Study finds genetic method for identifying hundreds of disease agents 'promising'

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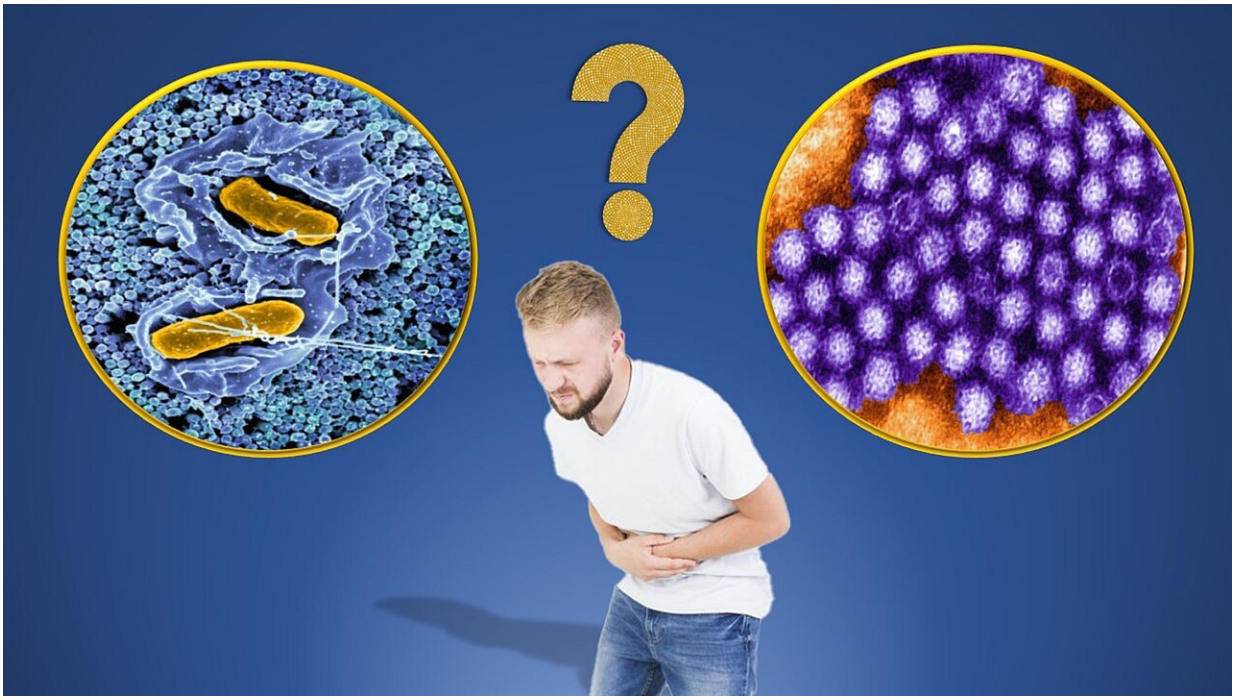

Diagnosing the source of a patient's illness—in the pictured scenario, a gastrointestinal disorder that may have a bacterial (Salmonella on left) or viral (norovirus on right) cause—may one day be simpler and more accurate using next-generation sequencing (NGS) than with current methods. A recent Johns Hopkins Medicine study found that NGS genetic diagnostic systems show promise but need refinement. Credit: Graphic created by M.E. Newman, Johns Hopkins Medicine, using public domain images.

In the pursuit of accurate diagnoses for illnesses, doctors have traditionally used multiple methods—including culturing patient samples on a wide variety of media, reviewing countless medical records and analyzing clinical data using complex mathematical algorithms—to try to identify the bacterium, virus, fungus or other pathogen responsible for an infection. The hunt is often slow and laborious, and the processes used may not be broad enough in scope to find specific disease agents.

One solution may be next-generation sequencing (NGS), according to the findings of a recent study by Johns Hopkins Medicine researchers. NGS enables clinicians to simultaneously sequence multiple strands of DNA found in patient samples and use that analysis to rapidly and accurately identify a single pathogen—from among hundreds of suspects.

