Clinical Chemistry

Reducing the Noise in Plasma Metagenomics to Further Define Clinical Utility

Article:
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Clin Chem 2025; 71(7): 728–30. https://doi.org/10.1093/clinchem/hvaf065

Guests: Dr. Erin Graf from the Mayo Clinic in Phoenix, Arizona and Dr. Jennifer Bosquez, who is starting her medical microbiology fellowship at Northwell Health in New York.

Bob Barrett: This is a podcast from *Clinical Chemistry*, a production of the Association for Diagnostics & Laboratory Medicine. I'm Bob Barrett. Metagenomics, the evaluation of circulating microbial DNA in human plasma samples using next generation sequencing, can detect pathogens that may not have been considered by the clinical team or may be difficult to grow using traditional laboratory culture methods. Retrospective analysis of tens of thousands of metagenomic sequencing results has indeed revealed unambiguous clinically actionable findings in several cases, allowing providers to rapidly adjust their plan of care.

However, these clearly actionable findings are vastly outnumbered by the ambiguous or uninterpretable detection of commensal or opportunistic bacteria. Do these findings indicate true infection, breakdown of the mucosal barrier, or simply contamination during specimen collection, processing, or testing? How can clinical laboratorians eliminate the guesswork and harness the power of metagenomic sequencing to maximize its positive impact.

An editorial, appearing in the July 2025 issue of *Clinical Chemistry,* highlights an original research article that identifies sources of error in metagenomic sequencing and lays the foundation for interlaboratory coordination to improve the clinical utility of this testing.

In this podcast, we welcome the editorial's authors. Dr. Erin Graf is an Associate Professor and Co-Director of Microbiology at the Mayo Clinic in Phoenix, Arizona and her research interests include diagnostic stewardship and utilization of advanced molecular diagnostics. Dr. Jennifer Bosquez is a recent PhD graduate in Microbiology from the University of Arizona and is starting her medical microbiology fellowship at Northwell Health in New York and we will start with you, Dr. Bosquez. Your editorial is entitled "Reducing the Noise in Plasma Metagenomics to Further Define Clinical Utility." Let's start with the basics here. What exactly is plasma metagenomics and what do you mean by noise?

Jennifer Bosquez: Hi, Bob. Great question to start us off. So, plasma metagenomics is the analysis of circulating microbial DNA from patient blood samples using the power of next-generation sequencing to potentially identify the presence of bacteria, fungi, viruses, or parasites. This diagnostic tool has been particularly useful in picking up fastidious or vector borne infections that would otherwise be difficult to diagnose using conventional methods.

Now, when we refer to noise, in this context, we're talking about signals or detections that are either misleading or unclear in their clinical significance. As bacteria and fungi are ubiquitous in the environment, including on all human surfaces, contamination during pre-analytical, for example, sample collection and analytical stages, for example, from the person performing the tests, or in laboratory reagents, or sample-to-sample cross-contamination, are all possible sources of noise.

In addition, plasma metagenomics might take up DNA from organisms that are common skin or gut commensals such as *E. coli* or *Staphylococcus epidermidis* and other types of bacteria. These detections could reflect true infection or simply translocation across a damaged barrier like the gut lining. And unfortunately, because there are no FDA approved assays or bioinformatic pipelines for plasma metagenomics, there's often no easy way to validate whether these findings are actually clinically meaningful.

This puts physicians in a difficult position when deciding whether or not to act on them. So, reducing that noise is really about figuring out how to distinguish true infections from background signals or irrelevant detections.

Bob Barrett: So, just to follow-up on that, in your editorial, you discuss a recent *Clinical Chemistry* publication, "A Comprehensive Assessment of Metagenomic cell-free DNA Sequencing for Microbe Detection." What's the significance of that publication and can you give us the major takeaways?

Jennifer Bosquez: Yes, absolutely. So, this study is really important because it's the first large-scale multicenter quality assessment of plasma metagenomic sequencing. These researchers used extremely intelligently designed mock samples that mimiced the degraded and low abundance nature of real plasma cfDNA spiked with clinically relevant potential pathogens at varying concentrations to robustly survey analytical sensitivity. And they sent these mock samples to over 130 independent labs to see how well each lab could detect these pathogens. And what they found was really encouraging. Despite major variability in methods used by these labs such as DNA extraction methods, to sequencing, to the bioinformatic

pipelines used, the overall accuracy was really high and false positives were very rare.

