Clinical Chemistry 65:1
000–000 (2019)

Q&A

The Microbiome and Men's Health

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Research on the characterization and measurement of the human microbiome in health and disease is exploding. The field is fast moving with evolving methods of measurement of microbial composition, diversity, and abundance, and with the development of strategies to modulate the human microbiome to improve health. A previous Q&A in *Clinical Chemistry* focused on diagnostic and monitoring applications and methods. In this Q&A, a panel of 4 experts in microbiology, microbial ecology, and epidemiology provide (*a*) their perspectives on research questions and key findings about the role of the microbiome in men's health, and (*b*) their expectations for the near-term and future translation of technologies to measure and modulate the microbiome for clinical and population application to enhance men's health and well-being. The target audience for this Q&A comprises scientists who do not study the microbiome, those who study the microbiome but not in the context of men's health, and young investigators who would like to study the microbiome especially with the goal of improving men's health and well-being.

What is the state of the science in "measuring" the microbiome with validity and precision in men?

Jacques Ravel

Jacques Ravel: Sex as a biological factor is now being considered in research studies, mostly because of a push from the NIH, but remains understudied. Men's health, in general, has not been addressed from a microbiome perspective. Although the gut microbiome has been extensively studied, studies of other body sites and organs, including those relevant to men, are lagging.

One of the major challenges for the microbiome field, irrespective of whether focused on men, stems from the fact that studies examining an extensive range of diseases have established only associations between microbiota composition and structure, and disease states; they have not established cause and effect. Many studies are cross-sectional, which means that they cannot distinguish whether composition of the microbiota changes and is followed by the disease state or whether the disease state occurs followed by a change in the microbiota. Cross-sectional associations could be used for diagnostic purposes, but clinical studies that clearly establish the diagnostic potential of the microbiome still need to be performed. These limitations make measuring the microbiome for precision health challenging. The issue of cause and effect is a recurring theme throughout this Q&A.

Cindy M. Liu

Cindy Liu: A similar set of laboratory approaches is used to characterize the microbiome of a wide range of body sites, including those of male-specific organs. These methods typically include 3 major steps: (*a*) cell lysis and total nucleic acid purification, (*b*) bacterial DNA sequencing (amplicon-based or unbiased), and (*c*) postsequencing data processing. Each of these steps can add nonbiological variability in the microbiome measurement.

Jacques Ravel: As Dr. Liu mentions, technical challenges in measuring the microbiome using these methods persist. These methods are semiquantitative and afford a survey of only the most abundant microbes, metabolites, gene transcripts, and functions that make up the micro-

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Received August 23, 2018; accepted October 8, 2018.

biome. Without comprehensive and reproducible quantitative measures of the microbiome, validity and precision will be limited.

Cindy Liu: Although there is no consensus on a gold standard method(s), efforts are underway to standardize. At this time, using controls, such as known mixes of bacteria and negative controls for the extraction and PCR steps, is probably the best strategy for microbiome researchers to use for quality control and internal validity in their studies.

Jacques Ravel: Interestingly, no microbiome studies have been performed that look at men's health throughout the life span. In contrast, microbiome studies addressing women's health across phases of the life course are prevalent, including studies of the urogenital and gut microbiota during pubertal or menopausal transitions, and the effect of menstruation and sex hormones on the microbiota. Yet, based on animal studies, we know that sexual maturation and hormones affect gut microbiota development. Studies in male and female mouse pups show that the development of gut microbiota diverged. This led to the development of the term "microgenderome" and a paradigm shift that highlights the role of sex differences in host–microbiota interactions that appear to be relevant (so far) for autoimmune and neuroimmune conditions. The bidirectional communication between the gut microbiome and brain, for example, has emerged as a factor that influences immunity, metabolism, neurodevelopment, and behavior, and it appears to be different in males and females. However, applied and mechanistic research that considers sex interactions when examining the composition and function of human microbiota is still desperately needed.

Noel T. Mueller

Noel Mueller: Although research on sex differences in the human microbiome remains relatively sparse, a small but emerging body of literature on the microgenderome shows not only sex differences in the microbiome but also sex-specific health effects of several early-life microbial perturbations. A landmark study showed that sex differences in the microbiome may arise after puberty, driving hormone-dependent regulation of immunity, and that transfer of the microbiota from mature male mice to immature female mice results in elevation of testosterone, metabolomic changes, and protection from type 1 diabetes. Since this study, several studies in humans show sex differences in the microbiome may arise even before puberty, as well as sex-specific differences in the impact of antibiotics and delivery mode on risk of childhood chronic diseases. Future longitudinal research in humans, from birth through puberty, is needed to further elucidate when sex differences in the microbiome emerge and whether they are a determinant or consequence of sex hormone changes and chronic diseases.

