Standards Seekers Put the Human Microbiome in Their Sights

Alla Katsnelson

NIST and others work to develop a reference standard for the human gut microbiome.

One day in the future, doctors may add a step to your routine checkup. In addition to measuring your blood pressure, putting a stethoscope to your chest, and running some blood tests, they may examine your poop. The stool sample you provide, loaded with the bacteria and other microbial matter that populate your gut, could divulge whether you have a particular disease or provide clues about your diet, stress levels, or other health markers. On the basis of that stool sample, doctors might prescribe medicines like the ones we already have—drugs that target specific proteins in our guts, for instance. But they might also give you medicines that rebalance the types and amounts of bacteria in your gut microbiome.

Dozens of companies are racing to develop drugs (or microbes prescribed as drugs) that target the microbiome and that change its composition, aiming to treat a wide range of conditions, including recurring bacterial infections, autism, depression, Parkinson’s disease, asthma, cancer, and more. So far, no such drugs have been approved by regulatory agencies, although a few are close. And academic laboratories are still trying to work out some basic questions, including the full lineup of microbial species inhabiting the gut and how they interact with each other to affect health. A few hundred microbial species inhabit the human gut, and the population fluctuates in complicated ways. But because the methods and tools to make measurements of the microbiome vary widely, different laboratories’ efforts to characterize this horde of tiny organisms are often not comparable.

Now, researchers are pushing to create standard tools for studying the gut microbiome—tools that would allow different laboratories to compare their results, apples to apples. One resource that would be especially helpful, experts say, is gut microbiome reference material—some known set of gut microbes, as close as possible to those in an actual human gut—that could be used as a yardstick for aligning results across the field. The US National Institute of Standards and Technology (NIST) has set out to create this and other tools that could serve as standards for microbiome measurements. Such tools will be crucial for gaining a full picture of the microbiome’s basic features, as well as getting a handle on how to modulate it for therapies.

A complex ecosystem

“The human gut microbiome has been described—and I believe rightfully so—as the most complex ecosystem on Earth,” says Scott Jackson, who leads the Complex Microbial Systems Group at NIST. “It raises measurement challenges that no one has figured out how to address yet.”

Jackson’s employer is the US agency responsible for pulling uniformity out of the chaos of the world’s natural variation. NIST is in the measurement business, setting quality and reference standards for an enormous range of stuff, including steel pipes, peanut butter, cigarettes, and human plasma. Companies rely on these standards to ensure the safety and uniformity of all kinds of products. And as interest surges around the potential for improving human health via the gut microbiome, researchers from industry

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and academia, as well as from the US Food and Drug Administration, have prodded his lab at NIST to take up the especially thorny challenge of creating standardized and validated methods and materials for studying the gut microbiome.

NIST is not alone in tackling these problems. A subset of microbiome researchers has had them in its sights since the field began to take off a decade ago. A few years ago, a consortium of microbiome researchers called the Microbiome Quality Control project had 15 independent laboratories genetically sequence a common set of gut and oral microbiome samples. Although the set of detected microbes differed significantly among the 18 people who provided the samples, suggesting that relative differences between individuals remained intact from lab to lab, each step of the analysis injected substantial variation, too, the group reported in 2017.

Typically, when companies develop a drug, they must demonstrate its identity, as well as its purity, potency, and stability. But what those terms mean in the context of the microbiome, where the “drugs” may be communities of microbes, is still being defined. The methods so far available for identifying the microbial species within these complex communities yield highly variable results.

That variability likely won’t stymie the path to approval of the first microbiome-based drugs, if the pioneering companies developing them can show the FDA internally consistent data that demonstrate safety and efficacy. But as the second, third, and fourth drugs progress to the clinic, regulators will need solid ways of comparing them with the second, third, and fourth drugs progress to the clinic, the second, third, and fourth drugs progress to the clinic, each step of the analysis injected substantial variation, too, the group reported in 2017.

Persistently, consistently variable
To some extent, the methodological woes bedeviling microbiome science simply represent a new field’s inevitable growing pains. When researchers first began sequencing the human genome en masse about a decade ago, they similarly had to contend with lots of inaccuracy, as well as uncertainty about what specific variations in the genome’s sequence reflect about people’s health. As the technology improved, the problems diminished, though they never fully disappeared. But the human genome has only 23 chromosomes and two copies of each gene. “The microbiome has an infinite number of possibilities” in terms of the kinds of microbes that could be present, Gohl says. No matter how good the research tools get, he predicts, it will be difficult to eliminate all the imprecision.

One of the biggest sources of variability in pinning down the cast of characters in the microbiome is the step in which researchers extract DNA from all the bacteria in a sample for sequencing, Gohl says. Some types, like Escherichia coli, break apart and release their DNA with just a little detergent, whereas others require special enzymes or mechanical force to break open their membranes and give up their genetic material. That means no single approach will extract a uniform cross section of microbial DNA from a given sample.

But even before researchers extract DNA, they must consider how a sample is collected. Is it preserved at the point of collection or carried fresh to the lab? Is it an actual stool sample or the toilet tissue used to wipe? Different DNA profiles also arise from different procedures for sequencing samples and from differences in the bioinformatic tools for identifying which microbes are present.

