Vertical Flow Assay Being Developed to Detect Biological Threats at the Point of Need

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NEW YORK (360Dx) – Researchers at the University of Arizona and University of Nevada are working to develop a vertical flow immunoassay for the detection of pathogens that pose a threat to national security and public health.

As part of a contract with the US Department of Defense, they are developing the point-of-care immunoassay — in which a fluid sample is diffused through a nitrocellulose membrane — as an alternative to a standard method involving the culturing of samples to identify

threatening biological agents. The researchers described the development of their multiplexed biothreat immunoassay recently in the journal *Talanta.*

So far, they have used their assay to accurately identify outbreaks of melioidosis, a fatal disease whose causal agent — *Burkholderia pseudomallei* — is found in soil and water. It is highly prevalent in Southeast Asia and Northern Australia and is on the US Department of Health and Human services growing list of pathogens that pose a threat to public security.

The research was sponsored by DoD's Chemical and Biological Defense program through its Defense Threat Reduction Agency (DTRA) for an undisclosed amount.

According to Frederic Zenhausern, one of the study authors and a director of the Center for Applied NanoBioscience & Medicine at the U of Arizona, Phoenix, the DoD was interested in the development of a multiplexed panel that simultaneously detects several biothreat agents. The U of Arizona group, which has expertise in immunoassay detection, teamed with the U of Nevada School of Medicine, Reno, which has experience in biothreat assay testing and validation, to focus "on biothreats that cause diseases with a very high mortality rate often because they are detected too late, and on developing a rapid test with the capability of detecting these biothreats earlier and with increased sensitivity," Zenhausern said.

The standard process of identifying these pathogens — microbiological cultivation — involves growing media cultures derived from samples of blood, blood, urine, pus, and sputum in laboratories. The approach, however, tends to have low sensitivity and can take up to a day or more to complete. Further, it can be hampered by low levels of bacteria in clinical samples, the researchers said.

The standard approach can also result in misdiagnosis or gross underreporting of events, the researchers said, adding that infections produced by these biological agents can quickly progress to septicemia and death if left untreated.

While microfluidic detection technologies don't normally scale well with miniaturization, the method developed by Zernhausern and his colleagues retains its high performance when it is scaled down, the researchers said. As a result, the platform can be developed to provide "a visual inspection at a larger scale or can be interfaced with a smartphone readout at a smaller scale and be designed to provide a very quick point-of-care device that could be deployed in different environments," he said.

According to the researchers, the device can provide very high sensitivity in less than 10 minutes.

Gerald Kost, director of the point-of-care testing center for teaching and research at the University of California Davis School of Medicine, said that the researchers have presented a novel approach to pathogen detection.

"[T]hey sum up the technology as theoretically sound, capable, low cost, easy to use, and stable at room temperature, but identify several future advances needed to bring a practical pathogen detection device to the point of care," he said.

Kost said it is noteworthy that there is a trade-off of membrane pore size and sample flow speed for optimization. Potential pathogen detection applications for the platform "would be numerous, and perhaps the complexities of the physical parameters can be worked out

to deliver a suitable and also badly needed multiplex device for biothreats."

Igor Medintz, a US Navy senior scientist for biosensors and biomaterials, said "The paper-based immunoassay system described by the Zenhausern research team demonstrates a lot of the characteristics desired from a biothreat detection technology that can meet many of the current needs."

He said that the ability to incorporate antibodies against different agents in a modular fashion is particularly promising. "Hopefully, this design can be exploited to develop a panel of multiplexed assays that would screen across a wide range of biothreat agents or be tailored to what is of concern at a given location," he said.

How it works

The vertical flow immunoassay employs a nanoporous nitrocellulose membrane encapsulated in a stainless steel filter. A sample diffused through the membrane is pre-functionalized with capture antibodies that are part of a sandwich assay, which generates a colorimetric signal when it detects the presence of target antigens.

In the *Talanta* study, the scientists targeted the vertical flow immunoassay at *B. pseudomallei* surface capsular polysaccharide, an envelope of loose gel surrounding a cell that's shed by the bacterium during infection and associated with the virulence of the pathogenic bacteria.

