Johns Hopkins Researchers Get $4M to Develop Sepsis ID, Phenotypic Antibiotic Resistance Platform

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NEW YORK (GenomeWeb) – Researchers at Johns Hopkins University have been awarded new funding to develop a microfluidic assay for sepsis that both identifies pathogens and performs single-cell phenotypic antimicrobial resistance testing within three hours directly from patient blood samples.

The award from the National Institute of Allergy and Infectious Diseases provides about $4 million over five years, principal investigator Jeff Tza-Huei Wang of Hopkins' Institute of NanoBioTechnology said in a recent interview. Wang, a professor of mechanical and biomedical engineering, previously developed a handheld microfluidic device along with a test for chlamydia.

Rapid diagnosis of bloodstream infections can be lifesaving given that the mortality rate for sepsis increases nearly 8 percent for every hour of delay to initiation of antimicrobial therapy.

Although the sepsis testing market has become highly competitive, commercially available molecular tests are limited by the ceiling of multiplexing, Wang said, and this is a problem considering there are more than 150 different pathogens known to cause sepsis.

Panel tests, such as BioFire's BCID, primarily provide pathogen ID and can only test for about 30 pathogens, although a system under development by Qvella can identify around 80 pathogens.

Probe-based panel tests are also designed to look for specific pathogens and so are not agnostic or unbiased. A platform using combined PCR/electrospray ionization mass spectrometry (PCR/ESI-MS) called Plex-ID, rebranded as Iridica by Abbott, can test agnostically, but involves a number of instruments and has an overall large footprint.

"For sepsis diagnosis, the capacity of traditional PCR is limited," Wang concluded.

Sequencing-based metagenomic methods can be used to identify pathogens agnostically, and researchers at the University of California have already launched a clinical metagenomic next-generation sequencing-based test for meningitis and encephalitis, for example. The trend is such that the US Food and Drug Administration has issued a draft guidance on next-generation sequencing-based diagnostic devices for infectious disease.

Although next-generation sequencing is getting faster and more portable, the throughput cost is still not at a level that enables near-patient use, Wang said.

Wang sees the diagnostics gap as even more serious than just the ID piece. In the age of antimicrobial superbugs, complete diagnostic information needs to include not just the identity of the pathogen but also...
information on its ability to resist treatment, he said.

Currently, resistance can be determined either with molecular testing or by applying antimicrobials to cultures of the pathogen. Genetic tests are sensitive, but they can miss new mutations conferring resistance. Phenotypic testing, on the other hand, is a gold standard. Drugs can be tested directly on the cultured pathogen to see if they work, and the method can also be used to determine dosage.

But, "culture is time consuming. It takes a couple days, or maybe weeks, depending on the pathogen," Wang said.

A new assay, the PhenoTest BC Kit, which runs on Accelerate Diagnostic's recently FDA-cleared Pheno System, can perform identification and antibiotic susceptibility testing. But the major advantages of the JHU microfluidic single-cell device over the Accelerate Diagnositics system, according to Wang, are the speed – the Accelerate system requires 8 to 10 hours for reporting the susceptibility result – and the ability to test directly from whole blood.

To address these issues, the JHU prototype device uses DNA melt curve analysis to analyze variable regions of the 16s gene using fixed flanking primer sites in conserved regions. "Using this approach, we are able to identify the pathogens independent of probes," Wang said.

The resistance profile is then determined using a single-cell approach. Specifically, the group uses a microfluidic digital array with nanoliter-sized chamber volumes. Each array can process millions of reactions. "We digitize it, so that one chamber contains one bacterium or no bacteria," Wang said, similar to the concept of digital PCR, but with single cells. The chambers can be loaded with antimicrobial drugs and then the behavior of the single bacteria can be measured.

"If you want to see if one bacterium grows or doesn't grow in a PCR vial, there is no way to do it, you have to go to culture," Wang said. But using the single-cell approach, whether the bacteria replicate from one to two can be quickly observed with optical measurements. Doubling time of E. coli takes 15 minutes, while Staphylococcus aureus takes 30 minutes, for example. "We don't need to wait for a colony to form," he said. The growth of the bacteria can also be determined using single-cell PCR and detecting a doubling of CT value.

Wang and his colleagues published a proof-of-concept study describing the general method in Analytical Chemistry last year. The group will validate the device at JHU as well as in collaboration with Sam Yang at the Emergency Medicine Department at Stanford University.