In a paper first posted online June 13, 2022, in the American Society for Microbiology's *Journal of Clinical Microbiology*, the researchers compared the pathogen detecting ability of an NGS system—the Respiratory Pathogen Infectious Diseases/Antimicrobial Resistance Panel (RPIP)—with a previously studied NGS system and standard of care (SOC) diagnostic methods for samples obtained with bronchoalveolar lavage. This is where a bronchoscope is passed through the mouth or nose into the lungs, followed by a fluid wash that is collected for examination.

The researchers believe their study is among the first to compare NGS and SOC diagnostics for respiratory pathogens.

"We evaluated the two NGS diagnostic techniques, one of which was the RPIP, and found that in both cases, the ability of NGS to identify specific pathogens was nearly comparable to the battery of diagnostic tests clinicians have been using for decades," says study senior author Patricia Simner, Ph.D., M.Sc., associate professor of pathology at the

Johns Hopkins University School of Medicine. "Although this shows great promise for the RPIP and NGS diagnostics in general, we feel more work is needed to further refine the technology before NGS can be considered equal to or better than current SOC methods."

In their study, Simner and her colleagues first evaluated the diagnostic ability of metagenomic NGS, a previously studied workflow process during which all DNA obtained from a bronchoalveolar lavage is sequenced—including genetic material unique to the patient (the "host read" or "human read") and the sought-after pathogen (the "microbial read"). Removing the host DNA enable clinicians to concentrate their search on the remaining genetic material to hopefully find the microbial read and ultimately, identify the cause of the patient's illness.

In the second part of their experiment, the researchers assessed a different NGS approach using the RPIP system called targeted NGS. In this method, everything in the patient respiratory sample is sequenced as with metagenomic NGS, but capture probes—tiny fragments of single-stranded DNA that correspond structurally to the DNA of specific pathogens—are used to enhance the searching ability.

"Using NGS to find the genetic signatures of pathogens is akin to searching for information on a specific topic in a library with a tremendous number of books," explains study lead author David Gaston, M.D., Ph.D., a former pathology fellow at the Johns Hopkins University School of Medicine now at the Vanderbilt University Medical Center. "With metagenomic NGS, you have to read through all of the books to uncover the ones that reference the topic. But with targeted NGS, you first ask the librarian to pull those volumes most likely to include the topic and then conduct a more focused, more promising search."

The researchers found that the effectiveness of both the metagenomic and targeted NGS varied with the type of organism sought. They report

that both NGS methods successfully identified viruses, with herpes viruses the most readily detected. Results for bacteria and mycobacteria (which include the organism causing tuberculosis) approached the level of SOC diagnostics, but dropped off as the number of organisms decreased—even with use of the capture probes in targeted NGS. Neither NGS method detected fungi well.

Overall, the researchers found that the RIPP targeted workflow agreed with traditional diagnostics 66% of the time. More specifically, they noted a 46% agreement for targeted NGS to detect pathogens of clinical importance and an 86% agreement for showing that pathogens were absent.

Along with its potential to accurately identify more than 300 pathogenic organisms from a bronchoalveolar lavage, the researchers feel that targeted NGS also shows great promise for one day being able to reveal some 1,200 genetic markers in pathogens that indicate which organisms are most likely to resist antibiotics.

"Overall, the current accuracy of both metagenomic and targeted NGS is nearing that of current diagnostic procedures and that's a major takeaway from our study," says Gaston. "We found that NGS can detect a lot but not all pathogens, and that in some cases, both NGS methods could identify pathogens that traditional diagnostics would have missed."

"There are currently pros and cons for the use of NGS as a microbiological diagnostic tool," says Simner. "For example, the RPIP targeted workflow requires more time and reagents but needs less bioinformatic analysis of the resulting data. On the other hand, metagenomic NGS is less technically demanding but requires more complex analysis."

Based on their findings, the researchers feel both the metagenomic and

targeted NGS workflows can currently be considered adjuncts with, but not yet replacements for SOC diagnostic techniques. With more refinement, they believe, NGS systems may one day become the standards for respiratory pathogen diagnosis.

More information: David C. Gaston et al, Evaluation of Metagenomic and Targeted Next-Generation Sequencing Workflows for Detection of Respiratory Pathogens from Bronchoalveolar Lavage Fluid Specimens, *Journal of Clinical Microbiology* (2022). DOI: 10.1128/jcm.00526-22

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