Jacques Ravel: Agree. Ultimately, if sex differences exist in the microbiome, especially in non–male-specific body sites like the gut, we would still need to demonstrate that they are relevant to health and disease.

What were the major contributions of the 10-year-long Human Microbiome Project (HMP)[6] to understanding its influence on men's health?

Cindy Liu: NIH's efforts to promote and support microbiome research include funding projects through the HMP and standard funding mechanisms. NIH microbiome efforts have shed light on the potential role of the microbiome in a wide range of health conditions, including those affecting both men and women, such as cardiovascular disease, inflammatory bowel disease, obesity, and infectious diseases, just to name a few. I think it is fair to describe the process of microbiome research as iterative, in which many hypotheses have been generated through descriptive and cross-sectional studies as Dr. Ravel mentioned. These should be followed up with empirical studies and large observational studies in humans to verify the many hypotheses generated in the past 10 years.

Jacques Ravel: In my view, the HMP, despite its scale and contributions to uncovering the role of the microbiome in human health, has done little to address the specific role of the microbiome in men's health. However, I agree with Dr. Liu that it is a good launching pad for studies to address specifically men's health. For example, the HMP was designed to sample many human body sites, and although the urogenital microbiome was sampled in women, unfortunately and surprisingly, no such samples were taken from men. An almost equal number of men and women were included, but sex was not heavily considered in the analyses.

[6] Nonstandard abbreviations: HMP, Human Microbiome Project; STI, sexually transmitted infection.

With respect to men's health, what are the next major national and international microbiome efforts underway or in planning?

Jacques Ravel: I do not know of any male-focused projects that are being planned that would examine from a holistic point of view the composition, structure, and function of the human male microbiome in relation to health or disease. Most efforts are small, have limited breadth, and are often focused on the penile, anal, or urethral microbiota in men (e.g., role of antibiotics on the penile microbiome and its role in HIV susceptibility). These studies are mostly focused on the link between these microbiomes and risk of sexually transmitted infections (STIs), or on the influence of preventive measures such a PrEP (preexposure prophylaxis for persons at very high risk of HIV) on the anal microbiome in men who have sex with men (although there is no evidence that a "healthy" and protective anal microbiome—which is yet to be defined—offers protection from an STI). Studies have evaluated the effect of circumcision on the penile microbiome (Dr. Liu led these studies), as the procedure in Africa has been shown to successfully reduce acquisition and transmission of HIV. Circumcision reduced bacterial bioburden, leading to reduced inflammation. A quantitative approach to studying the microbiome was key to identifying an association, but the studies have yet to establish a direct causal link between change in penile microbiota and HIV risk reduction.

Cindy Liu: For the male genital microbiome, I think the next major efforts will come from citizen science projects. We are working on one called "Your Private Biome." This project will allow women and men to learn more about their own genital microbiome while contributing to our understanding of the diversity and dynamics of the genital microbiome. Citizen scientists can also enroll with another person, and they will be able to do their own experiments to see how life changes could alter their microbiome.

Noel Mueller: We are working on an international multicohort collaboration examining sex differences in the gut microbiota and their production of short-chain fatty acids, which may contribute to the differential risk of cardiometabolic diseases between men and women. Work is still needed to determine whether the microbiome plays a role in the etiology of prostate cancer and, more fundamentally, in the regulation of testosterone and other hormones. There is also a lot of interest in the role of the gut microbiome in liver diseases, including nonalcoholic steatohepatitis, a major risk factor for liver cancer.

What has been discovered about the influence of the microbiome on men's health and aging?

Jacques Ravel: Advances in understanding microbiome influences on health include a better understanding of the far-reaching impact of the gut microbiome on distant body sites, including male-specific sites, such as the prostate. The gut microbiome appears to be associated with many inflammation-linked diseases, not only in the gut but also at other body sites (e.g., cancer, rheumatoid arthritis, STIs). However, we do not understand how this happens from a mechanistic standpoint.

Noel Mueller: Some studies suggest that perturbations in gut microbiota may initiate an inflammatory cascade that manifests in inflammatory bowel diseases. One of the most exciting findings in this space is that differential production of butyrate, produced by butyrate-producing microbiota, may play a mediating role in the inflammatory processes involved. This is a rapidly evolving area of research worth keeping an eye on.

