Interview with Dr. David Quig

Chris Kresser: I'm really excited to welcome Dr. David Quig from Doctor's Data as a guest in the ADAPT Level One Framework program.

David Quig received his master's degree in human nutrition from Virginia Tech and his PhD in nutritional biochemistry from the University of Illinois. He was then a postdoctoral fellow at Cornell University for five years prior to serving as senior cardiovascular pharmacologist with a major pharmaceutical company for seven years. For the past 29 years, he has performed and published research pertaining to nutrition and chronic disease. He regularly gives presentations at international and national biomedical conferences. Dr. Quig is currently vice president, scientific support, for Doctor's Data, Incorporated.

I asked Dr. Quig to come speak with us about stool testing. As you know, one of the stool tests I'm going to be teaching is the Doctor's Data Comprehensive Stool Analysis with Parasitology assessment. Dr. Quig is one of the most knowledgeable people I know about stool testing in general, all of the different methodologies that are available, and the pros and cons of each, and so I wanted to invite him to come on and talk about that with us in some more detail. I think you're really going to enjoy the conversation and hopefully learn a lot, so let's get to it.

Dr. Quig, welcome. It's a pleasure to have you on the show. Thank you so much for joining us.

David Quig, PhD: Thank you for having me.

Chris Kresser: I'd like to begin by just doing a bit of an overview of the three primary methods of stool testing. Since we're talking to clinicians here, folks are going to be familiar with culture-based stool testing certainly because that's been the dominant paradigm of stool testing for decades, but I think it would be really helpful to maybe start with a 30,000-foot overview of the three main techniques and then get into some detail about the pros and cons of each because, of course, that's really the important background here when we start to talk about the advantages of the Doctor's Data tests that I'm going to be teaching to students and including in the training.

David Quig, PhD: Yeah, I think it's really important to realize that for every single laboratory assay, there are pros and cons. That's just the nature of the beast, but I think it's a fascinating time to be in clinical microbiology and especially related to the gastrointestinal microbiome. The three primary methods for evaluating the microbiome would include the full genome sequencing, PCR, and then culture, now with proteomic identification.

I'll start with what is coming on the horizon, the full genome sequencing. When I first started reading about this, it was next-generation sequencing. Then it was next-next-generation sequencing.

Chris Kresser: Right.

David Quig, PhD: It's absolutely a fascinating area, and I can't even describe how it's done. The analogy that I like to use is it's like taking 10 boxes of puzzles that each have 10,000 pieces in them, dumping all of them out on the floor, somehow magically all of the 10 different puzzles get put back together again. It's just mind boggling, the technology there.

Full genome sequencing provides a very high throughput evaluation of nearly the entire gastrointestinal microbial DNA content, and the biggest advantage there is that it even can identify the DNA for microbes that cannot be cultured.

Chris Kresser: Right.

David Quig, PhD: Having said that, it still has a depth bias, and that is very minority species present at very low concentrations of the total microbial DNA are potentially missed, but at the rate of progression of this methodology, I think that that's going to be overcome rather quickly.

Now, one of the problems with this method right now—and it's why I say it's really on the horizon—is that currently the results are not clinician friendly. It's like reading a phonebook.

Chris Kresser: Yeah.

David Quig, PhD: It's probably worse than reading just a printout from 23andMe about all the different SNPs. But I have great confidence that informatic systems will soon be able to compress the results to be much more clinically interpretable. Having said that, however, culture-based testing is still required to identify strains and most importantly for direct susceptibility testing against antibiotics and natural and botanical agents because there we're looking at the phenotypic expression. There's no way to just look at the genes of a microbe and look at the antibiotic-resistant genes. There are hundreds and hundreds of those, and you don't know whether they're turned on or turned off, so we still have to fall back on culture for susceptibility testing. But the bottom line is, for the horizon, full genome sequencing will eventually complement culture in assessing the microbiome and is progressively replacing PCR-based methods.

Chris Kresser: Mm-hmm.

David Quig, PhD: Which leads us to the second primary way to look at the microbiome, and that's through PCR. I think the most important take-home message here is that not all PCR-based methods are equal. Basically, PCR, the polymerase chain reaction, involves amplification of a single copy or a few copies of a piece of DNA that's been captured by a probe, and that amplification generates thousands to millions of copies of a particular DNA sequence. Because of that huge amount of amplification, that's much room for error in that process.

