Study Shows Potential of Thermo Fisher OpenArray qPCR Platform in Multiplexed Blood Screening

Jun 16, 2017 | Ben Butkus

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NEW YORK (GenomeWeb) – A research-use-only blood-borne pathogen panel developed using Thermo Fisher Scientific's OpenArray multiplexed real-time PCR platform shows promise for flexible and cost-effective blood screening of both common and emerging infectious organisms, according to the results of a new study.

In a paper published this week in the *Journal of Molecular Diagnostics*, researchers from the US Food and Drug Administration, Thermo Fisher, molecular diagnostic provider Diatherix Laboratories, and nonprofit blood donor testing firm Creative Testing Solutions demonstrated that the panel — designed to simultaneously test for 17 viruses and 13 bacteria and protozoa — was able to detect pathogens from human blood donor samples with an accuracy of about 95 percent.

The new study, led by Robert Duncan, an investigator with the FDA's Center for Biologics Evaluation and Research, builds on similar research conducted by Duncan and collaborators at FDA and Life Technologies (now part of Thermo Fisher) and published in early 2014.

That study showed proof of principle for the OpenArray as a flexible platform for molecular screening of infectious agents in blood, but only interrogated a handful of pathogens. The latest study upped the ante significantly and included viruses such as HIV-1, HIV-2, Dengue, chikungunya, and influenza; bacteria including *Escherichia coli* and *Staphylococcus aureus*; and protozoa such as *Leishmania donovani*, *L. infantum*, and *Babesia microti*.

The researchers first cultured the 30 pathogens in the laboratory and mixed them with normal blood samples or plasma to mimic blood from an infected individual, primarily to assess limit of detection and assay specificity. Then the investigators obtained 92 donor blood specimens and tested them using the panel, correctly identifying 94.8 percent of infectious agents.

Limitations of the study included the fact that the clinical blood samples that were tested only contained a handful of viruses actually included on the panel, so the assay's ability to discriminate between some of the

rarer pathogens was not really tested. In addition, the platform fell a bit short when it came to subtyping HIV strains.

Nevertheless, the study provides further evidence that Thermo Fisher's OpenArray platform is ideal for developing a commercial blood-screening platform that could be modified relatively quickly and inexpensively to respond to emerging infectious disease threats to the blood supply.

OpenArray is based on a metal plate the size of a microscope slide containing an array of 3,072 "through-holes," each of which is loaded with individual TaqMan assays and contains 33 nanoliters of PCR reaction mixture. The panel runs on Thermo Fisher's QuantStudio 12K Flex PCR system, which is designed to run several of Thermo's PCR reaction formats and is for research use only.

Previous work has shown that PCR assay performance in OpenArray through-holes is equivalent to microplates but with an approximately 150-fold lower reaction volume and the ability to profile multiple targets using the same sample.

This architecture in and of itself is cost-effective since less sample and PCR chemistry needs to be used to run multiple assays. In addition, the fact that OpenArray is a spatial multiplexing technology — i.e. individual PCR reactions are run in separate microfluidic "through-holes" as opposed to in a single tube or reaction chamber — means that adding new primers and probes to the panel will require significantly less testing and validation.

"The clear advantage of the OpenArray platform for this type of application is the ease of assembling singleplex TaqMan assays into the panel, having technical assay replicates and/or an additional assay for each detected target, limited or absence of assay cross-reactivity, and Cq for target detection within a linear range of PCR amplification," the researchers wrote in their paper.

There is currently no commercial test that achieves the same level of multiplexing in blood samples as is described in the new study. There are singleplex and multiplex molecular assays for pathogens such as HIV, hepatitis B, and hepatitis C available from companies such as Roche, Abbott, and Hologic. But the increasing prevalence of recent emerging pathogens such as Zika, Ebola, chikungunya, and dengue viruses demands corresponding flexible and expeditious methods for testing the blood supply.

In a statement, Duncan noted that "all blood for transfusion must be tested for infectious agents. The increasing number of agents that may infect blood and the recognition that some of them only pose a risk in certain areas or certain times means that new methods to streamline blood testing must be developed. The major feature of [OpenArray] — the ability to test for multiple infectious agents at the same time — could be an answer to that need."

