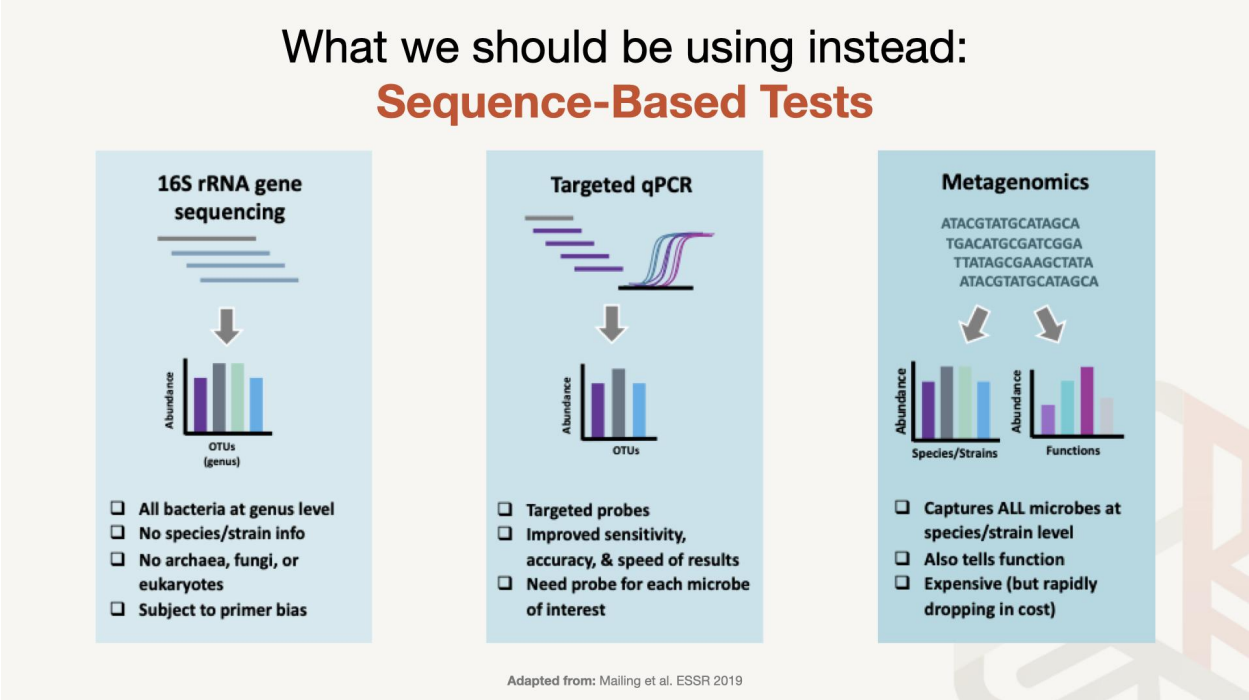
Gut: Stool Testing - Part 2



Tracey O'Shea: So what should we be using instead? I think ultimately, sequence-based tests are the preferred method of testing. There [are] pros and cons, though, to each method of testing, and there just isn't a perfect stool test yet. This is why most commercial labs or practitioners are combining different methodologies and using different lab companies to offer the most robust data.

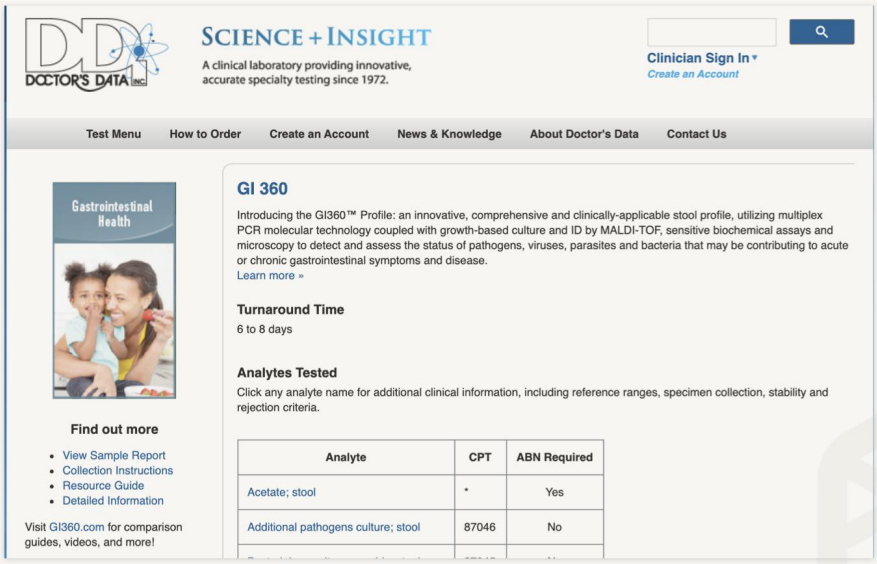
Culture methods are simply unable to truly quantitate microbes or analyze many anaerobic [gastrointestinal] (GI) microbes at all. The DNA and RNA sequencing methods, including the most common 16S approach, offer the ability to test for a wide array of organisms at [a] higher taxonomic level in a cost-effective manner. But they lack the ability to quantitate and report to the clinician just how much of anything is actually present in a specific patient. These sequencing techniques are most appropriately used in research settings where the goal is to look across a wide array of organisms and to determine the relative populations to one another within a subject sample. So this is why test results for 16S sequencing and metagenomic and metatranscriptomic sequencing-based methods are reported as a percentage of the total DNA found versus an absolute quantitative amount of organism DNA found.

