RNA profiling pathogens

By Tim Fulmer, Senior Writer

Massachusetts researchers have developed an RNA-based method for the rapid detection of pathogens in clinical samples.¹ The team is now designing an integrated diagnostic platform that contains a comprehensive set of bacterial, viral and fungal probes to help pinpoint specific pathogens and their degree of drug resistance more efficiently than conventional diagnostics.

Most hospitals rely on culture-based methods to diagnose infectious diseases and determine drug resistance. Culturing infectious agents typically takes two to four days, and determining a pathogen's drug resistance profile can take up to a month. The long delay necessitates the use of broad-spectrum antibiotics to treat the early stages of infection and also can increase a patient's risk of death because the optimal treatment regimen remains unidentified.

The primary strategy to replace culture-based methods has been to use pathogens' genome sequences as sources of unique biomarkers that can be readily detected in patient fluids using standard PCR and DNA sequencing.

Although such approaches can help detect the presence of a particular pathogen, they do not provide insight into drug resistance because in many cases the DNA mutations that cause a particular form of resistance are unknown.

Now, a team of researchers from the **Broad Institute of MIT and Harvard** and from **Harvard Medical School** has zeroed in on RNA as a potentially better diagnostic tool than DNA for infectious diseases. The group was led by Deborah Hung, an infectious disease physician at **Brigham and Women's Hospital** and **Massachusetts General Hospital** and a researcher at Broad.

Just like a DNA signature, an RNA profile contains sufficient information to accurately identify the presence of a pathogen. In contrast to DNA analysis, RNA profiling can help determine whether a pathogen is drug resistant because antibiotic exposure triggers stress-induced changes in the RNA profile of a drug-sensitive pathogen, whereas little or no change is seen in the profiles of resistant pathogens.²

To test this idea, the team first designed a set of fluorescent oligonucleotide probes that targeted mRNA sequences unique to

Mycobacterium tuberculosis and to three different Gram-negative pathogens: *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

Using these probes, the researchers detected and distinguished each of the four pathogens in pure culture and in complex mixtures containing eight additional pathogens. Next, they designed probes that identified nonbacterial pathogens, including viruses (influenza, HSV-2 and HIV-1), a fungus (*Candida albicans*) and a parasite (*Plasmodium falciparum*), suggesting that the mRNA detection platform was applicable across a broad range of infectious agents.

The team then tested whether the approach could also be used to determine drug susceptibility of pathogens.

Following a 10-minute exposure of wild-type and ciprofloxacin-resistant *E. coli* strains to the antibiotic, the researchers saw changes in mRNA levels of a subset of genes. The result was an mRNA ciprofloxacin-susceptibility signature in the wild-type strains. Two

other antibiotics, gentamicin and ampicillin, also elicited unique mRNA drug-susceptibility signatures in *E. coli*.

The researchers then looked for such signatures in other organisms. They found a ciprofloxacin-susceptibility signature in *P. aeruginosa* and *M. tuberculosis*. The latter also had isoniazid- and streptomycin-susceptibility signatures.

Finally, the researchers looked at whether they could detect mRNA signatures in clinical samples.

In 34 urine specimens collected from patients who had tested positive for a urinary tract infection, the method identified all 17 *E. coli*-positive samples. In a second set of 13 *E. coli*-positive urine samples, the method differentiated ciprofloxacin-sensitive and ciprofloxacin-resistant *E. coli* strains. The bacterial loads detected in these clinical samples were 10⁵–10⁹ cells/mL.

The findings were published in the *Proceedings of the National Academy of Sciences*.

"Our method can potentially identify infectious pathogens within three to four hours, compared, for example, to the two to three days typically required for diagnosing a MRSA infection or the three weeks needed to diagnose tuberculosis," said Hung.

According to Jeremy Bridge-Cook, SVP of the assay group at **Luminex Corp.**, one advantage of the method is that "direct detection of mRNA provides information that could indicate the presence of active infection by live, metabolizing organisms," which stands in contrast to approaches that analyze genomic nucleic acids.

Luminex markets molecular diagnostics for infectious disease that use the company's PCR-based xTAG technology, including the xTAG Respiratory Viral Panel (xTAG RVP) and the xTAG Gastrointestinal Pathogen Panel (xTAG GPP).

Mark Perkins, CSO of the **Foundation for Innovative New Diagnostics (FIND)**, added that by focusing on the mRNA signature,

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“you could test for resistance when the knowledge of resistance-associated mutations is incomplete or when the resistance mechanisms are known but difficult to detect with conventional platforms.”

FIND is developing DNA-based molecular diagnostics for a range of infectious diseases including malaria,³ salmonella⁴ and tuberculosis.⁵

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Getting realistic

The Broad-Harvard team plans to continue working “to define the most robust mRNA signatures for a wide variety of drug-sensitive and drug-resistant pathogens. That work is essential to ensure we arrive at a set of signatures that

clearly distinguishes the various organisms,” Hung said.

She added: “On the engineering side, we are designing a benchtop device to house the diagnostic that can potentially be used in any doctor’s office as well as in the developing world. The long-term goal is to have a device that can quickly analyze a urine or saliva sample and indicate the general type of infection—bacterial, viral or fungal—as well as the specific infectious species within those types.”

Tom Lowery, VP of diagnostics R&D at **T2 Biosystems Inc.**, said that to prove the clinical relevance of the approach, it will be important to show the method enables the detection of pathogens present at cell counts much lower than those tested in the paper.

T2 is developing an NMR-based molecular diagnostic platform to detect different *Candida* species in whole blood from candidemia patients.

According to Garth Ehrlich, professor of microbiology and immunology and executive director of the Center for Genomic Sciences at the **Drexel University College of Medicine**, “The problem of most previous molecular diagnostics was that their coverage was too narrow. So, if the diagnostic showed a negative result, it wasn’t because someone wasn’t infected but because your assay didn’t cover that infection.”

In addition, Ehrlich said the researchers will have to show that their RNA-based method “can differentiate log-fold differences in

concentrations of different bacterial species that might occur in polymicrobial infections.”

Ultimately, to deal with samples that include multiple infectious agents, it will be necessary “to create very large mRNA probe sets that do not interfere with each other and collectively cover all potential pathogens within a domain—for bacteria and fungi that is hundreds of species each,” said Ehrlich. “On top of that, it will be necessary to identify the antibiotic-sensitivity signals for each of those pathogens.”

Ehrlich and colleagues used a mass spectrometry-based approach to show that the adenoids of children undergoing adenoidectomy serve as reservoirs of polymicrobial biofilms.⁶

Hung agreed that a key next step is to identify RNA signatures that are sufficiently robust to identify low levels of a particular pathogen in the presence of other pathogens in clinical samples. She declined to provide additional details.

The *PNAS* findings are covered by patents that are available for licensing from the Broad Institute.

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