, double-blind, placebo-controlled) clinical trials are therefore critical to test microbiome-based therapeutics. Unfortunately, the mix-up of microbial strains, underpowered studies, and the lack of clear clinical outcomes currently limit the usefulness of some microbiome-centered clinical trials as it becomes difficult for the medical practitioner to draw conclusions from the published literature.[2] Also, host variables relating to their physiology, lifestyle, and dietary characteristics create confounding factors in microbiome-based clinical studies that are difficult to block.[30] Upcoming clinical studies that generate vast quantities of data per human subject are faced with additional data storage and data analysis challenges.

Both targeted and untargeted microbiome-directed interventions are underway to become clinically relevant.[9] Fecal microbiota transplantation (FMT) has been rediscovered as a means to directly change a patient's gut microbial composition and to convey a health benefit.[17,31] Patients that suffer from recurrent episodes of *Clostridioides difficile* infection (CDI) could benefit from FMT to achieve a modified microbiota composition and lower the numbers of toxigenic *C. difficile*.

While a unified regulatory framework is still lacking in the EU, the FDA has currently categorized Fecal Microbiota Products as medicinal products (biologics), which is regulated by the Center for Biologics Evaluation and Research (CBER). Currently, two fecal microbiota products have been approved.[10] Rebyota was the first fecal microbiota product approved by the FDA for the prevention of recurrence of CDI in individuals 18 years of age and older, following antibiotic treatment for recurrent CDI. Similarly, VOWST has recently been approved with the same indication. The two products have in common that they are derived from human donor stool, but their manufacturing process is different. Whereas Rebyota represents a fecal microbiota suspension for single-dose rectal administration, VOWST contains purified fecal microbiota spores and is intended for oral administration with multiple doses. As such, these two products are therapeutics examples in a spectrum that is being established from least (e.g., Rebyota) to most (e.g., VOWST) manipulated fecal material for CDI treatment.[18] Undoubtedly, the approval of these two fecal microbiota products establishes an important precedent in the field.

Live biotherapeutic products (LBPs) are medicinal products that contain live microorganisms.[26] This category excludes fecal microbiota products. Compared to chemical drugs, where the chemical formula of the drug is well-defined, biologics are much more challenging in their clinical development. The regulatory context for LBPs is still not defined, but since it bears resemblance to cellular therapy products (FDA) and advanced therapy medicinal products (EMA), good manufacturing processes seem critical for these therapies to be accepted by regulatory instances.[6,26] A guidance document has been established by the FDA to formally establish the LBP category and contains recommendations for clinical trials that may be of benefit to the public health.[11]

## CLINICAL STUDIES

Since the human microbiome is more and more recognized as an important factor in different disease types, it is unsurprising to see the increase in scientific reports. Whereas the number of scientific publications that concern fecal transplantation, or the human microbiome/microbiota in general has been on the rise since the 2000's (76,665 Pubmed identifiers since 1990), the number of clinical studies that involve investigation of the human microbiome really started only from the 2010's **(Figure 1)**. To date 3460 studies have been registered on ClinicalTrials.gov. There's

however a clear bias in favor of studies conducted in Europe (34%) and in countries of the Anglosphere (US 28%, CA 5%, GB 4%), next to China (10%), Taiwan (2%), and the Republic of Korea (1%).

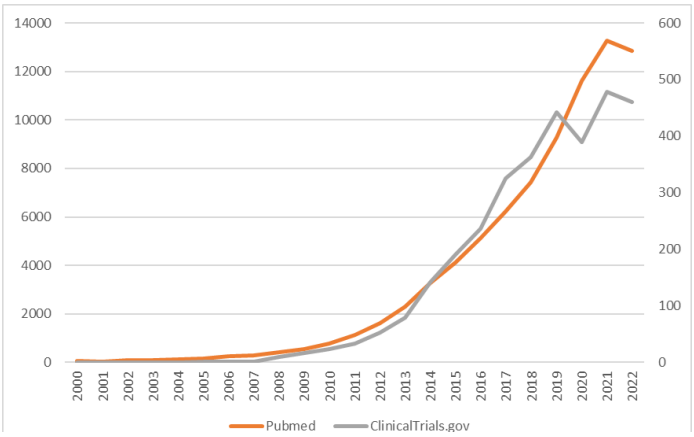


**Figure 1: The rise of clinical studies involving the human microbiome in recent years (Pubmed, left y-axis, and ClinicalTrials.gov, right y-axis)**