When the goal is to use the test diagnostically on an individual subject and then apply that information to make clinical decisions, much like we're doing in a Functional Medicine practice, then the most appropriate methodology we think is [polymerase chain reaction] (PCR), and a quantitative method such as qPCR is ideal and preferred. This is generally performed on a curated target list of clinically relevant organisms where the clinician is provided information not only about what is present, but also how much of each organism is there. [There's a] pretty rapid turnaround time with PCR methodology that's conducive to a clinical setting, whereas a lot of culture-based methods may take a couple [of] weeks to maybe even a month, respectively, depending on the lab.

Stool Testing Methods	**Fully quantitative***	**Highly sensitive detection** ***(Measures very low levels of organisms)***	**Each analyte individually validated**	**Provides only Clinically Relevant Organisms**	**Rapid turn around time** ***(within days)***	**Identifies bacteria, parasites, fungi, and viruses down to the strain level**	**Identifies genes involved in microbial function**
qPCR / rt-PCR	+++	+++	+++	++	+++	+++	++
Standard PCR	–	++	++	++	+++	++	++
Shotgun Metagenomic Sequencing	–	+	–	–	–	++	+++
Metatranscriptomic Sequencing	–	+	–	–	–	++	+++
16S Sequencing	–	+	–	–	–	–	–
Culture + MALDI-TOF MS	–	–	+	+	+	–	–
Microscopy	–	–	+	+	+	–	–

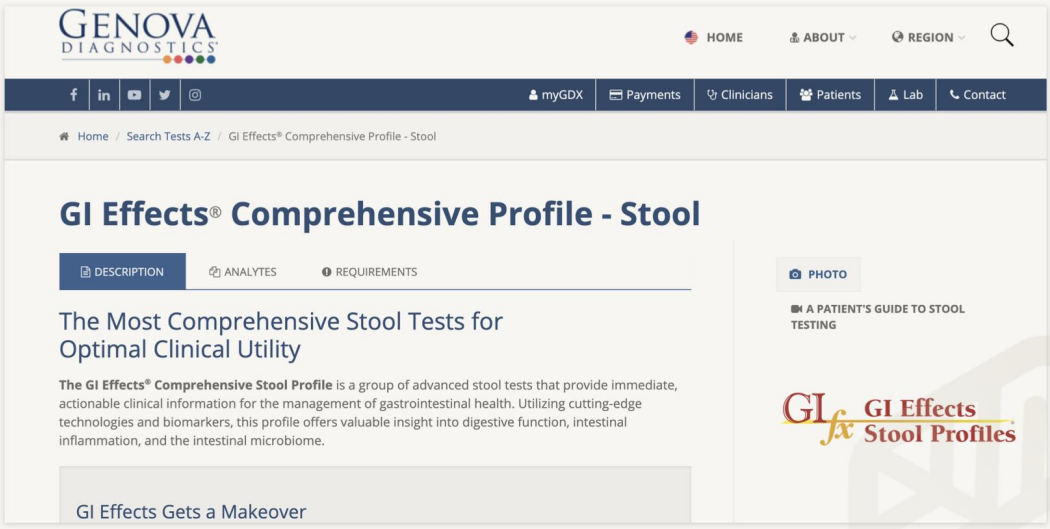
Adapted from: https://www.townsendletter.com/article/450-diagnostic-stool-testing-methodology/

Here's a nice comparison chart from the *Townsend Letter* for your reference. It gives the pros and cons and different [methodologies] that are included, [and] some that aren't. It's a nice reference if you're trying to make some sense out of all of this.