Now, when I say that not all PCR is the same, there's some fantastic PCR out there, but the old-school, antiquated PCR-ELISA method is very different from the current state-of-the-art, real-time, multiplex PCR platforms, which have been validated for the classic pathogens. They're excellent for, is a particular pathogen there, but as with any PCR, it's limited based on the number of probes that are available.

Now, the PCR-ELISA method that's been around for a while, it's vintage 1990s methodology. It's typically in-house developed, non-peer-reviewed or non-peer-validated methodology that's fraught with false negatives. It also has the depth bias in that it can only detect down to about 104 colony-forming units per milliliter of DNA extract, and it also has contamination issues. I'm sure that you've seen the recently published paper about one such PCR-ELISA method that failed miserably in having 100 percent false negatives.

Chris Kresser: Yes, for pathogens.

David Quig, PhD: Yeah.

Chris Kresser: Fifty-six percent false positive for parasites and 6 percent false positive for yeast. I did see that.

David Quig, PhD: Actually with the yeast it was like flipping a coin. It was 50 percent.

Chris Kresser: That's troublesome because that technology had kind of gained a little bit of a foothold in the functional medicine community, and I know a lot of practitioners are still using it … or were using it. I guess there are some changes that have been made, but it's just really important to get this information out there and to continue to refine our understanding and knowledge of this so we can give patients the best case possible.

David Quig, PhD: Absolutely. That's everyone's goal. The bottomline for PCR is that intensively validated real-time, closed, multiplex PCR platforms are definitely excellent for high-throughput analysis, but again, they're limited in the number of probes and microbes that can be detected. So when we're thinking from a much more functional medicine-type perspective, the most comprehensive way to evaluate the beneficial, expected, and commensal microbes still relies on culture using a wide array of culture conditions. In traditional medicine, they want to know, are any of the eight diarrhea-causing bacteria present, period. They don't care about the beneficial.

Chris Kresser: They're looking for the known pathogens like Yersinia or C. diff or Salmonella.

David Quig, PhD: Right.

Chris Kresser: So in 10 years maybe we'll have accurate, affordable, and clinically relevant and actionable DNA sequencing of the microbiome. I'm just throwing that number out there, but I like some of the analogies you use, like reading the phonebook. The information you get back from it

may be interesting from a research perspective but not really clinically that actionable, which is kind of the same boat that we're in with a lot of the SNPs at this point. We know that they're important, we know that there's probably a lot of hidden information in there that we're going to be able to make use of at some point, but at the moment, we're just scratching the surface, and to some extent, it's kind of that saying of a little bit of knowledge is a dangerous thing because you get people extending or amplifying the implications of those findings above and beyond what we can really rely on, based on the scientific literature.

Let's talk a little about culture, which has kind of been in some ways lately viewed with some disdain as an old-school method, but let's talk a little bit about some of its advantages, too.

David Quig, PhD: Yeah, I'm glad you put it that way because one of the most inaccurate statements out there—and it's thrown about even by full-blown authentic microbiologists—is that the vast majority of gastrointestinal bacteria cannot be cultured. Since I've been working on this stuff and working very closely with some of the world's renowned microbiologists, I ask them about this question. What do you mean they cannot be cultured? They all came to the same conclusion that a more accurate statement would be that most gastrointestinal bacteria are not routinely cultured.

Chris Kresser: Ah.

David Quig, PhD: And that's primarily because most labs are only using three different culture conditions. How can you expect so many different species of bacteria to grow on three different plates? And most of the time they're not even doing anaerobic culture.

Chris Kresser: Right.

David Quig, PhD: Really, to drive that point home that they're not routinely cultured, I'd just love for your audience to google the term "culturomics." At a Swiss lab, a gentleman called Lagier has done heroic work with a technique called culturomics. What culturomics is incorporates a vast array of culture conditions coupled with a proteomic method for identifying what is cultured, and as you read through some of these papers, you'll find that using this culturomics technique, they've actually been able to detect more species from a single stool specimen than they were able to using 16S rRNA pyrosequencing.

Chris Kresser: Wow.

