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Q&A

# Microbiome-Based Diagnostics: Ready for Applications in Laboratory Medicine?

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Laboratory medicine is ripe for a holistic approach to human disease that includes evaluation of human and microbial cells. Advancements in our understanding of the human microbiome are leading to new ideas regarding the diagnosis and management of human diseases. Nucleic acid sequencing, mass spectrometry, and immunoassays will facilitate the application of microbiome science to medical microbiology and clinical chemistry. Biomedical advances in the human microbiome will enlarge the scope of laboratory medicine and result in new diagnostic and disease monitoring strategies. Collaborative efforts within the International Human Microbiome Consortium and specific large-scale research initiatives such as Metagenomics of the Human Intestinal Tract and the Human Microbiome Project have documented differences in microbial composition and function in healthy and diseased states.

Diagnostic applications of the microbiome can be divided into two categories: diagnosis of infectious diseases and monitoring of microbial components of noncommunicable chronic diseases. The diagnosis of human infections and selection of antimicrobial agents may be refined in the context of the microbiome. Rather than focusing solely on identification of the etiologic agent(s) of infection, clinical laboratories could evaluate the microbiome at a specific body site of interest. For example, rapid detection of *Clostridium difficile* in stool specimens in cases of recurrent *C. difficile* infection may be tested in parallel with stool-based 16S rRNA gene sequencing to evaluate the extent of dysbiosis or cooccurrence of other enteric pathogens. Microbial metabolites may provide useful microbial biomarkers to monitor effective treatment in chronic infections such as recurrent *C. difficile* associated disease. These parallel evaluations could aid medical decision-making regarding selection of antimicrobial agents or fecal microbiota transplantation (FMT).[6]

Advances in microbiome discovery are also altering the field of human microbiology for noncommunicable chronic diseases with a microbial component. For example, specific changes in microbial composition and patterns of intestinal dysbiosis are leading to novel considerations of microbiome-based applications for disease stratification and management in inflammatory bowel disease (IBD). Specific gut microbes that may contribute to neoplastic progression in colorectal cancer have also been identified and may provide a novel diagnostic and therapeutic angle on cancer prevention and early disease management. Such findings may change the nature of colorectal cancer screening and early detection. Disease-specific metabolites might be produced by human cells, microbial cells, or both (cometabolites). In terms of metabolic disorders such as prediabetes and type 2 diabetes, microbial metabolites (e.g., branched chain amino acids) may serve as microbial biomarkers for disease prevention or mitigation. Target genes, proteins, or metabolites within human microbes may be considered as part of holistic laboratory medicine. In this Q&A article, four experts share their views on this important topic.

***Can you identify or describe exciting frontier(s) in the next 5–10 years of human microbiome science?***

**Joel Dore:** Building a full understanding of a human as an ecosystem is the most exciting frontier I wish to propose for the immediate future. We are microbial from birth, with underlying fundamental and constant crosstalk between our human and our microbial constituents. Deciphering infec-

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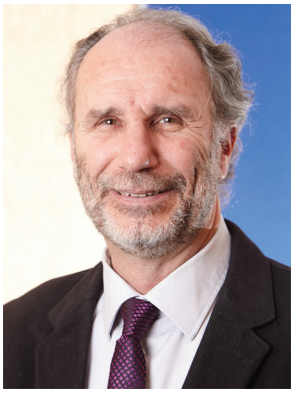
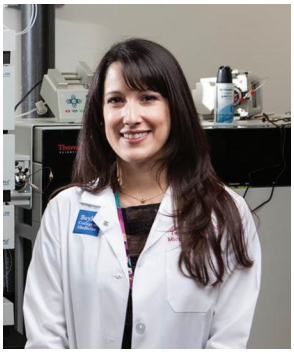
[6] Nonstandard abbreviations: FMT, fecal microbiota transplantation; IBD, inflammatory bowel disease; ASD, autism spectrum disorder; GI, gastrointestinal.