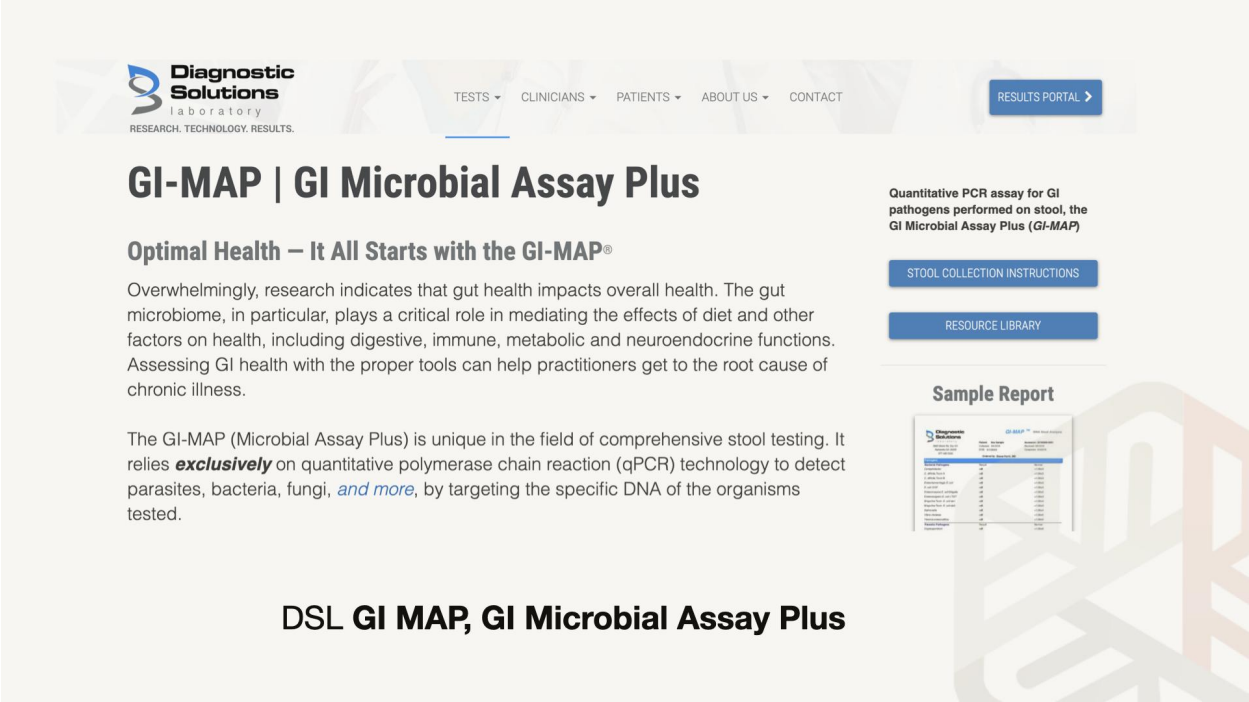
Doctor's Data **GI360™ Profile**

Now, let's take some time to discuss the different lab companies that we've been using in practice. I'm going to go over the three main companies that we've been using, and then discuss why we're using them and how we're utilizing them and the interpretation. This is the Doctor's Data GI360. It utilizes multiplex PCR technology coupled with the [matrix-assisted laser desorption/ionization time-of-flight] (MALDI-TOF) proteomics. It identifies and characterizes [the] abundance and diversity of more than 45 analytes [and] evaluates [the] composition of gut flora. It also can identify the presence of pathogenic viruses, bacteria, and parasites. [It] also give[s] you something called a dysbiosis index, which is a calculation with scores from one to five based [on] the overall bacterial abundance and profile within the patient sample as compared to a reference population. Other valuable information [it provides] includes biomarkers for digestion, absorption, inflammation, and immune status; [assessment of] short-chain fatty acid production; and biomarkers of overall health such as stool pH, white blood cells, red blood cells, mucus, occult blood, and more.



Genova Diagnostics **GI Effects**® **Comprehensive Stool** Profile

[The] Genova [Diagnostics] GI Effects Comprehensive Stool Profile uses a combination of PCR, culture, and microscopic methods. It also evaluates the composition of gut flora. There [are] biomarkers of digestion that include pancreatic elastase, fecal fat, [and] markers of inflammation and immunology, like calprotectin fecal secretory [immunoglobulin A] (IgA). It can identify the presence of pathogenic viruses, bacteria, and parasites using a combination of culture and PCR testing. It also recovers live organisms like yeast and bacteria for susceptibility testing and add-on biomarkers that can include *Campylobacter*, [*Helicobacter pylori*] (*H. pylori*), lactoferrin, zonulin, and a few others.