David Quig, PhD: Now, granted, they were using up to 200 different culture conditions in this research setting, but it just really drives home my point that the statement that they can't be cultured is far from the truth.

Chris Kresser: So it's not a question of what you can see; it's a question of how intensively you're looking.

David Quig, PhD: Right. And full-blown culturomics like I just described is not practical for routine analysis.

Chris Kresser: Right.

David Quig, PhD: I just use it to illustrate my point that there's so much more that can be grown than is currently routinely done.

Nontraditional microbiology using a greater array of culture conditions better addresses the question that we're all begging, and that is, what is present or absent, as opposed to, is it present or absent, which is what you get when you use a PCR-based technique.

Chris Kresser: Right.

David Quig, PhD: Some of the pros of culture-based testing: It actually has greater sensitivity for those microbes that can be cultured. You can detect down to one colony-forming unit per gram of stool. Just by comparison, some of the PCR-based techniques are two to three orders of magnitude—that's a hundred to a thousand times—less sensitive. The culture-based techniques are really important for something that drives me crazy a couple of times a week, and that is that it identifies insufficiency dysbiosis and/or bacterial imbalance. You can have very significant GI as well as systemic symptoms when you have insufficiency dysbiosis even in the absence of dysbiotic microbes.

The reason I say it drives me nuts is when clinicians call and they say, "Well, you didn't find any dysbiotic bacteria. You didn't find any yeast or parasites. What's wrong with this test? It just doesn't find anything?" Well, you look at the beneficials and they're tanked—insufficiency dysbiosis. And then you see this huge pile of commensals, which just really indicates a severely disrupted microbiome. That in and of itself can result in very significant clinical symptoms, like I said, both in the gut and systemically.

Chris Kresser: Sure.

David Quig, PhD: Inflammation and immune dysfunction.

Chris Kresser: Yeah. Intestinal permeability and the whole nine yards.

David Quig, PhD: Absolutely.

Chris Kresser: We know a lot now about the protective effect of those bacteria and what can happen when they're insufficient. Lower levels of butyrate, which controls the inflammatory response and plays an immunoregulatory role, so, yeah, I'm with you on that.

David Quig, PhD: It's not so much a clinical pearl as it's a reality that you have to really understand that entire mucosal immune barrier and the regulation of inflammation and the immune response is so highly dependent on those beneficial bacteria. Sometimes that's all that you really have to deal with. Everybody is trying to stamp out or eradicate a pathogen, but it's not always the pathogen.

The other thing about culture is that it's absolutely required to do direct susceptibility testing, which is a phenotypic expression. Again, you have all these antibiotic-resistant genes, but you have no idea whether they're turned on or turned off, and none of that evaluation has ever been validated for use in the stool analysis.

Of course, there are some cons with culture. One of the cons is that most commercial labs do not routinely use an extensive array of culture conditions, and so you're only going to find what you're fishing for, basically. But having said that, even with nontraditional culture, there absolutely are fastidious microbes for which ideal culture conditions have yet to be established. I think the really fun part about this is this is where you have the marriage with next-generation sequencing or full genome sequencing, where, as they find at least the genetic material for these species, then you're going to work backwards and say, "OK, let's figure out the best way to grow this stuff so that now we can do the really exciting stuff to me as a biochemist, and that is to look at the metabolomics. What are these things actually doing? What ticks them off? What makes them happy? What are they secreting? How are they affecting inflammation and the immune system? At some point, it's going to really feed into the "old-school culture," where you're really looking at the phenotypic expression, which you can't do by just looking at a pair of genes. Obviously after an extensive wide array of culture conditions, you have to be able to positively identify the species that were grown, and that's where we enter the fascinating revolution in clinical microbiology, the proteomic method of MALDI-TOF MS, which is, of course, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight mass spectrometry.

Chris Kresser: Acronyms sure come in handy sometimes!

David Quig, PhD: Yeah, the acronym comes in handy there! My friend MALDI identifies microbes by their signature high-abundance proteins, and those proteins are mostly the highly conserved proteins, primarily ribosomal proteins, and the signature mass protein spectra for a given microbe or an unknown microbe is compared to spectra in a reference database or a library. The matching-up of the spectral peaks to the peaks in the reference library provides a rapid identification of bacteria, yeast, and fungal species from an array of anaerobic and aerobic culture, and the amazing thing is that you can ID an unknown in less than a minute. The old automated biochemical techniques took between six and 24 hours to identify a single microbe, where we're talking on the order of 40 seconds here now.