The microbiome has also been associated with metabolic diseases such as obesity and type 2 diabetes. Chronic low-grade inflammation initiating in the gut may also underlie these conditions. Many of the epidemiologic studies on this topic, however, have been cross-sectional, relatively small, and not adequately controlled for potential confounding factors. For example, metformin, a drug used to treat persons with diabetes, may affect the microbiome, thus possibly confounding the observed microbiome association with diabetes.

Jacques Ravel: Despite all of this exciting work, again, sex as a biological factor, for the most part, has not been considered in studies of common conditions related to aging. And, as far as men-specific health and aging, aside from the microbiome and STIs in men, unfortunately, there is not that much work at present that is functional in nature and causal, beyond very basic, insufficiently powered, associative studies.

Cindy Liu: As Dr. Ravel alluded to, there is an active area of male-specific microbiome research on the influence of the microbiome on transmission and infection with infectious agents. It has been known for some time that a particular vaginal condition characterized by anaerobe-dominated microbiome—bacterial vaginosis—is associated with a wide range of negative health outcomes, including HIV risk, in women. What we know now is that men could have many of these bacteria, too, on their penis, especially uncircumcised men, and that having these anaerobes may also be associated with HIV risk in heterosexual men.

It is worth noting that another aspect of men's health and microbiome is sex differences in the micro-

biome and associated health implications. For example, we know that men are at higher risk for *Staphylococcus aureus* colonization and infection, and our data suggest that microbiome profiles differ between men and women who are *S. aureus* carriers. In particular, male *S. aureus* carriers have higher absolute abundances of *S. aureus*. I think this is interesting for 2 reasons. First, it is helpful when microbiome composition matches epidemiologic observations. Second, it highlights the importance of conducting absolute abundance-based studies when comparing groups, such as men and women.

What are the chief mechanisms by which microbiota influence men's health?

Dominique S. Michaud

Dominique Michaud: As Dr. Ravel mentioned, there is emerging evidence that gut dysbiosis, which is known to affect local immunity, such as inducing inflammatory responses and altering the gut epithelial barrier, may also be affecting immunity at distant sites, such as in the lung. How different sites share an immunological link driven by microorganisms, increasingly referred to as the gut–lung axis or gut–brain axis, for example, is still at early stages of research, but the data are compelling and of great interest, given that they imply that modifying the gut microbiota could affect the immune response at distant sites to reduce infection or disease.

Noel Mueller: Gut microbiota help to break down polysaccharides in food to produce short-chain fatty acids, which are metabolized by the colonocytes or other organs. The products of microbial fermentation help to fortify a strong intestinal barrier, preventing translocation of inflammation-inducing molecules like lipopolysaccharides, and they may be involved in metabolic programming through epigenetic effects. The gut microbiota is also involved in the production of certain vitamins (B vitamins) and minerals, as well as in metabolism of drugs (xenobiotic metabolism).

Cindy Liu: Host–microbe interactions that lead to inflammation could change a man's susceptibility to diseases. In the genital mucosa (i.e., foreskin), this interaction could have implications for a man's risk of becoming infected by sexually transmitted pathogens such as HIV. Interactions among microbiome constituents could influence whether a man becomes colonized by opportunistic pathogens like *S. aureus* and *Escherichia coli*, which can increase the host's risk for subsequent infections. In nature, bacteria almost always exist as complex bacterial communities, and over time, bacteria have evolved different ways to ensure their survival. The mechanisms for microbial interactions that determine the presence of opportunistic pathogens could include, for example, indirect (e.g., resource competition for nutrients and oxygen) or direct (e.g., antimicrobial peptides) competition.

Jacques Ravel: In my view, explanations in the literature given so far lay in the realm of overinterpretation of often poorly powered studies. The field still suffers from lack of reproducibility, and the science is not supporting any of these proposed mechanisms as "causative."

How does the female, or more specifically the vaginal, microbiome influence men's health, including across the course of life?

Noel Mueller: Considering our first major exposure to microbiota comes at birth, for vaginally delivered babies the vaginal microbiota may be the bugs to pioneer colonization of the infant gut. These pioneer microbiota may train the immune system and contribute to break down of foods including oligosaccharides found in human breast milk. The roles that these microorganisms play in long-term health are still being discovered and may include risk of developing obesity and immune-mediated diseases later in life in both men and women.