With the advent of human microbiome analysis in clinical studies also comes the challenge of sampling and transport. As opposed to other sample types, such as human blood etc, for which dedicated sampling devices have been developed for many years now, the development and validation of such sampling devices for the human microbiome is still in its infancy. The human gut microbiome, being the best studied, has received the most attention since it also became clear that larger cohorts studied longitudinally are needed to understand the dynamic nature of the human gut microbiome. Although cryopreservation is still considered the gold standard, other preservation protocols may be useful, as long as they preserve the microbial signature of a sample.[29] In particular, room temperature transport vials are sought since they relieve the burdens associated with cold chain management. Recently, the first FDA-approved fecal sampling device called OMNIgene•GUT Dx has been approved using the *de novo* pathway.[12] This represents yet another important precedent for clinical studies. Also, new technologies are being developed to sample the entire gastrointestinal tract.[7]

## MEDPACE CAPABILITIES

NGS assays are comprised of a wet-lab and a dry-lab (bioinformatics) component, who are tightly integrated.[5] At Medpace, we oversee the whole process, from sampling to DNA extraction, sequencing, and bioinformatics analysis **(Figure 2)**. This approach has distinct advantages compared to a distributed testing process with third-party labs, since it allows rapid internal feedback between the scientists, both at the bench and on their computer, to optimize and even customize the protocols, depending on the Sponsor's needs.

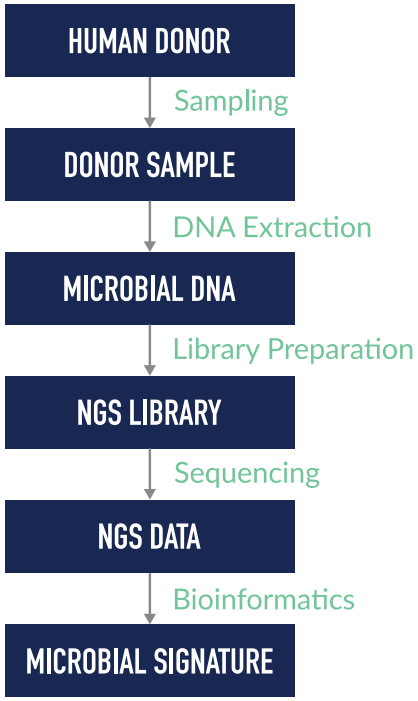


**Figure 2: From sample to analysis using the Medpace NGS protocol**

Medpace has the capability to help Sponsors from sample to analysis. By organizing the logistics of sample transport to our laboratories, we can streamline the whole process and allow samples to arrive in the lab in the best conditions. Our processes for sample processing are standardized according to standard operating procedures (SOPs) and these entail wet-bench and dry-bench components, which allow us to obtain reliable and reproducible results during the duration of the clinical study. Our molecular department is specialized in the extraction of nucleic acids from clinical samples, their amplification (if necessary) and sequencing using next-generation

sequencing technologies. 16S rRNA gene sequencing and shotgun metagenomics sequencing can be performed depending on the needs of the Sponsor. Whereas the former allows us to survey the gut microbial composition based on the amplification and sequencing of a taxonomic marker gene, the latter provides a metagenome that can provide more precise information on the presence of microorganisms and their functions. Finally, strain-specific assays can be developed using qPCR to accurately quantify the abundance of your strain of interest.

## CONCLUSION

Clinical studies involving the human microbiome are on the rise since its implication in many different non-trivial disease types, from gastrointestinal to neurological, and chronic metabolic diseases, such as obesity and type 2 diabetes, is increasingly being recognized. While much can be learned from *in vitro* systems and studies in model organisms, translation of these findings is limited. Well-designed clinical trials are critical to test microbiome-based therapeutics. The recent FDA approval of two fecal microbiota products (Rebyota and VOWST) and of a fecal sampling device (OMNIgene•GUT Dx) represent important precedents for a field that is slowly maturing, but for which the expectations set forth are high. Human microbiome analysis using next-generation sequencing provides a way to investigate the microbiome composition in a culture-independent way. It can be performed in a targeted or untargeted way depending on the Sponsor's needs and represents therefore an interesting avenue to complement clinical outcomes.