Chris Kresser: Wow. That's incredible.

David Quig, PhD: You have to see it in action. I'll just kind walk quickly through it. After culture, the technician just takes a portion of a colony-forming unit from an array of different conditions, embeds that portion of the colony-forming unit in a saturated matrix, and that saturated matrix on a target plate stabilizes and crystallizes the proteins and imparts positive charge. Then that matrix is dried, and then the dried matrix with the portion of the colony-forming unit is zapped with nanosecond pulses of laser, which actually causes ionization and vaporization of intact proteins. Vaporized proteins fly through an electronic field, and it's under vacuum, so they're flying through the field inversely proportional to their mass. So the small proteins reach the mass spec before the midsize and larger proteins, and the mass spec will then give you a printout. The mass spec protein profile for the unknown is immediately sent to the computer, which then matches it up with the spectral peaks that are contained in the reference library.

Chris Kresser: There are 1,400 species now in that library, right? Somewhere around there?

David Quig, PhD: Yes. It's phenomenal. And that's really a good point because the size of the reference database or library really does matter. There are limited libraries used by many labs, but they currently only contain about 400 spectra for gastrointestinal bacteria and yeast. A different approach is to use a much more expansive library, a much more functional approach, to use an extremely comprehensive open-reference database, which, as you say, currently contains about 1,400 GI species, and it's growing on a monthly basis. We're just in the beginning of this. I remember when we started with this, it was only about 800, and it's growing in leaps and bounds.

Chris Kresser: I can really understand why the developers of this earned a share of the Nobel Prize in Chemistry. Is that right? In 2002?

David Quig, PhD: Yes, they did.

Chris Kresser: Yeah, it seems like a pretty revolutionary development in this field.

David Quig, PhD: Yeah. I call it space age. It's Star Trek-y to me.

Chris Kresser: Yeah! Talking to some of the challenges with the DNA methodology that we talked about before, the possibility of false negatives and false positives, with MALDI-TOF you have something like 99 percent accuracy for identification of normal flora at the genus level and 100 percent ID rates of the more common pathogens and then 99 percent concordance across eight international labs. Those are pretty impressive numbers in terms of specificity and reproducibility.

David Quig, PhD: Right. In addition to that, if you don't get a high enough score for a match to what is in the reference database, the manufacturer of the equipment can actually have that particular pure isolate that's been MALDI-TOF'd and do full gene sequencing to come up with what it is and then add that to the database. That's part of the process of adding to to the library.

Chris Kresser: Mm-hmm.

David Quig, PhD: But as I said, every method has pros and cons.

Chris Kresser: Yes.

David Quig, PhD: The biggest pro is that it has the potential to rapidly—as I said, less than a minute—identify just about anything that you can grow in culture if you're using the vast reference database, and currently, like I said, it not only has the ability to identify 1,400 different species, but important to me is the fact that we can now identify 170 different species of yeast, including 80 different candida species. That was unheard of five years ago.

Chris Kresser: Yeah.

David Quig, PhD: Another pro is that it eliminates the need for subculturing. Typically one would culture on one particular medium and then pick colonies and subculture them, which takes a lot of time. Now you can pick a colony-forming unit from a primary culture and zap it, and you don't have to wait around. There are several hundred published papers that validate the methodology, and probably the most impressive was the publication that showed the incredible reproducibility across labs. They looked at 480 different specimens in eight different international labs, and they got 98 percent concordance for identification of the bacterial species.

Chris Kresser: Wow.

David Quig, PhD: I don't think you can say that for measuring insulin across eight different labs!

Chris Kresser: Right! No. I was going to say that's much higher than what's accepted as a valid, reproducible test.

David Quig, PhD: Yeah. Some of the cons would be if a lab is not using the most extensive reference database, you only have the potential to identify about 400 different species, which isn't a whole lot more than you could using the old automated biochemical timely methods. Then another con would be that currently it does not include viruses or parasites, which, of course, you're not going to be growing in culture anyhow.