tious disease mechanisms in the battles between pathogens and human cells resulted in ways of controlling infections that threatened humankind. While medicine has claimed victory in the battles against major infections, for several decades medicine has been at a loss to deliver therapies in the field of chronic nontransmissible diseases. One in four persons is affected, and we are only beginning to understand what we suspect to be microbiota-driven disorders (e.g., IBD, nonalcoholic steatohepatitis, and obesity). Dysbiosis may be detected via the presence of microbial signatures or alterations in the symbiotic relationships between man and microbes. Chronic nontransmissible diseases stem from a lack of recognition of a human as an ecosystem. These diseases derive in large part from harsh environmental conditions imposed on our microbiome in recent human history. Human nutrition, exposure to xenobiotic compounds, and perinatal management are examples of domains with drastic changes during the past 2–3 generations. These environmental challenges go far beyond what our genetics can cope with, leaving the burden on the microbiota. If, as it seems, altered human–microbe symbiosis is the underlying feature, building a full understanding will indeed be key for basic scientific knowledge but even more so for the future of mankind. It will take an integrated view to listen to this unique dialogue between human cells and microbes, which supports our health and well-being.

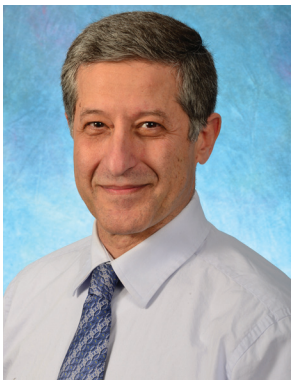


**Francisco Guarner:** The development of precision tools for manipulating the intestinal microbial ecosystem in humans is probably the most exciting frontier. There is no doubt about the wide and deep impact of microbial colonizers of the gut on a number of body functions in the host. It was impressive that gut microbes can affect brain injury after stroke. Changes in the composition of the gut microbiota can reduce the volume of brain infarcts substantially after occlusion of the cerebral artery, basically by modifying the ratios of regulatory T cells and Th17 cells, lineages that are induced in the small intestine by different microbial taxa. This finding was demonstrated in an animal model, but the experiment opens important avenues for the induction of immunoregulatory pathways in humans via changes in the gut microbiota. This approach offers potential for treating intestinal and systemic inflammatory processes. A major challenge in modern society is the persistent increase in the incidence of chronic noncommunicable diseases, most of which result from a pathophysiological background of exacerbated inflammation.

The task of developing such tools is not easy. Probiotics and prebiotics tested so far seem to have limited effects on chronic conditions. On the other hand, FMT has proven to be very useful for prevention of recurrent *C. difficile* diarrhea, but provokes disparate outcomes when tested in patients with chronic inflammatory conditions, from resolution to exacerbation of the underlying disease. This is not surprising since many unknown microbes are introduced with the fecal inoculum; some of them may mitigate inflammation but others may induce Th17 cellular responses. For the future, precision tools are badly needed to obtain predictable responses. Ultimately, targets for such precision tools will include immunological processes and inflammation, in addition to enteroendocrine cells, gut sensory functions, and the whole array of gut–brain axis communication.



**Ruth Ann Luna:** As we unravel the interconnectivity of the inherent microbiome with various organ systems and its impact on human performance, we will pursue exciting pathways for treating human disorders. Due to the known axes-based interactions within the human body, including gut–lung crosstalk as well as communication along the gut–brain axis, evidence is mounting regarding the ability to manipulate symptoms manifesting in remote organ systems by inducing microbial shifts in the gut. One of the most striking recent examples of this strategy in an animal model is the study in killifish at the Max Planck Institute for Biology of Ageing in Cologne, Germany, in which older fish were fed the gut contents of young fish, which dramatically increased longevity and increased activity levels in the older fish. While animal models of disease have repeatedly shown global benefits associated with microbial manipulation therapy, clinical studies are now replicating these effects in many patient populations. Following multiple probiotic supplementation studies in mouse models that resulted in resolution of gastrointestinal symptoms and improvement in behavioral symptoms associated with autism spectrum disorder (ASD), the first FMT study in pediatric ASD produced significant improvements in gastrointestinal symptoms and captured global improvements in many of the core symptoms of ASD while reporting no adverse events. The stage is set for future microbial therapy trials in ASD.