When they did occur, they were usually traceable to wet bench contamination, especially during nucleic acid extraction. This is really significant because it shows that at least from a technical standpoint, most of the noise we worry about, it doesn't seem to be coming from the labs themselves, and that really shifts the focus to biological or host-derived factors like mucosal barrier damage as potentially more likely sources of ambiguous results.

So, the study not only builds confidence in the reliability of lab workflows, but it also highlights the need for standardized proficiency testing and better biological understanding of what contributes to ambiguous signals. And it's a really strong foundation for moving forward towards more consistent clinically meaningful interpretations of this really powerful technology.

Bob Barrett: Okay, thank you for that, Dr. Bosquez. Dr. Graf, you've been sitting patiently waiting here. How do these findings impact the field of plasma metagenomics? In other words, what's next?

Erin Graf: Thanks, Bob. As Dr. Bosquez so eloquently put it, these findings really give us confidence that many of the steps and the processes of metagenomic sequencing that we used to worry about being the sources of noise like the reagents, for example, have now been tightly controlled after over a decade of refinement. And as we keep working towards standardization and harmonization of metagenomics, the data from this study show that at least some of the steps in the process, for example, maybe the reagents used across different extraction platforms, may not be as critical to standardize compared to things like the bioinformatic pipelines and the post-analytic cut-offs that we used to determine clinical significance.

But what we really need are true clinical trials, particularly for plasma since it is so prone to noise, as Dr. Bosquez mentioned, from mucosal spillover. So, we need clinical trials from the types of patients who are often cited as the use cases for metagenomics, namely immunocompromised hosts, and these hosts inherently have a higher degree of mucosal barrier damage and therefore "noise in plasma." Many of the initial clinical trials for plasma metagenomics use normal or healthy hosts as their negative controls. And so, we really need to understand the expected noise from the plasma of an immunocompromised host population as a negative control.

Bob Barrett: These clinical trials seem promising. What exactly do you think would be needed to achieve something like that?

Erin Graf: Yeah, I think a multicenter study perhaps similar to what was done in the *Clinical Chemistry* Diao et al. publication could be achieved using plasma samples from a large cohort of immunocompromised hosts who are deemed by clinical experts to not be under investigation for infection. So, in a sense, they're negative controls but in the right patient population.

Of course, the difficulty here becomes the detections in these hosts could either reflect noise as we described, or perhaps early signals for a true infection that will later manifest. So, this is where the clinical trial component comes in. These patients would need to be longitudinally monitored to assess whether an infection ultimately develops or whether they remain to be asymptomatic.

Further, clinical trials in which symptomatic hosts are randomized to have plasma metagenomics testing or have only standard of care testing, and then comparing outcomes and other metrics such as hospital length of stay, are still really needed to accurately define the clinical utility of this methodologic approach.

Bob Barrett: Well, finally, Dr. Graf, is there anything else related to the use of metagenomics as a clinical diagnostic tool that we should be on the lookout for?

Erin Graf: Yeah. Plasma and cerebrospinal fluid, or CSF, which we haven't talked about today, have been the most common sources for metagenomic testing due to their "sterility" and I say air quotes around sterility because as we mentioned, the mucosal spillover of microbial DNA really complicates the presumed sterility of plasma.

Well, now, we're actually seeing more interest in other sample types. For example, lower respiratory tract sampling such as a BAL fluid, or a bronchoalveolar lavage fluid, and that type of specimen is frequently contaminated by upper respiratory tract colonizing organisms as well as fungal spores that are inhaled. So, ideally, before metagenomic sequencing hits primetime in lower respiratory tract sample types, we would have clinical trials similar to what I just described, randomizing patients and accurately assessing the full clinical impact. So, Bob, I'll just end by saying that hopefully, we can find ways to collaborate as a field to create such trials for our patients.

Bob Barrett: That was Dr. Erin Graf from the Mayo Clinic in Phoenix, Arizona and Dr. Jennifer Bosquez from Northwell Health in New York City. They wrote an editorial in the July 2025 issue of *Clinical Chemistry* describing a metagenomic sequencing

quality improvement study. They've been our guests in this podcast on that topic. I'm Bob Barrett. Thanks for listening.