Chris Kresser: Right.

David Quig, PhD: But there are real-time multiplex PCR platforms to identify viruses and parasites.

Chris Kresser: Mm-hmm. One of the issues as a clinician that I'm often up against is— this is a little bit of a tangent, but I'm just curious since we're talking about the subtypes and the possibility of getting even more granular with this stuff—Blastocystis hominis, as I'm sure you're aware, some of the more recent, more advanced DNA sequencing technology has determined that it's pretty common even in the stools of … I always say "healthy" with air quotes because you never know.

When they're talking about healthy subjects in these studies, I don't really know what that means. Anyhow, in so-called healthy subjects, I've seen some recent studies indicating that Blasto is present in something like 75 percent of people, which then leads to the question of, why does Blasto cause problems for some people and not for others? And of course, you have to consider things like the host environment, which is probably the biggest factor, but—I know the research literature is mixed on this—some people believe that subtype of Blasto plays a role, and other people don't, but do you see a time where we'll be able to more easily identify the subtypes of organisms like that if we find out that one subtype is more pathogenic than others so that a clinician might be able to make a more informed decision about whether to treat?

David Quig, PhD: Yes. From my reading, the consensus is that there are about 17 different subtypes of Blastocystis hominis, and I know that there's a lab in Europe that specializes in subtyping the Blastocystis, but I think for right now, if you see many, many moderate Blastocystis on a comprehensive stool analysis report, you just have to use your clinical skills, and there are no symptoms, then don't treat it. If there are significant symptoms, then treat it.

Chris Kresser: Yeah.

David Quig, PhD: But someday it's going to become much more widely available and affordable to do the subtyping.

Chris Kresser: Right.

David Quig, PhD: I think that's more the issue than there being an argument whether Blastocystis, per se, can cause problems. I think we have to get down with a fine-tooth comb and say, "OK, which subtype is it?"

Chris Kresser: Yeah, I agree with that. It's like saying, "Does food cause problems?" Maybe not quite to that extent, but our understanding has evolved a lot from this kind of idea of … now we have the old friends hypothesis and the idea that some of these commensal organisms that we co-evolved with, not only may they not be harmful, they may actually even play some protective role. It's a little bit more nuanced and complicated than we originally thought it was, I think.

David Quig, PhD: Absolutely.

Chris Kresser: Let's circle back. We've talked about these different three methods of stool testing, and let's talk a little bit more specifically about the Doctor's Data CSAP, the Comprehensive Stool Analysis with Parasitology test, that I'm teaching in this course and which technologies you've chosen and are including and how that works out in that profile.

David Quig, PhD: Basically working with one of the world's renowned microbiologists, we're sticking with the cutting edge on microbiology at the moment, using nontraditional microbiology. We do a total of 10 different anaerobic, aerobic, and microaerophilic culture conditions. That's a

huge step up from your traditional three plates where you're not even looking at anaerobes. Then we follow that with the state-of-the-art for clinical microbiology ID post-culture using the MALDI-TOF proteomic method and absolutely use the largest library available, so we're looking at 1,400-plus potential species versus hundreds. Again, I can't stress enough how important it is to be able to identify 170 different species of yeast, especially 80 candida species. And we're still doing the microbiology because, again, the susceptibility testing is done on every patient's dysbiotic bacteria and yeast, using both natural and botanical agents as well as antibiotics for those who choose to use antibiotics. We also offer enzyme immunoassays for Shiga toxins, and that would pick up the E. coli O157 and Shigella. Then we also have authentic genetic platforms for the analysis of what's known as the PaLoc gene that encodes for toxins A and B produced by C. difficile as well as genetic platforms for other pathogens. Our approach is basically to stick with what is sound and proven in the scientific literature.

Chris Kresser: This test is something that I've been using probably over two years now, maybe three years now, and have just found it to be a really helpful tool in the arsenal of doing a full functional medicine workup, even for patients without gut symptoms. Of course, I'm going to be talking about this at length in the training, to the point where you'll probably get tired of me saying it, but just the ability to identify things like insufficiency dysbiosis, which is really, really common, in my patient population, at least, and to feel confidence in terms of what we see in terms of dysbiotic pathogens and then things like parasites, is excellent. I've found it to be the most useful comprehensive stool test that I've used, and I've pretty much used them all, as you can imagine, as someone who's been doing this for a while and who's kind of experimental and investigative by nature.

