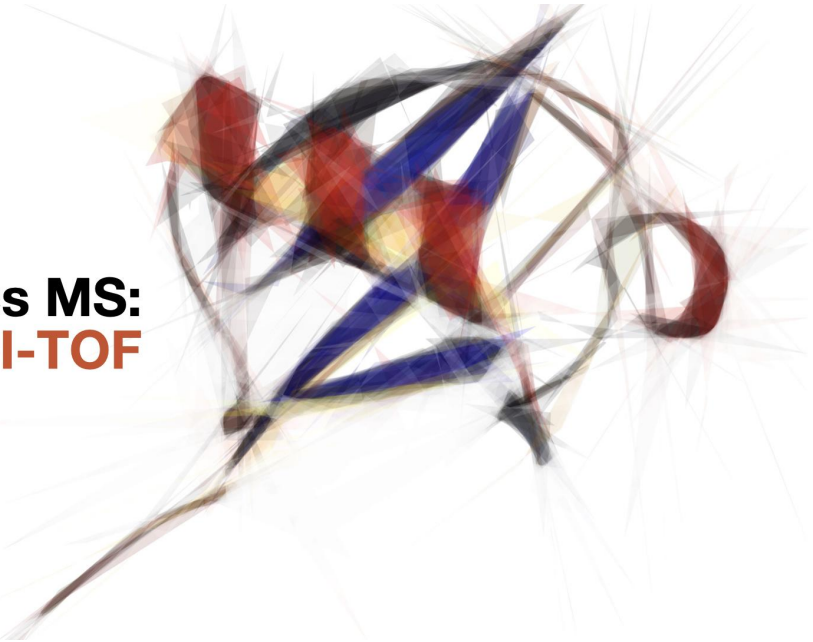
Gut: Stool Testing - Part 1

Tracey O'Shea: Hey, everybody. In this presentation, we're going to talk all about stool testing. There are two primary categories of techniques for stool testing. The first category is culture-based techniques that include both high-complexity culture and proteomics-based [mass] spectrometry stool testing. You'll often find the high-complexity culture and the proteomics-based [mass] spectrometry combined together in a lot of laboratory testing. So that's usually how you'll see those come together. The second category is molecular or sequence-based techniques that include DNA [polymerase chain reaction] (PCR) testing, 16S rRNA gene sequencing, and whole genome sequencing.

How [does] proteomics work? Proteomics-based [mass] spectrometry stool testing uses proteomics or the study of proteins for identification and characterization of proteins in cells, tissues, or organisms. These microorganisms have unique ribosomal fingerprints that can be identified from those ribosomal proteins. So these signature proteins, also known as protein fingerprints, are matched against this open, rapidly expanding database that holds over 1400 species at this point, for really quick identification of bacteria, fungi, yeasts, and pretty quick turnaround, less than 40 seconds or so. High-complexity stool culture combined with mass spectrometry of microbial proteins is [a] proteomics-based [matrix-assisted laser desorption/ionization time-of-flight] (MALDI-TOF) method. It can rapidly identify over 1400 microbial species without having to purify or isolate them.

Proteomics MS: MALDI-TOF



MS = mass spectrometry

The MALDI-TOF method has also been shown to have 99 percent accuracy in the identification of common commensal microbes, 100 percent accuracy in identification of common pathogenic species, and high reproducibility across labs. But this method earned a share in the Nobel Prize in Chemistry in 2002. It ranked third in the Cleveland Clinic's list of medical innovations that would likely improve patient care in 2013, and we see that it has [done so]. It was also approved by the [U.S. Food and Drug Administration] (FDA) pretty quickly around that time. So it was a pretty big innovation, and we're happy to have it, especially in the stool testing world.