**Yehuda Ringel:** The major contribution of human microbiome research in the next 5–10 years will be in identifying and highlighting diseases, or subsets of diseases, in which the microbiome plays an important etiopathomechanistic role. New insights regarding the contribution of the microbiome to the abnormal physiology and symptomatology of specific disease conditions could lead to major changes in the way we understand certain disease conditions, particularly gastrointestinal diseases. For example, in IBD and functional gastrointestinal disorders, we may see a shift from phenotypic clinical diagnoses and classifications, which are currently based on identifying and characterizing abnormal physiology and clinical symptomatology, to etiologic and pathomechanistic classifications based on new knowledge of the role of the microbiome in these diseases. This knowledge is likely to also lead to the development of new diagnostic tests and therapeutic approaches. For example, we may be able to identify IBD conditions/subsets with altered, dysbiotic microbiomes and differentiate them from IBD conditions/subsets with eubiotic microbiomes that may be mostly related to altered immune function. These findings could lead to new treatment approaches since certain patients may be more suitable for therapeutic strategies targeting the intestinal microbiome while others may benefit more from current interventions targeting immune system dysfunction, or a combination of the two.

***Can you describe an example of diagnostic applications of the microbiome in human disease?***

**Joel Dore:** Chronic conditions for which microbiome signatures associated with the disease have been documented are numerous today, and recurrent features are emerging. Many disease states are associated with a reduction in microbiome diversity. An emerging diagnostic application is the potential for a prediction of the response to cancer immunotherapy. Less than 50% of the patients will see an improvement of their cancer condition upon treatment. Yet it was recently evidenced that the presence of specific bacteria in the microbiome will prime an immune response favoring the removal of cancerous cells and reduction of tumors. Transferability of response/nonresponse to laboratory animals was demonstrated, and bacterial strains with adjuvant potential were isolated. The impact of such discoveries ranges from companion diagnostics development to changes in clinical practice. Examples would be the avoidance of antibiotics that would remove adjuvant bacteria and the introduction of live biotherapeutics. Similar developments may be expected in other clinical conditions such as cirrhosis in patients awaiting liver transplantation, especially when they do not respond to steroids. Finally, since we may consider symbiosis of humans and microbes as a healthy reference, it would seem relevant to examine models of diagnostic value combining features of both humans and microbiota. In an era of emerging big data and machine learning, the limitation will not be the challenge of a combinatorial approach but rather the identification of most relevant features that will be the key to health monitoring of a human as an ecosystem. Microbiome signatures may be far more discriminant in their predictive value than any single or even combination of human genetic markers. High-impact diagnostic applications of the microbiome that yield a predictive value will require longitudinal prospective studies. Few such markers are available to date.

**Francisco Guarner:** Human studies have found different variants of gut dysbiosis in several conditions, including IBD, metabolic disorders, chronic liver disease, and some functional bowel disorders. However, the progress in developing useful diagnostic applications based in microbiome testing is still behind expectations because of unresolved issues. First, taxonomic changes identified in different studies focusing on a particular disease are not fully consistent. Different technological approaches and sample populations, as well as confounding factors such as diet, drugs, or colonic transit time, may account for the relative lack of consistency. Second, the lack of a defined "healthy range" for the composition of the human gut ecosystem makes this issue challenging to address because of the large degree of variation between healthy individuals. Thus, current approaches for identifying microbial markers for diagnostic purposes need to rely on comparisons with parallel control groups of healthy individuals. Commonly detected dysbiotic changes do not seem to be disease-specific. The most common finding in such exploratory studies is a relative reduction of bacterial diversity or loss of microbial gene richness, which has been associated with several metabolic or inflammatory conditions. Poor diversity or richness is linked with reductions of fermentative taxa (short-chain fatty acid producers) and overrepresentations of bacteria with lipopolysaccharides (endotoxins) that can promote intestinal inflammation. Interestingly, loss of microbial gene richness in the intestine seems to be correlated with derangements of metabolic and inflammatory parameters such as increased adiposity, insulin resistance, and serum concentrations of C-reactive protein. In any case, diagnostic markers for defining dysbiosis still need to be properly validated.