I'd like to move forward and talk about one other topic that's maybe a little bit controversial or just misunderstood, perhaps, and that's secretory IgA, fecal sIgA, or sIgA. Then I want to finish up by talking about the trio of inflammatory proteins—calprotectin, lactoferrin, and lysozyme—which can be really, really helpful in differentiating between functional bowel conditions and inflammatory bowel disease and just detecting inflammation in general.

Let's start by talking about fecal sIgA and what it can and can't tell us and some of the myths and truths about.

David Quig, PhD: From what I've read, there's a lot yet to be learned about what regulates the secretion of the secretory IgA and other very mundane aspects to a clinician with respect to the biochemistry and physiology that's going on, but I see nothing that suggests that it's OK to have zero secretory IgA in any organ that has mucosal tissue, anything that has wet tissue. Secretory IgA is absolutely the first immunological response to enteric pathogens, and it absolutely provides immune exclusion. If it wasn't so important, why would nasty critters like some of the candida species secrete a secretory IgA-specific protease enzyme, as do some bacteria? These things have weapons of their own, and one of their weapons is to knock out secretory IgA. Teleologically that says to me that, hey, it's important stuff, and it's always there. If it's not actively involved in binding an enteropathogen and hastening its removal from the body—I like to look at it like a biological

chelator—it's at least surveying what's going on and hanging around the beneficial bacteria and helping them not only thrive and colonize and proliferate, but also keeping them from overgrowing. It's all about balance there.

The other important thing about secretory IgA is it's absolutely essential in your innate antiparasitic protection, in that it's involved in eosinophil activation. It's also very important to downregulating proinflammatory responses to pathogenic bacteria and other antigens. It quenches the proinflammatory cytokines.

One thing important about secretory IgA, sometimes you'll be looking at a report and you don't see any dysbiotic bacteria, yeast, or parasites, but you see significantly elevated secretory IgA, which is an appropriate immune response, and you have to keep in mind that with a gastrointestinal virus, secretory IgA can remain elevated for up to four to six weeks after a virus. That's the first thing I ask. If I don't see any dysbiotic microbes or parasites, I ask if the person has had a gastrointestinal flu, as people call it.

I don't think it's as controversial from the clinical perspective, and I just don't understand how there are some laboratories around the world that are saying the reference range for secretory IgA is zero to 800. I don't know of an immunologist that would say that it's OK to have zero secretory IgA. It reminds me of the paper I read from Lancet, of all places. It was a letter to the editor, and it was back in, like, 1976. There was an MD that had written about this young six-year-old boy that just had chronic, massive candida overgrowth. That admission right there was huge.

Chris Kresser: Yeah, that's unusual.

David Quig, PhD: But he went on further to say that this child had an inherited secretory IgA deficiency, and he was going to be plagued with yeast for life.

Chris Kresser: Whoa.

David Quig, PhD: And this was 1976.

Chris Kresser: Yeah.

David Quig, PhD: So I don't think it's as controversial. Reading some of that literature gets so technical that it gets confusing, but I think it's pretty clear that we really need the stuff.

Chris Kresser: Yeah, I think that's pretty clear when you look in the literature. What I would say just from what I've read is what's less clear is whether low or high sIgA indicates anything specifically on its own or whether it is a marker that tracks with certain conditions and improves with treatment. That's typically what I see.

David Quig, PhD: Right.

Chris Kresser: I'll see low or high sIgA in people who have parasites or fungal overgrowth or dysbiosis or IBD inflammation, some combination of all of those things, and then when we treat them and start addressing those conditions, then the sIgA starts to normalize. But of course, then there are people who have inborn or hereditary deficiencies. If we see super-super-low sIgA, then I'll often do a test for total immunoglobulins just to make sure that they're producing any IgA anywhere outside of the gut. Thanks for clearing that up because I think the more research comes in, the more we actually find that we need it and the more of a red flag low sIgA is.

David Quig, PhD: There's one case study that I often present, and again, it's a young boy, an autistic child. He had four different species of candida at 3+ or 4+. His secretory IgA was 1.6 milligrams per deciliter of stool. Then it begs the question, is it all the antisecretory IgA proteolytic enzymatic activity from the candida knocking it down?