Validation of MALDI-TOF

99%
+ ID of **normal flora that can be cultured** at the genus level

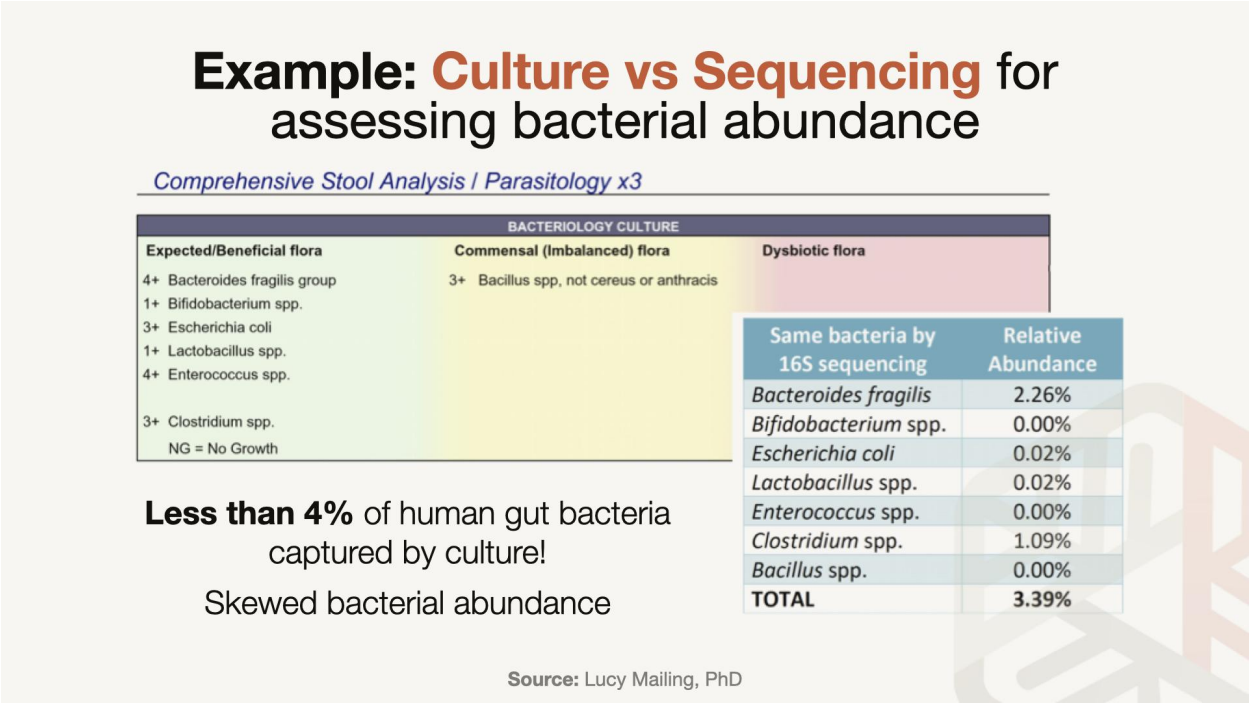
100%
+ ID of ***Salmonella***, ***Campylobacter***, ***Vibrio***, ***Yersinia enterocolitica*** species

99%
Concordance across **8 international labs**

Several studies have validated the MALDI-TOF method. One looked at 605 stool samples, 304 different colonies from selective cultural media, and in terms of enteric pathogens, there was 100

percent positive identification of *Salmonella*, *Campylobacter*, *Vibrio*, and *Yersinia enterocolitica* species. These are all microorganisms associated with acute diarrheal illness. For normal flora, there was a positive identification of 236 pure isolates, 99 percent and 97 percent genus and species level. It is important to note, though, that most Functional Medicine labs outside of a research setting are not culturing this amount of microbes. That's why you're seeing such a smaller number of commensals being reported in these stool reports. They have the capability of purifying the isolates, but they just don't have that many primers and they're testing for a lot smaller number.