Jacques Ravel: Dr. Mueller is currently leading studies to evaluate whether reconstituting exposure to vaginal microbes in babies born by cesarean section leads to better outcome for conditions like obesity and allergies, which are thought to be associated with birth by cesarean section. At a later point in the course of life, the penile and urethral microbiota can be seeded and colonized by vaginal bacteria after intercourse. Most often, in uncircumcised men, the penile microbiota comprise strict anaerobes often found in the vaginal microbiota and associated with a "dysbiotic" condition characterized by the lack of beneficial *Lactobacillus* species. In circumcised men, exposure to vaginal microbes appears to have less of an effect on the penile microbiota, as vaginal anaerobes do not survive on the skin, although potentially, vaginal microbes may affect the urethral microbiota (no data are available). Although this dysbiosis can affect the risk of acquiring an STI in uncircumcised men, it also affects the health of future women partners, as these microbes could be transferred to these new partners, and leads to dysbiotic states that carry risks to acquiring an STI.

Cindy Liu: Specifically, whereas *Lactobacillus* species are common in the vaginal microbiome, they are not com-

monly seen among the sexually active men we have studied. However, evidence suggests that sexual partners share the strict anaerobes that are associated with bacterial vaginosis. The implication of this sharing is that these anaerobes may be associated with increased risk for the transmission of sexually transmitted pathogens. This sharing potentially also has relevance for bacterial vaginosis management. We still need to determine if/how much reintroduction of anaerobes through sexual activity plays a role in recalcitrance (i.e., resistance to treatment) and persistence, a common feature of bacterial vaginosis.

How does the male microbiome influence the microbiome of other men and thus men's health?

Jacques Ravel: With respect to men who have sex with men, there are very few data that would support a role of the anal microbiota in disease susceptibility. There are also no data on the impact of the penile microbiota of a sexual partner on the anal microbiota. A lot more studies are needed in this area.

Fecal microbiota transplant has proven to be successful in treating patients with stubborn Clostridium difficile infections, a major cause of hospital-associated diarrhea. What are the opportunities for analogous strategies to manage other infections and to modulate other health states in men?

Noel Mueller: Fecal microbiota transplants have shown efficacy for managing other conditions, including chronic nutrition-related conditions like metabolic syndrome and type 2 diabetes. There is interest in using fecal transplants to manage inflammatory diseases of the gut, although the evidence has been less convincing than for treating patients with *C. difficile* infection. Some believe the reason that fecal transplants are so effective against *C. difficile* is because patients have had their microbiome completely ablated by antibiotic treatment, allowing for transplantation of a new microbial ecosystem. Other conditions in which fecal transplants have been less successful may not have had the level of antibiotic pretreatment.

Dominique Michaud: Identification of bacterial strains that may be particularly virulent for certain disease conditions, perhaps when combined with environmental exposures (e.g., cigarette smoking) or health conditions (e.g., diabetes), may be able to offer opportunities to target pathogens for disease prevention in certain populations. However, it is also possible that for certain diseases, a constellation of bacterial factors or bacterial products will be associated with disease risk—in those situations, it may be that we can target bacterial activity (by either increasing or decreasing certain metabolites) to modulate health states in men.

Recent studies support the belief that the gut microbiome can modulate response to cancer immunotherapy. What is the mechanism and what are the implications for patients who are candidates for these therapies?

Jacques Ravel: Cancer development has been linked to the presence of some bacteria, such as *Fusobacterium* species, and colon cancer. However, mechanisms are still unknown. Does the microbiome cause cancer or does cancer cause the microbiota to change, for example, by attracting certain species? Although certain microbes have been linked to cancer, cancer therapy itself could induce negative changes in the microbiome. Interestingly, some cancer treatments are ineffective in mice that are germ-free, meaning that some drugs require aspects of the microbiome to be effective. While we are learning a lot about the role of the microbiome in cancer and cancer treatment, these studies are at the preclinical stage, and it is premature for providers to consider the microbiome in treatment plans.

Dominique Michaud: I agree with Dr. Ravel that this research is in early stages. Modulating the microbiome may be an attractive way to improve treatment efficacy and may also prove to be effective in reducing side effects. Although many questions remain about causality, recent animal models have demonstrated that *Fusobacterium* species can promote tumor progression, arguing against its presence in colon tumors as merely an opportunistic infection. If increasing tumor progression is considered as causal, then bacteria may well turn out to have a causal role in carcinogenesis.

Does research support the notion that men can alter their microbiomes to enhance their well-being rather than merely treating poor health?