Chris Kresser: Exactly.

David Quig, PhD: Or does this child have an inherited deficiency in his ability to put together secretory IgA, or total IgA, for that matter?

Chris Kresser: Right. Or even some combination of both. That's what I meant by the causality not totally being clear, but nevertheless, you can still look at it and say this is a sign of a problem and that there's a significant compromise in immune tolerance in the gut. I like what you said earlier about the role of it kind of … the way I've described it to some extent is kind of as the mother of beneficial bacteria in the gut, where it plays a nurturing role in maintaining a healthy gut ecosystem. As we know, even in the popular media now, I think, it's amazing how many people now have heard of the microbiome and the importance of beneficial bacteria, so I don't think that's controversial in the slightest at this point.

Let's finish up by talking about some of the inflammatory proteins because that's something that's definitely unique about your test and especially recently with the addition of calprotectin, which is even more specific, I think, at least according to some of the papers that I've read.

David Quig, PhD: Yeah, it's really interesting. We look at what we call our triad of inflammatory protein markers. They're all released into the lumen by neutrophils in response to some inflammatory condition. Lysozyme is a nonspecific marker of inflammation, and by nonspecific, I mean it'll be significantly elevated with the presence of dysbiotic bacteria, yeast, or parasite. It will also be markedly elevated with inflammatory bowel disease.

Chris Kresser: Right.

David Quig, PhD: On the other hand, lactoferrin and calprotectin have much, much greater clinical specificity with respect to inflammatory bowel disease, so with an enteropathogen only, you'll see elevated lysozyme, whereas with true IBD you'll see all three of the proteins elevated in stool. Now,

if you look at the sum total of the literature on the polyclonal assayed calprotectin and lactoferrin, they're equivalent. Really there's no difference.

However, recently there has been developed a monoclonal calprotectin assay that has superior sensitivity, and so to beef up our lactoferrin and lysozyme markers, we've just added the monoclonal calprotectin to the whole inflammation panel just so that we're covering all the bases. It's more sensitive. I've seen it light up incredibly well. Basically, our standard trio of the inflammatory proteins, the quantitative lactoferrin, some labs use a qualitative lactoferrin as a positive indicator. We prefer to go with the more quantitative assays. The monoclonal calprotectin assay and the lysozyme in combination allow the practitioner to really comprehensively and differentially assess organic versus just enteropathogen-induced or some other acute cause of gastrointestinal inflammation. Just yesterday I consulted with a clinician, and she was barking up the wrong tree. She noticed that, yes, there was some insufficiency dysbiosis. There were two species of yeast at 1+, which a lot of people have without having any overt or obvious problems.

Chris Kresser: Yes.

David Quig, PhD: Then, as I went down the report, I looked at the inflammatory panel. Lactoferrin, calprotectin, and lysozyme were unbelievably elevated. This was a two-year-old child.

Chris Kresser: Aye yai yai.

David Quig, PhD: She told me about the protocol. She had him on all these natural botanical and antimicrobial things, and I said, "Why? You should follow this up in about four weeks, and then if you have another positive on, say, calprotectin and/or lactoferrin"—which can be ordered stand alone; you don't have to order the whole test—"then it is recommended that that person be referred to a very competent gastroenterologist and undergo further evaluation."

Chris Kresser: A colonoscopy. Yeah, I have to say we've diagnosed at least five or six people with IBD that didn't know that they had it before using your test. The path that we generally take is we'll evaluate the markers using the evidence-based ranges for differentiating between IBD and functional bowel disorders. Then depending on the age of the patient and their situation, we might redo the test in a month, but I like to also include a blood panel that covers some of the newer antibodies, like pANCA. LabCorp has a good antibody panel for Crohn's and inflammatory bowel disease, and if they have any positive results there and their C-reactive protein is elevated and their sed rate is out of range, then we'll refer them for a colonoscopy. We've done that a few different times, and these are typically patients that don't have the classic presentation. They don't have bloody diarrhea, and so their primary care physician—and sometimes even their gastroenterologist—doesn't think to do a colonoscopy or a more complete screening for IBD because they just don't fit the classic profile.