## FULL-SERVICE CLINICAL DEVELOPMENT

Medpace is a scientifically-driven, global, fullservice clinical contract research organization (CRO) providing Phase I-IV clinical development services to the biotechnology, pharmaceutical and medical device industries. Medpace's mission is to accelerate the global development of safe and effective medical therapeutics through its high-science and disciplined operating approach that leverages local regulatory and deep therapeutic expertise across all major areas including oncology, cardiology, metabolic disease, endocrinology, central nervous system and anti-viral and anti-infective.Necum aperum etur atis reptae

## REFERENCES


1. Aragón, I. M., Herrera-Imbroda, B., Queipo-Ortuño, M. I., Castillo, E., Del Moral, J. S.-G., Gómez-Millán, J., Yucel, G., & Lara, M. F. (2018). The Urinary Tract Microbiome in Health and Disease. European Urology Focus, 4(1), 128–138. https://doi.org/10.1016/j.euf.2016.11.001

2. Brüssow, H. (2019). Probiotics and prebiotics in clinical tests: An update. F1000Research, 8, 1157. https://doi.org/10.12688/f1000research.19043.1

3. Busi, S. B., de Nies, L., Habier, J., Wampach, L., Fritz, J. V., Heintz-Buschart, A., May, P., Halder, R., de Beaufort, C., & Wilmes, P. (2021). Persistence of birth mode-dependent effects on gut microbiome composition, immune system stimulation and antimicrobial resistance during the first year of life. ISME Communications, 1(1), 8. https://doi.org/10.1038/s43705-021-00003-5

4. Castillo, D. J., Rifkin, R. F., Cowan, D. A., & Potgieter, M. (2019). The Healthy Human Blood Microbiome: Fact or Fiction? Frontiers in Cellular and Infection Microbiology, 9, 148. https://doi.org/10.3389/fcimb.2019.00148

5. College of American Pathologists (CAP) Molecular Pathology Checklist (2022). Part of the CAP accreditation program.

6. Cordaillat-Simmons, M., Rouanet, A., & Pot, B. (2020). Live biotherapeutic products: The importance of a defined regulatory framework. Experimental & Molecular Medicine, 52(9), 1397–1406. https://doi.org/10.1038/s12276-020-0437-6

7. de Vos, W. M., Tilg, H., Van Hul, M., & Cani, P. D. (2022). Gut microbiome and health: Mechanistic insights. Gut, 71(5), 1020–1032. https://doi.org/10.1136/gutjnl-2021-326789

8. Durack, J., & Lynch, S. V. (2019). The gut microbiome: Relationships with disease and opportunities for therapy. Journal of Experimental Medicine, 216(1), 20–40. https://doi.org/10.1084/jem.20180448

9. Fan, Y., & Pedersen, O. (2021). Gut microbiota in human metabolic health and disease. Nature Reviews Microbiology, 19(1), 55–71. https://doi.org/10.1038/s41579-020-0433-9

10. FDA-Approved Fecal Microbiota Products. https://www.fda.gov/vaccines-blood-biologics/fecal-microbiota-products, accessed May 15, 2023.

11. FDA. Early Clinical Trials with Live Biotherapeutic Products: Chemistry, Manufacturing, and Control Information (FDA, 2016).

12. FDA. Device Classification Under Section 513(f)(2)(De Novo). https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/denovo.cfm?id=DEN200040, accessed May 15. Decision Date 11/03/2021.

13. Galloway-Peña, J., & Hanson, B. (2020). Tools for Analysis of the Microbiome. Digestive Diseases and Sciences, 65(3), 674–685. https://doi.org/10.1007/s10620-020-06091-y

14. Gilbert, J. A., Blaser, M. J., Caporaso, J. G., Jansson, J. K., Lynch, S. V., & Knight, R. (2018). Current understanding of the human microbiome. Nature Medicine, 24(4), 392–400. https://doi.org/10.1038/nm.4517

15. Gilbert, J. A., Quinn, R. A., Debelius, J., Xu, Z. Z., Morton, J., Garg, N., Jansson, J. K., Dorrestein, P. C., & Knight, R. (2016). Microbiome-wide association studies link dynamic microbial consortia to disease. Nature, 535(7610), 94–103. https://doi.org/10.1038/nature18850

16. Grice, E. A., & Segre, J. A. (2012). The Human Microbiome: Our Second Genome. Annual Review of Genomics and Human Genetics, 13(1), 151–170. https://doi.org/10.1146/annurev-genom-090711-163814

17. Gupta, S., Allen-Vercoe, E., & Petrof, E. O. (2016). Fecal microbiota transplantation: In perspective. Therapeutic Advances in Gastroenterology, 9(2), 229–239. https://doi.org/10.1177/1756283X15607414