Dominique Michaud: We have only scratched the surface to uncover how and when altering the microbiome may beneficially affect men's (and women's) overall health and well-being. At this time, it is difficult to know the direction of associations observed between the oral or gut microbiome and disease, which is needed to support intervention. For example, is periodontal disease itself causing dysbiosis in the oral cavity and gut, or are underlying immune states, metabolic conditions, and/or genetic predispositions leading to periodontal disease development and simultaneously affecting changes in the microbiome? To answer the directionality of the associations, we will need to conduct studies to examine causality using experimental (animal) models, but we also need human observational and intervention studies to fully explore the impact and role of the microbiome on human health. Once we better understand the biological

pathways, we can design interventions to address health, including men's well-being. As far as reducing cancer risk or increasing cancer survival is concerned, it is too early to know whether these will be in the form of dietary interventions, antibiotics targeting particular pathogens, or "pills" to replenish beneficial bacteria. Although there is mounting evidence that taking general antibiotics will alter the gut microbiome drastically, it is unclear what the long-term effects are in adults, given that an individual's microbiome will reestablish to preantibiotic profiles after several months.

Jacques Ravel: Known therapies that alter the microbiome, such as taking antibiotics as Dr. Michaud mentioned, should be kept for specific pathogens, as their effect on one's microbiome is unpredictable and could have other negative health effects (e.g., antibiotic resistance). There is no evidence yet, nor any recommendations that would be specific to men, to reduce risk of any chronic diseases, besides healthy eating and living.

Noel Mueller: One of the main functions of the microbiome is to break down the constituent molecules of the food we digest. Thus, it is no surprise that different dietary patterns are associated with different microbiota composition. For example, eating a high-fiber diet has been shown to significantly increase the abundance of butyrate-producing bacteria in our guts. Butyrate is a primary energy source for our colonocytes, helping to maintain healthy intestinal barrier function. To maintain a healthy gut microbiome, we should consider eating a diet rich in vegetables and fruits that are high in indigestible fibers, as well as beans and nuts. Certain oats and grains can also be an excellent source of nutrition for gut microbiota. We should avoid highly processed foods that contain preservatives, simple sugars, and salt, which recently was shown to alter gut bacteria. Preliminary research suggests that processed meats and artificial sweetener are likely not beneficial for the gut microbiota.

However, evidence is still lacking as to whether the microbiome changes that result from lifestyle interventions are causally related to health. To address this question, we are conducting human intervention trials to measure microbiome changes in relation to cardiometabolic health. If we observe a change in both, we plan to test whether these are causal by using postrandomization, experimentally treated gut microbiota in germ-free mice experiments.

How generalizable are microbiome research findings and microbiome-altering interventions across men?

Dominique Michaud: Studies are reporting substantial differences in gut microbiome by race/ethnicity and geographic location, more so than by sex or age in adults. It will be critical to address these differences in population studies that are collecting samples for microbiome analyses in relation to disease, both internally (when including >1 race/ethnic group) and externally (when determining generalizability).

Noel Mueller: There is a dearth of data on the generalizability of microbiome research findings on human health across diverse populations. There is reason to believe that a diet that is evolutionarily adaptive for one population's microbiota may not be a good fit for the microbial ecosystem of another population. It looks like genetics plays only a small role in intraindividual differences in microbiota. Furthermore, the gut microbiome seems to predict cardiometabolic diseases independently and as strongly as genetics. There has been considerably less research on the topic of the gut microbiome with epigenetics. More diversity in study populations is certainly needed to determine whether findings are generalizable.

Jacques Ravel: To follow on from Dr. Mueller, this also means that sample size needs to be large, and by targeting diverse study populations, a broader set of confounders, including social, environmental (including diet), and economic factors, must be accounted for.

How do we move from observation to consensus of causation of the influence of the microbiome on men's health and well-being?

Dominique Michaud: There are many barriers in studying the microbiome and human health and disease, and new ones are being identified as we forge forward at a rapid pace. These need to be overcome to move from observation to establishing causation. Studying the microbiome involves addressing complexities beyond study design issues that we typically have to address in studying human disease, such as reverse causation (microbiome changes because of disease) and confounding. The microbiome is a diverse, dynamic ecological concept that differs drastically from studying 1 organism at a time, as was typically done in the past because of technological constraints. As Dr. Liu mentioned, these barriers in methods are now being addressed by collaborative groups who are providing protocols to standardize procedures and separately evaluating different methods for measuring the human microbiome.