**Ruth Ann Luna:** In the clinical laboratory, we are rapidly moving toward utilizing microbiome characterization as a diagnostic tool. This emerging application is not meant to be a standalone assay, but rather a companion diagnostic to be used with routine clinical laboratory testing and clinical history. These evaluations could be highly useful in difficult-to-treat cases as well as for longitudinal monitoring during treatment. For instance, in a child with no organic cause of GI symptoms and in the absence of any identified pathogen via routine methods, a significant deviation (e.g., complete absence of *Bacteroides sp.*) from the typical microbiome in age- and sex-matched healthy controls could encourage the treating physicians to continue to pursue GI-based interventions. With regard to treatment selection, there is evidence of the utility of multiomic profiling (i.e., integrating the microbiome and metabolome) in predicting successful outcomes. With respect to nutritional intervention in irritable bowel syndrome, distinctive features of the intestinal microbiome have been correlated with the ability to respond to a low fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAP) dietary intervention in children. Similarly, longitudinal characterization of the microbiome could yield information related to prognoses, and this approach has been used for monitoring of pediatric recurrent *C. difficile* infections in patients undergoing FMT. In the respiratory tract, changes in microbial composition associated with the subsequent development of bronchopulmonary dysplasia in ventilated neonates have been identified. Microbiome characterization would also be highly useful in the ongoing management of chronic diseases, such as monitoring the respiratory microbiome in patients with cystic fibrosis, with special emphasis on the manifestation of potentially chronic bacterial infections.

**Yehuda Ringel:** Regardless of the degree to which the microbiome is found to play a pathomechanistic role in disease conditions, the human microbiome remains a dynamic community that is sensitive and responsive to a variety of host states, genetic phenotypes, and environmental factors. Identifying patterns of microbial communities and/or their metabolites may allow for prediction of host disease susceptibility, extent of disease progression, and response to therapy. Diagnostic applications that target microbial communities or metabolites may enable individually tailored treatment approaches.

***As we consider new therapeutics based on the microbiome, can you elaborate on new drug targets in human disease?***

**Joel Dore:** Numerous changes in our everyday life and clinical practice may compromise our well-being "as an intact ecosystem." Disruptions to the homeostasis of human–microbe symbiosis can result from unbalanced diets, treatments to cure infections, attempts to mitigate degenerative and autoimmune diseases, and surgical interventions. The question pertains to what can be done to restore health and promote symbiosis. In conditions so severe that alterations of the symbiosis might be viewed as a life-threatening event, being able to restore the human ecosystem, or should we say "a" human ecosystem, seems most appropriate. This is the basis of FMT, be it allogeneic—from a healthy donor—or autologous—from a sample collected in anticipation from the recipient. In these cases, restoration of microbial ecology may be important for preserving GI function, potentiation of immunotherapies, and prevention of colonization by drug resistant pathogens.

**Francisco Guarner:** Microbiome research presents opportunities for novel drug development and delineation of potential targets. Some microbial factors induce regulatory T-cell subsets in animal models. A better knowledge of microbe–host interactions will uncover mechanisms and host receptors to be targeted by synthetic drugs. Another area of opportunity relies on the investigation of microbes in processing dietary-derived phytochemicals with biological functionalities. For instance, some plant-derived flavonoids have been shown to stimulate host energy expenditure by enhancing thermogenesis in brown adipose tissue of mice, and microbial metabolism of these substances may modulate various physiological effects. Thus, studies investigating the role of microbe-modified flavonoids on energy expenditure offer opportunities for novel drug development. Another interesting observation is that lower urinary concentrations of enterolactones generated by microbial metabolism of dietary lignins are associated with an increased risk of depression in perimenopausal women. This observation may lead to the discovery of novel pathways in the microbiota–brain axis and potential drug targets.

**Ruth Ann Luna:** While serotonin has been targeted in the treatment of a variety of psychiatric conditions, it has not been specifically targeted in the treatment of the core symptoms of ASD, for which extremely limited Food and Drug Administration approved pharmacologic interventions currently exist. With known interactions between the central and enteric nervous systems via the gut–brain axis and the predominant production of serotonin by the gut, it remains a viable and promising target for the resolution of both GI and behavioral symptoms in ASD. Specific microbes associated in children with ASD and accompanying GI symptoms have been correlated with tryptophan and 5-hydroxytryptamine. Commonly reported sleep difficulties, at least partially resolved (i.e., decreased sleep latency) by melatonin supplementation, further support the role of serotonin in ASD symptom-

atology. Although both hyperserotonemia and hyposerotonemia can induce ASD-like phenotypes in animal models of ASD, these findings also support the treatment potential associated with the serotonin pathway.