I don't know if you saw this. There was a study that recently came out—I have all these research alerts set up—but it was a group of patients, a case report, whose only observable sign or

symptom of IBD was oral lesions. They didn't have any gastrointestinal symptoms at all. It was sores in the mouth, basically, but they had an active inflammatory process, and when they ran the serum markers, they were positive, and then when they did a colonoscopy, they saw active disease in the intestines. So really, I can't emphasize enough how important it is to have these markers on the test because you'll be catching people who have no idea that they have this inflammatory process going on.

David Quig, PhD: Yeah. I just also a week before had a case of a 36-year-old female who went to her clinician because she was having pain in her abdomen. They did a stool analysis. Well, in the meantime, before she got her results back, she was rushed to the emergency room.

Chris Kresser: Wow.

David Quig, PhD: She was doubled over in pain. They scoped her and they found out that she … sure enough, when we got the test results back, it was the highest levels of lactoferrin, calprotectin, and lysozyme that I've ever seen. This is a 36-year-old woman who previously, like you said, didn't know.

Chris Kresser: Yeah.

David Quig, PhD: What triggers this, it's analogous to the late-onset celiac disease, where people have been consuming gluten all their lives. They may have the genetic predisposition, the HLA-DQ2 and DQ8 or portions of it, but it's not until their fourth decade where something triggers, something changes. It's just fascinating.

Chris Kresser: Yeah. Fascinating and tragic because that person … for example, they have the genetic predisposition and they have some kind of trigger, maybe an intensely stressful event or maybe a gastrointestinal infection or exposure to mold or something like that, and they start producing antibodies to gluten. But like I was saying with IBD, maybe they don't have the classic diarrhea or IBS type of response. Maybe they start to develop eczema or a dermatitis skin condition.

David Quig, PhD: Right.

Chris Kresser: Maybe they start to develop some brain fog and they're not thinking as clearly. They go in to their doctor, and their doctor's like, "Oh, you know, you're getting older. Ha ha ha, we all forget things. It's just normal aging." And they say, "OK, I guess so. Everyone just kind of talks about that, so I guess I'm just aging." What their doctor didn't find out is that they had started producing antibodies to the myelin sheath or to glutamic acid decarboxylase. Then 20 years later they develop dementia and early-onset Alzheimer's, and again people are like, "Oh, bad luck. You must have had bad genetics. Geez, there sure are a lot of people getting Alzheimer's now." Who's going to make that connection to the gluten or undetected inflammatory bowel disease? That's the biggest tragedy for me, and so I'm really excited to be able to hopefully get these more accurate

and sensitive tests and knowledge about them out there so that we can save lives, really. That's what it comes down to.

David Quig, PhD: Yeah, and the quality of life as well. Absolutely.

Chris Kresser: Yeah.

Well, Dr. Quig, thank you so much for joining me. I think this is really going to help a lot of people to understand the ins and outs of stool testing. As a clinician myself, I know how we're certainly not taught this stuff in school. Then we get out there into the world and it's a bit like it is … not necessarily to the extent with pharmaceutical companies, but a lot of, I think, what we learn initially as clinicians is from what we find maybe from someone that we respect, that is a mentor to us, or then literature from a particular lab, and maybe we don't have time to really follow it up and do the exhaustive research ourselves and figure out whether what we're being told is legitimate and is actually a representation of what's in the evidence-based, peer-reviewed literature. It's nice to have that perspective here and know that these methods are. It's really important for me. Everything that I recommend I want to have that kind of backing behind it, and I spend a lot of time reading the research, so it's great to be able to have a lab to work with that has that same approach.

David Quig, PhD: Oh, it's wonderful. I wouldn't be here if it wasn't that way!

Chris Kresser: Yeah, I bet.

All right, well, thanks again for joining us. I appreciate your time. If clinicians want to get set up with you and learn a little bit more about your approach and what you have to offer, I assume they can just go to your website and contact someone on your staff through the normal channels?

David Quig, PhD: Absolutely.

Chris Kresser: Great. Thanks again.

David Quig, PhD: Thank you, Chris.

Chris Kresser: Take care.

David Quig, PhD: All right, take care.

Chris Kresser: Bye.

David Quig, PhD: Bye.