Noel Mueller: The gut microbiome as a therapeutic target is intriguing because, unlike the human genome, it may be modified through lifestyle and pharmacologic interventions. Although cross-sectional studies, animal experiments, and small short-term clinical trials have provided important insights into the role gut microbiota

play in metabolic pathophysiology, rigorous randomized controlled trials are needed to assess causal relationships.

Dominique Michaud: Unfortunately, as it pertains to cancer development and risk, randomized controlled trials are unlikely to be feasible, given long latency periods (between exposure and cancer diagnosis), costs of randomized controlled trials with long follow-up periods, and early life exposures that may be altering risk. Observational studies have been critical to understanding causes of cancer, and collecting samples for microbiome research should be a priority, in conjunction with working on mechanisms in animal models.

Jacques Ravel: The field needs to further explore bacterial activities using approaches like RNAseq, which also affords access to the specific genetic makeup of particular microbes. Understanding the fine genetic differences between bacteria is critical. The field tends to forget that a given bacterial species may come in many different strains. Just because we identify a bacterium as *E. coli*, this does not mean that all *E. coli* are equal. Each strain could have a different functional potential because of genetic differences, hence influencing health differently. Associating aspects of health (e.g., glucose, temperature, hormones) with microbial functions and activities is key to uncovering the mechanisms by which the microbiome influences health, including men's health. The field would also benefit from the development of improved "wearables" (wearable electronic measurement devices) to monitor aspects of health with frequent measurements, along with frequent measurement of microbial composition and activities.

Cindy Liu: As my colleagues stated, to make progress, we have to improve study design, methods, model systems, and ways to incorporate imprecision into microbiome reporting. I fully agree that we should move from small, cross-sectional microbiome studies toward larger longitudinal or intervention studies. Basically, I think the knowledge gained from small microbiome studies is not additive. We need to work toward methods standardization among researchers. A major component of moving toward establishing causation will be to conduct empirical studies in which controlled experiments can be performed to test specific hypotheses. Of course, there will be limitations to model systems as well, whether in animal or in vitro models. However, the process can be iterative, in which hypotheses are generated and tested in model systems, with the hypotheses then refined again in another observational study, and so forth.

Dominique Michaud: To move toward causation, we need to work collaboratively and across disciplines to (try to) avoid making mistakes made in other research fields driven by new technologies (e.g., metabolomics) by addressing all the methodological issues in addition to the study design and confounding issues (as is now required by NIH).

Jacques Ravel: I agree with Dr. Michaud, multidisciplinary approaches are critical and should include disease (topic) experts, epidemiologists (clinical trial design), data scientists/statisticians/modelers, computer scientists, ecologists, and technology experts (data acquisition and generation), and even ethicists. Technology experts (e.g., wearables development) need to be better integrated into large projects associating health and the microbiome.

Noel Mueller: I believe epidemiology is still underrepresented in microbiome science. There are many data scientists doing wonderful things with machine learning. However, epidemiologists have much to contribute to considerations of study design and bias, and to how to ask the right questions to provide answers that are clinically and policy relevant.

Cindy Liu: I believe we need more engineers and chemists who can work on developing tools for better microbiome sampling and storage methods, better ways for lysis of bacteria and fungi, and also better in vitro model systems.

What are the opportunities for young investigators to get involved in and even lead research on the microbiome and men's health?

Dominique Michaud: Young investigators must obtain proper training in conducting microbiome analyses before embarking on microbiome research. The learning curve is steep, as there are many complex issues that arise in the measurement of microbiomes. Methods are evolving fast and require substantial expertise and knowledge to navigate. Identifying knowledgeable and experienced collaborators, as well as working across disciplines, will be crucial for young investigators who want to lead research in microbiome studies.

Cindy Liu: There has been a lot of progress in sequencing and data processing but relatively less progress in the upstream processing steps. High-throughput, cost-effective ways to achieve total bacterial cell lysis and maximize nucleic acid yield could have a big impact on our ability to do the large-scale studies needed to move the field forward, in addition to being able to better deal with low-biomass samples, which are common in some body sites. This is a great area for young investigators to get involved in.

Jacques Ravel: The picture painted above shows that very few data are available about the human male-specific microbiome and men's health. There is a major push at NIH, as there should be, to study sex differences in health, opening opportunities for young investigators to study the microbiome and aspects of men's health.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

Authors' Disclosures or Potential Conflicts of Interest: *No authors declared any potential conflicts of interest.*

Previously published online at DOI: 10.1373/clinchem.2018.287284