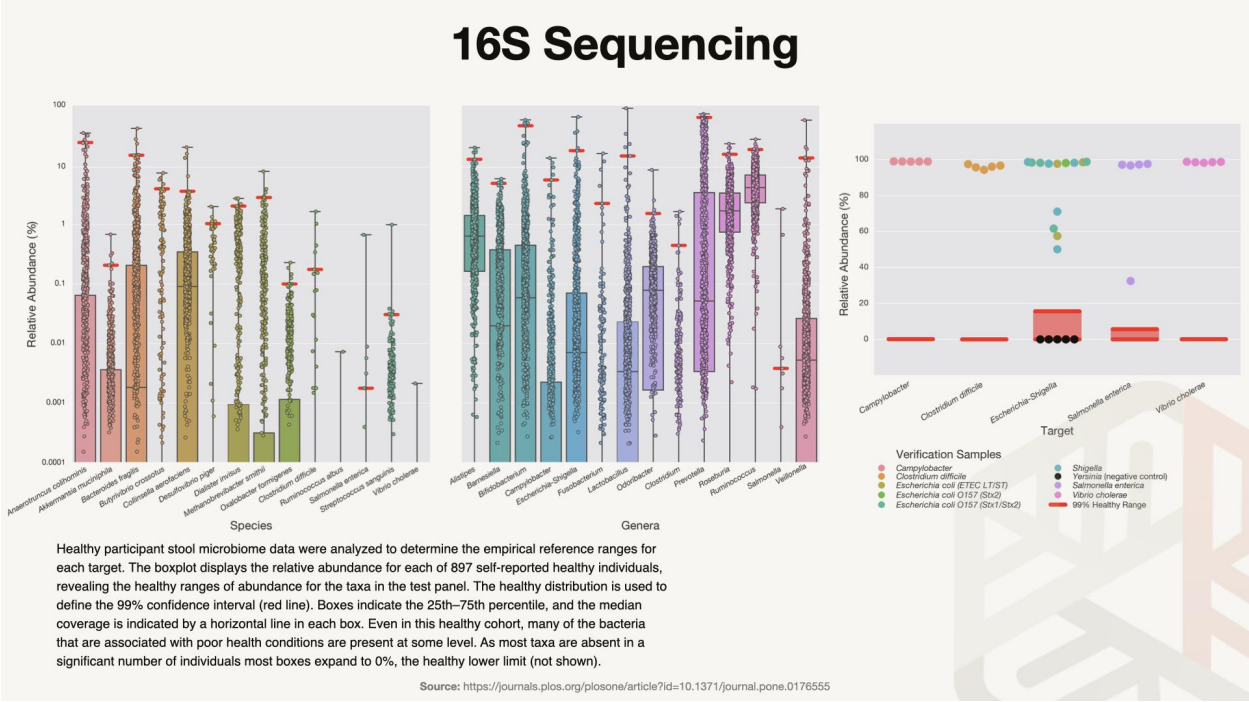
It's also important to note that this methodology can accurately identify the microorganisms that are oxygen tolerant and the ones that are able to be cultured. And even though they're accurately doing that, it still typically accounts for less than 5 percent of total bacteria. So while it's accurate once cultured, the limitation with the MALDI-TOF is the ability to report abundance, and to culture a large number of organisms. This can dramatically skew the results of abundance of microbes. Overall, the MALDI-TOF is extremely accurate for potentially pathogenic microbes, but it can give a false idea of the relative abundance within the stool sample.



Here's an example of results from culture versus sequencing from the same stool sample that shows the discrepancy between culture and sequencing that we were mentioning before. First, let's look at the culture-based results in the top left. Some of you probably are very familiar with

this report. It provides [these] semi-quantitative data on a scale from NG being no growth to four plus. So when you run this same sample by 16S ribosomal RNA gene sequencing, the 16S returns all of the bacteria in the gut. And when we match up the culture-based results and look at the relative abundance, we find that only less than 4 percent of the commensal bacteria were actually captured by culture. So it skews the relative balance of bacteria. You can see the *Enterococcus* is listed at four plus, yet, it really only makes up less than 0.01 percent of the entire ecosystem. So if this was a four plus in the pathogenic category, you might be wrongly treating something that makes up less than 0.01 percent of the overall ecosystem. So it's just something to pay attention to and consider when we're looking at culture-based lab stool testing.