18. Hoffmann, D. E., Palumbo, F. B., Ravel, J., Rowthorn, V., & von Rosenvinge, E. (2017). A proposed definition of microbiota transplantation for regulatory purposes. Gut Microbes, 8(3), 208–213. https://doi.org/10.1080/19490976.2017.1293223

19. Hooks, K. B., & O'Malley, M. A. (2017). Dysbiosis and Its Discontents. MBio, 8(5), e01492-17. https://doi.org/10.1128/mBio.01492-17

20. Lloyd-Price, J., Abu-Ali, G., & Huttenhower, C. (2016). The healthy human microbiome. Genome Medicine, 8(1), 51. https://doi.org/10.1186/s13073-016-0307-y

21. Marchesi, J. R., & Ravel, J. (2015). The vocabulary of microbiome research: A proposal. Microbiome, 3(1), 31, s40168-015-0094–0095. https://doi.org/10.1186/s40168-015-0094-5

22. Mousavi, S. E., Delgado-Saborit, J. M., Adivi, A., Pauwels, S., & Godderis, L. (2022). Air pollution and endocrine disruptors induce human microbiome imbalances: A systematic review of recent evidence and possible biological mechanisms. Science of The Total Environment, 816, 151654. https://doi.org/10.1016/j.scitotenv.2021.151654

23. Nguyen, L. D. N., Viscogliosi, E., & Delhaes, L. (2015). The lung mycobiome: An emerging field of the human respiratory microbiome. Frontiers in Microbiology, 6. https://doi.org/10.3389/fmicb.2015.00089

24. O'Toole, P. W., & Jeffery, I. B. (2015). Gut microbiota and aging. Science, 350(6265), 1214–1215. https://doi.org/10.1126/science.aac8469

25. Reynoso-García, J., Miranda-Santiago, A. E., Meléndez-Vázquez, N. M., Acosta-Pagán, K., Sánchez-Rosado, M., Díaz-Rivera, J., Rosado-Quiñones, A. M., Acevedo-Márquez, L., Cruz-Roldán, L., Tosado-Rodríguez, E. L., Figueroa-Gispert, M. D. M., & Godoy-Vitorino, F. (2022). A complete guide to human microbiomes: Body niches, transmission, development, dysbiosis, and restoration. Frontiers in Systems Biology, 2, 951403. https://doi.org/10.3389/fsysb.2022.951403

26. Rouanet, A., Bolca, S., Bru, A., Claes, I., Cvejic, H., Girgis, H., Harper, A., Lavergne, S. N., Mathys, S., Pane, M., Pot, B., Shortt, C., Alkema, W., Bezulowsky, C., Blanquet-Diot, S., Chassard, C., Claus, S. P., Hadida, B., Hemmingsen, C., … Cordaillat-Simmons, M. (2020). Live Biotherapeutic Products, A Road Map for Safety Assessment. Frontiers in Medicine, 7, 237. https://doi.org/10.3389/fmed.2020.00237

27. Thomas, T., Gilbert, J., & Meyer, F. (2012). Metagenomics—A guide from sampling to data analysis. Microbial Informatics and Experimentation, 2(1), 3. https://doi.org/10.1186/2042-5783-2-3

28. Ursell, L. K., Metcalf, J. L., Parfrey, L. W., & Knight, R. (2012). Defining the human microbiome. Nutrition Reviews, 70, S38–S44. https://doi.org/10.1111/j.1753-4887.2012.00493.x

29. Vandeputte, D., Tito, R. Y., Vanleeuwen, R., Falony, G., & Raes, J. (2017). Practical considerations for large-scale gut microbiome studies. FEMS Microbiology Reviews, 41(Supplement_1), S154–S167. https://doi.org/10.1093/femsre/fux027

30. Vujkovic-Cvijin, I., Sklar, J., Jiang, L., Natarajan, L., Knight, R., & Belkaid, Y. (2020). Host variables confound gut microbiota studies of human disease. Nature, 587(7834), 448–454. https://doi.org/10.1038/s41586-020-2881-9

31. Vyas, D. (2015). Fecal transplant policy and legislation. World Journal of Gastroenterology, 21(1), 6. https://doi.org/10.3748/wjg.v21.i1.6

32. Yan, Y., Nguyen, L. H., Franzosa, E. A., & Huttenhower, C. (2020). Strain-level epidemiology of microbial communities and the human microbiome. Genome Medicine, 12(1), 71. https://doi.org/10.1186/s13073-020-00765-y