In a follow-up email, Duncan said that the features of the OpenArray platform were promising "because with over 3,000 separate chambers on a wafer the size of a microscope slide, a very high level of multiplexing was possible. The fact that the PCR reactions all occur in separate chambers means the usual concern about interference between reactions is removed. Such technologies may be beneficial in blood donor setting when screening for multiple infectious agents."

He also noted that FDA would not pursue the development of a panel for commercial use. "However, we encourage the development of such devices by industry," he said, adding that CBER sponsored a workshop in 2013 that brought together government agencies, the blood industry, device manufacturers, and academics to discuss ways of advancing this type of technology.

Collaborator Creative Testing Solutions may, however, be interested in new approaches to blood screening. Contributing authors from the company did not reply to a request for comment, but in a statement, Phillip Williamson, vice president of operations and scientific affairs for CTS, noted that "as the

largest independent blood donor testing organization in the US, Creative Testing Solutions welcomes the opportunity to work with the FDA and others on new research initiatives and clinical trials that will ideally improve blood donor testing and the safety of the US blood supply."

Diatherix, which specializes in molecular testing for infectious diseases, does not participate in the blood screening market but sees the value of OpenArray as a platform for building smaller panels for multiplexed infectious disease testing.

Prior to its acquisition by Eurofins in 2015, Diatherix was commercializing infectious diseases tests using a multiplex PCR technology called TEM-PCR, for which it acquired the patents rights from Qiagen in 2013.

Elena Grigorenko, vice president of R&D for Diatherix, first author on the recent *JMD* study and an inventor of the through-hole technology on which OpenArray is based, said that when she joined Diatherix from Life Technologies in 2013 she "saw the advantage of bringing other multiplex technology in house, such as OpenArray, which is considered spatial multiplexing, not necessarily running a single reaction with multiple pathogens. … I think our interest as a company is to look at a variety of multiplex technologies and to add to our existing portfolio."

Diatherix already offers an antibiotic-resistance panel that was developed in partnership with Thermo Fisher using OpenArray. Grigorenko said that her company could take a similar tack with multiplexed infectious disease panels.

"I think this paper demonstrates that this platform is sensitive, specific, flexible, and [tested] a large number of clinical samples, and … accurately identified the presence of different pathogens," she said. "If you look at FDA submissions, [this] more or less describes the workflow [for how] diagnostic tests are developed and validated in house."

The panel as described in the paper, or a modified version thereof, "can have potential as an RUO first, and later could be an FDA-approved test," she added. "The reviewers of the paper had some questions about why we included HIV, and that shows that the platform can be expanded to other clinical applications, not just simply screening blood for transfusions."

From Thermo Fisher's perspective, collaborative work such as the recently published *JMD* paper goes a long way toward helping the company continue to carve out a footprint in molecular diagnostics for its real-time PCR technologies, and particularly the OpenArray.

In August, Thermo Fisher said it had begun offering an OpenArray- and TaqMan-based RUO panel for women's health that targets multiple organisms responsible for urogenital infections including bacterial vaginosis, urinary tract infections, and vaginitis. A company executive also noted at the time that partnering with other companies to develop similar panels is a big part of the company's strategy.

This week, Josh Trotta, senior director for applied healthcare solutions in the genetic sciences division of Thermo Fisher Scientific, reiterated this approach.

"We see … increased demands from people to interrogate multiple areas in panel-based applications, where they can identify, in this particular case, viruses, bacteria, and protozoa," Trotta said.

"With our [women's health offering], customers in the states are really seeing the value of being able to kind of customize their specific panels to their applications," he added. "It continues to be well-received across the board, and people are continuing to look out for future … panels they might be able to build and deploy, as well. We will continue to work with groups like Dr. Duncan's and others where we can evaluate areas that are going to add value to market needs."

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FDA, BioTrove Collaborate on Blood Screening
GenomeWeb, 2009

Karolinska Institute, Thermo Fisher Collaboration Yields Improved Prostate Cancer Screen
GenomeWeb, 2015

Diatherix Buys TEM-PCR from Qiagen to Expand CLIA Lab Offerings, Possibly Develop MDx Kits
GenomeWeb, 2013

Applied BioCode, Diatherix Ink Partnership, Supply Agreement
GenomeWeb, 2016

Idaho Technology Launches FilmArray RP in Europe; Names Distributor
GenomeWeb, 2012

FDA, HHS Awards Diatherix Contract for PCR-based Pathogen Detection Methods
GenomeWeb, 2014