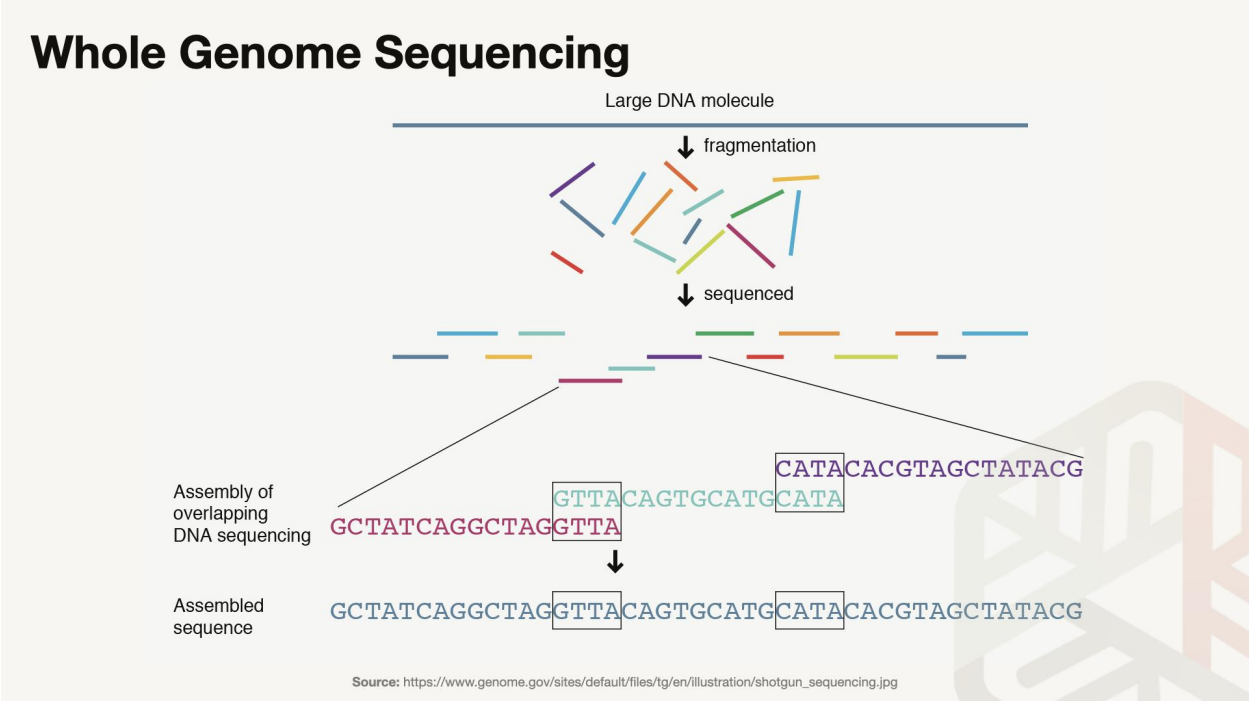
Let's talk a little bit about DNA PCR testing. The PCR technology amplifies DNA by creating a large number of copies of a particular DNA fragment in the presence of primers, one of the major steps involved in full DNA sequencing. Overall, PCR technology is very accurate for identifying microorganisms. But one potential limitation for PCR testing may be that it requires a specific primer for each microorganism, which dramatically increases the cost for the lab running the markers. So if a lab chooses to have a limited number of primers, that will often determine the actual number of microorganisms that that lab can test. DNA PCR may be able to provide susceptibility information, but the use of this clinically is still limited. I should note that at the time of this recording, Diagnostic Solutions [Laboratory] is the only known commercial stool lab that is reporting susceptibility information based [on] DNA and not culture and sensitivity.



Healthy participant stool microbiome data were analyzed to determine the empirical reference ranges for each target. The boxplot displays the relative abundance for each of 897 self-reported healthy individuals, revealing the healthy ranges of abundance for the taxa in the test panel. The healthy distribution is used to define the 99% confidence interval (red line). Boxes indicate the 25th–75th percentile, and the median coverage is indicated by a horizontal line in each box. Even in this healthy cohort, many of the bacteria that are associated with poor health conditions are present at some level. As most taxa are absent in a significant number of individuals most boxes expand to 0%, the healthy lower limit (not shown).

Source: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0176555

16S sequencing is similar to other sequencing methods, but only a single gene encoding 16S ribosomal RNA is sequenced. The 16S ribosomal RNA gene is common to almost all bacteria, so determining an organism's abundance when using the 16S gene is not as accurate as other sequencing methods because that 16S gene varies widely in copy numbers per genome. Some limitations to the 16S ribosomal RNA methodology include identification of bacteria at [the] genus level only. No species or strain information is available. There's no eukaryotic, fungi, or parasite testing, so it's not comprehensive in that sense. And it's also subject to primer bias, which can reduce the accuracy of the relative abundance of each microbe in the sample. Popular companies that you may have heard of are Thryve, American Gut, and Atlas Biomed. And we know at [the] time of [this] recording, uBiome is no longer in operation.



Whole genome sequencing, also known as metagenomics or shotgun sequencing, results in the precise and correct order of nucleotides of a given DNA fragment. This is the preferable technology for many target regions, or if you're having a high sample number when compared to qualitative PCR, mostly because of the primer issue that we mentioned previously, where you [must] have a single primer for each microorganism that you're testing. It is likely to provide more information on microbial richness and diversity of the sample. Currently, BiomeFX and Onegevity are the labs offering whole genome sequencing for commercial purposes. Our preference right now is for Onegevity, because of their techniques and reproducibility when doing side-by-sides.

And for now, we think that the raw data [are] likely reliable, but the clinical utility at this stage is still lacking and in our opinion still needs to be combined with other stool testing techniques to provide well-rounded results in data.

What this means is the raw data come out, you have the numbers, you have the diversity, [they] show you what's there, but the clinical utility of these results [is] still questionable, and I think we're moving in that direction and we will be there soon. I think it will eventually provide affordable and clinically relevant information.