Diagnostic Solutions [Laboratory] (DSL) has a comprehensive test called the GI-MAP. It relies exclusively on qPCR. So while the other labs that we talked about have a combination of methodologies, GI-MAP just uses qPCR to detect potential pathogens [and] commensal flora. It looks for bacterial, [parasitic], and viral pathogens. It's important to note that because this is a PCR test only, there is a little bit more clinical correlation required when deciding on treatment because the presence of DNA material or pathogenic strain does not always indicate illness as the levels of toxins being produced [are] not measured. So this may be, in some instances, where you really need to correlate clinical symptoms or follow up with toxin testing as needed, depending on which pathogen is identified.

I think there's just a little bit more critical thinking that might be involved when we're only using qPCR. Other markers include commensal, opportunistic, and dysbiotic flora, potentially pathogenic parasites, worms, and viruses. Biomarkers for intestinal health are also included here. I don't think the intestinal health section of the GI-MAP is as robust as the Genova or Doctor's Data test, but it does still provide you with some valuable information. There is antibiotic resistance that's measured using antibiotic-resistant genes versus the gold standard of culture and sensitivity. This is where I mentioned previously where there's this one lab that is using PCR technology to report antibiotic resistance, but they're really doing that based [on] the genes that exist and not necessarily with culture and sensitivity. And they do have *H. pylori* testing, using the

qPCR technology, and it also includes virulence factors, which we'll talk [about] a little bit more during the *H. pylori* section.

[For] GI testing, we've talked about pros and cons of different methodologies. I've just presented three different companies that we have used variously in different combinations. But I think it's really important to remember that this is a quickly changing landscape and that as practitioners, we always need to understand the strengths and weaknesses of available testing. And ultimately, that testing, while important, should rarely be viewed as the sole factor in clinical decision-making. We've seen huge changes in stool testing in the past five years, and I fully expect us to see even more in the next five. We're constantly adjusting our lab preferences to meet the needs of patients, and we need to stay flexible and adaptable.

Also, with many of the treatments that we're advocating for, like nutrition, botanical[s], probiotics, the specific pathogens, overall may be less important than the overall approach that we're using. In other words, if a lab incorrectly identifies a pathogen or misses something, but you also have another, enough markers that are indicative of imbalance, it may all point to [an] overall pattern of dysfunction. And then we're treating using botanicals to have a broad spectrum of activity, and we may achieve the clinical goal that we set out to even if the pathogen we end up treating isn't the one identified on the test.

So this isn't an issue unique to Functional Medicine or even natural medicine. For example, studies have shown that rifaximin helps patients with [irritable bowel syndrome] (IBS), even when they don't have [small intestinal bacterial overgrowth] (SIBO). Well, the question is why. And we're just not sure, but researchers suspect that other effects of rifaximin, like a beneficial impact on the colonic microbiome, may be playing a role. It's also important to not get too hung up on the idea that we can only get good treatment results if we use the best possible lab. Many practitioners taking this course for various reasons may not have access to one or more or any of these specific lab tests that we recommend. That doesn't mean you can't use other labs and still get great results with patients. In fact, I'm sure [for] many, as well as Chris, when they first started their practice 10-something years ago, these tests didn't exist; these methodologies weren't perfected, and he was still getting fantastic results with patients. So I don't think it's absolutely necessary.

I want to give you a little bit of background of how we've used these tests. As of about a year ago, we were mostly using Doctor's Data and Biohealth Labs. And then Biohealth Labs closed down. Other companies like the ones we previously mentioned start[ed] to produce new lab kits in these updated methodologies. So we then changed over to using [the] DSL GI-MAP for about a

year or two, with a few others sprinkled in there. And now, we're using a combination of three different labs that I just discussed. And I imagine that will continue to flux and change as we go.

We've done some split samples that overall seem pretty reproducible and reliable between GI360 and GI-MAP. Some small consistencies that we see [are] mostly with abundance reporting, and there were a few items that were completely missing off of those tests that were found, missing off of GI-MAP that we're finding in GI360. But again, [there are] pros and cons to each lab test and methodology. And we really have to take into consideration that there's probably variability that likely exists even within a single stool sample. Let's start discussing lab results and interpretation.