By increasing the flow speed up to 1.06 mm/s and reducing the membrane pore size to 0.1 µm, they improved the sensitivity by more than five times its value prior to the changes. The vertical flow immunoassay's limit-of-detection for capsular polysaccharide spiked in buffer solution was determined to be 0.02 ng/mL.

As a result, the researchers believe that the assay has potential as a point-of-care tool for detection of biothreat agents in clinical and resource-restricted conditions, and they are developing prototype multiplex assays to detect polysaccharide- and protein-based markers. In addition to detecting *B. pseudomallei* for meliodosis, their assay is being developed to detect *Bacillus anthracis* for anthrax, *Yersinia pestis* for plague, and *Francisella tularensis* for tularemia.

Currently about 70 biological agents have been identified by HHS as threats to humans and animals, the researchers said, and *Burkholderia pseudomallei* is among those considered a high priority biological threat. Meiliodosis infections vary in their severity, but they can manifest as septicemia that develops in patients within 24 to 48 hours after the onset of symptoms. If left untreated, the fatality rate for some of these infections are as high as 90 percent, the researchers said.

"The challenges faced in rapidly screening and diagnosing potential biothreats in resource poor environments around the world cannot be overstated," said Medintz, who is coauthor of a review of biothreat agent detection technologies published this month in *ACS Sensors.*

Further, the is a need for "reliable, simple, cheap, broad range, sensitive, and rapid diagnostics that can be applied in these same environments" and in developed countries, he said. "The ongoing Zika and Ebola outbreaks serve as examples of why such tests are so critical."

In developing the vertical flow technology, the researchers looked at modifying the concept of lateral flow detection — commonly used in POC pregnancy tests and HIV tests — in which a fluid sample flows by capillary force along a nitrocellulose, or paper, surface.

"Lateral-flow immunoassays are low cost and very useful as point-of-care devices, but when you need high sensitivity and multiplexing of biomarkers, the format starts to be a challenge," Zenhausern said.

Apart from the direction in which the sample flows, the researchers used a far smaller pore size in their device to capture more biomolecules.

That meant they had to address another issue: To achieve an adequate flow rate in lateral flow testing, the pore size of paper materials needs to be at least several micrometers, which can hinder the capturing of biomolecules, according to Peng Chen, one of the University of Arizona researchers developing the technology. To increase performance, large sample volumes are needed compared with the vertical flow format, and it is difficult to achieve multiplexing with lateral flow, he said.

To solve this and improve upon the lateral flow platform, their device leverages vertical diffusion of fluids through the membrane surface. Jian Gu, a researcher working to develop the platform, said that pore size in lateral flow nitrocellulose membranes are usually a few microns or larger in diameter and fluid must flow for a distance of 20 to 40 millimeters.

"The smaller nanometer pore size in our platform and shorter flow path — which is only 130 microns — enables delivering more sample to sensor, leading to higher performance," he said.

In their DoD study, the researchers sought to demonstrate the platform's multiplexing capability by conducting a proof-of-concept experiment to simultaneously detect *B. pseudomallei* and *Bacillus anthracis*.

The change to the fluidic regime enables greater capabilities for multiplexing, which has advantages when you are looking at rare targets, Zenhausern said, adding, "These tests are two orders of magnitude more sensitive than their equivalent in the lateral flow format."

Because the format is versatile and inexpensive, the research team is looking to expand its platform to detect Ebola, Zika, and Lyme disease, and is currently developing prototypes. As sample media, the group is looking at use of blood, urine, and saliva. The platform, if required, could do multiplex detection on a panel of up to 30 pathogens, Zenhausern said.

Zenhausern noted that his group is now optimizing the platform technology for manufacturing at high volumes. He said that the team is working with an undisclosed commercial partner that will help it take the platform and its assays through clinical validation in preparation for submission for US Food and Drug Administration clearance. Depending on the availability of adequate funding, he said, a platform could be commercially available within three years.

Further, under a contract with NASA, the group is looking to integrate detection of nucleic acids on the platform in developing a platform to test for radiation exposure to astronauts embarking on long-term missions.

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