**Yehuda Ringel:** Since this field is still in its infancy, interventional tools remain blunt and nonspecific in their effects (e.g., antibiotic therapy, fecal transplants). It seems that the development of more finely targeted microbiome-based pharmaceutical interventions for specific disease conditions is still on the horizon. However, interventions include broader microbiome-based approaches for promoting health and prevention of diseases. With the rapid increase of our knowledge of the composition and function of the human microbiome, we can effectively strengthen the "healthy microbiome" by increasing microbial diversity, stability, and resilience to various stressors. Our ability to identify microbial alterations that are associated with specific disease conditions may lead to the development of strategies for possible "correction" of dysbiosis. Another potential area of development may be microbiome-based adjuvant therapy for potentiating the effects of dietary interventions and "classic" drug therapy or preventing side effects.

***Can you explain your perspective on the relative importance of microbial composition vs function in human disease?***

**Joel Dore:** Every human microbiome is unique. Human intestinal metagenomics have told us that we share a core microbiome and yet differ in harboring individual or personal traits in our microbiome. Similarities in microbial function greatly exceed similarities of microbial composition between individuals. These observations emphasize the conservation of function and include microbial metabolites and proteins that act as signals between humans and microbiomes. Beyond metagenomics, growing attention is expected to be devoted to metatranscriptomics, metaproteomics, and metabolomics. In terms of diagnostics, what matters is the clinical sensitivity and specificity conferred by any given feature or set of features. Single molecules and single microbial genes, in addition to cocktails of molecules or combinations of genes, should be considered for new diagnostic tests.

**Francisco Guarner:** Evidence from studies of the human gut microbiota suggests that taxonomy may not lead to the identification of universally valid markers. Most studies have used 16S rRNA gene sequencing approaches to identify taxonomic features of microbial communities. The procedure is efficient for recognizing the proportions of sequences pertaining to each phyla and other taxonomic levels, but is not effective at providing information from community members at the species or strain levels. Species- or strain-level differences between individuals can be large, and most studies provide information on similarity distance indices between samples from individuals grouped according to their health condition. Most statistically significant differences detected with this approach have little biological meaning and no clear implications from a clinical point of view. Different taxa may have similar functions within the microbial ecosystem, and therefore 16S rRNA gene sequencing seems insufficient for describing the biological roles of microbial communities. Whole genome sequencing approaches will provide better information about human gut microbes and their functions. However, the procedure is still raw in terms of tools development for analysis of whole genome sequencing data. More research is needed for standardizing technologies for clinical practice.

**Ruth Ann Luna:** Several options for microbial manipulation and the treatment of human disease include antibiotics, prebiotics/probiotics, or complete community transplantation via FMT. The ultimate goal is to restore function to the targeted microbiome. The inclusion of metabolomics in our ongoing research has allowed us to remain focused on the functions of microbial communities, and an array of microbial community variation could serve similar functions. The complex interactions of environment, human genetics, and the microbiome together dictate function. The continued accumulation of a critical mass of data in specific patient populations throughout the world will allow us to further our understanding of the role of the functional microbiome in human health and disease.

**Yehuda Ringel:** The human microbiome is widely perceived and accepted as an important functional organ in maintaining health and preventing disease. In a similar fashion to human organs, the microbiome is composed of multiple functional units that work together to benefit the host. Recent research in this area has shown that multiple microbes, even from different genera, may perform similar functions. Substantial functional overlap exists among various microbes residing within humans. Changes in microbial communities depending on alterations of relative abundances of microbes may not result in harmful sequelae as long as other bacteria compensate for these changes. The core functions of the microbiome may be maintained. Identifying the myriad functions and interactions of these groups remains an important challenge in this field. Extensive advances in metagenomics have provided tools for characterizing the human microbiome and have placed the primary focus of research on microbial composition. We are likely to see an increasing emphasis on investigation of microbial function as techniques such as metabolomics become more powerful and

affordable. Ultimately, we will require both lines of investigation to advance this field; we must identify which microbiome-mediated functions are important in health and disease, and which microbes are performing these functions to effectively monitor and intervene with the biology of the human microbiome.

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