“A colleague and I tracked it—there are currently 97 different ways to analyze the same raw data, and they will give you 97 different answers,” NIST’s Jackson says.

That variation adds up in unpredictable ways, and it has undoubtedly muddled the literature. “That’s why you don’t get reproducible results,” says Rashmi Sinha, an epidemiologist at
the National Cancer Institute studying the link between the microbiome and cancer. For example, a news-making trio of papers published in January 2018 showed that people responded differently to a new class of immune-modulating cancer drugs depending on their gut microbiome composition. However, the three papers, each from a different lab, hit on somewhat different lineups of microbes responsible for the effect, presumably at least in part because their protocols varied.

So what to do? Many researchers in the microbiome community say it’s too soon for researchers to settle on specific shared techniques, because methods are still evolving in this young field, and what is considered state of the art is still in flux. Nailing down the tools too early hobbles a field’s growth and locks researchers into seeing only what the current techniques reveal, says Daniel McDonald, scientific director of the American Gut Project at the University of California San Diego.

**A fecal standard**

Instead of standard protocols, what the field really needs is a reference sample, many microbiome scientists say—standardized poop equivalent, containing as close as possible to a copy of a baseline community of microbes in the gut. Researchers could run such a reference sample alongside experimental samples—stool from, say, healthy people or those with a gut disorder. By getting a handle on how their results compare to the reference material, they could determine the biases in their study samples and thereby compare results across laboratories. “You need some ground truth, some way of knowing that two labs are getting the same answers,” Gohl says. “Having those reference standards is critically important for being able to do that.”

A few simplified versions of such microbial mixtures are already available for purchase. Companies like ATCC and Zymo Research sell reference standards containing 10–20 species of microbes, but that’s just a sliver of the hundreds of species inhabiting the gut, and it’s not clear the chosen ones are most important to human health. The Microbiome Quality Control project recently put the finishing touches on a larger, more representative mock-up of a gut microbiome community. About 5 years ago, Emma Allen-Vercoe, a microbiologist at the University of Guelph and a member of the group—along with Sinha and others—began developing a concoction made up of about 45 strains of microbes. The process proved more difficult than she anticipated, however.

For one thing, the microbial strains from which they were building the mixture turned out to be not as pure as they had thought. For another, to make their mixture more realistic they wanted to spike it with poop. That meant they had to use microbial species that were closely related to ones in the microbiome but not precisely the same, so they could be accurately quantified. Also, creating a stew of microbes as a reference material has an inherent drawback: your supply eventually runs out. It’s not possible to determine precisely how many of each kind of microbe you started with, and there is no way to control how they grow, so there’s no way to make another identical batch. “It’s one of those things that on paper, it sounds like, ‘Oh, this will be easy,’ and then when you actually sit down to do it, it gets really complicated,” Allen-Vercoe says. “I think we came to the conclusion that we can do our best, but we are never really going to have a ground truth.”

NIST launched its microbiome standards effort in 2016. One of its first acts was to partner with the pharma company Janssen. Working together, the firm’s Human Microbiome Institute and NIST have begun a large-scale study called Mosaic, in which hundreds of laboratories will run their microbiome-sequencing methods on a standard sample to get the widest possible picture of interlab variability. It took a couple of years to design protocols and make sample kits to ship to laboratories volunteering to participate, and it will take a few more to complete the study, but results from the first dozen or so laboratories are now rolling in.

Jackson’s team is modifying and testing the process that Mosaic used to make its reference material to create an even better assortment of poop that NIST can provide to scientists. Unlike Allen-Vercoe’s mock community, built from the bottom up, NIST’s gut microbiome reference standards will be made by starting with poop from four or five donors who vary in factors like age, sex, and health status; extensively characterizing those stool samples; and then homogenizing and stabilizing the poop. NIST’s supply won’t be ground truth either, Jackson concedes. “We can measure it 100 different ways and get 100 different answers, and nobody can point to one of those and say it’s right or wrong,” he explains. But it will provide a qualitative measure for laboratories to compare their results.

The researchers are now testing tubes of poop prepared in different ways to see which ones are most robust. For example, Mosaic samples were stabilized with a high salt buffer, but that salt content may interfere with mass spectrometry readings—a problem better avoided. “It’s a big commitment and we want to do it right,” he says. “We don’t want to spend years producing 1,000 units of this stuff and then find it’s no good because we overlooked something.”

Jackson’s team is also working on fabricated devices that researchers could use to perform systematic and reproducible experiments that perturb microbiome samples. This
spring, researchers at Harvard University described this type of gut-on-a-chip device, but Jackson envisions that NIST’s version will be simpler to use and thus more widely applicable.

Other microbiome researchers, meanwhile, are tackling other dimensions of standardization. For example, Sinha’s lab is investigating how different protocols for preserving microbiome samples affect experimental outcomes. An additional major issue that groups are diving into is the widespread inconsistencies in the way microbes are named and the way that species are defined. These are not impossible problems to solve, but they will require a concerted, community-wide effort, Sinha explains. “I think it’s doable,” she says. “People have to step back and say, ‘I know this is really painful, but it’s a grind that has to be done.’”

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