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Molecular diagnostics developer Akonni Biosystems has been awarded a $3.2 million Phase II Small Business Innovation Research grant from the National Institute of Allergy and Infectious Diseases to integrate PCR amplification with its existing TruArray gel-drop microarray in a single-chamber system.

Specifically, the SBIR award will fund the development of a combined RT-PCR/array platform to detect influenzas A and B and their antigenic subtypes, including antiviral-resistant types. According to the grant abstract, the performance goal is a sample-to-answer result in two hours or less with an input target concentration of 102 viral particles per mL for nasopharyngeal swab.

The aim of the integrated system is to reduce the complexity of infectious disease testing while lowering the cost, Darrel Chandler, Akonni's chief scientific officer and the principal investigator on the grant, told *PCR Insider*.

While the system will provide a level of multiplexing that is "more than conventional PCR, but not as high as microarrays in general," the expected cost "is on par with, and in some respects lower than, what hospitals and clinics are paying for real-time PCR," Chandler said.

While he declined to provide pricing details, he noted that the instrumentation required for the system would likely be "almost 10 times lower" than current array scanners and "not even close" to the price of current real-time PCR systems.

Chandler noted that the key advantage of the new platform is the ease of use compared to current microarray systems, which currently require a preliminary PCR amplification step followed by hybridization on the array. "The entire analysis chain is pretty hands-on and labor-intensive because all of those steps are manual and separate from one another," he said.

By combining the PCR and the microarray into a single chamber, "the user never has to manipulate amplified material in an open test tube format," he said.

"For microarray technologies, this [has been] one of the significant bottlenecks for adoption in the clinical marketplace," he added.

The integrated system also offers advantages over microfluidics-based PCR array systems from companies like BioTrove, Fluidigm, and Idaho Technologies, "which essentially have to split their sample into individual reaction wells" on the microfluidic device.

"What this means, practically, is that our technology is going to avoid the problem of the split sample, [which] becomes important when you're trying to detect genes or microorganisms at very low concentrations."

As an example, he explained that if the required limit of detection for an assay is 100 copies per reaction, in order to detect that in a platform that splits the sample over 1,000 wells, the initial concentration would have to be on the order of 10^5 copies.

"Our technology doesn't require that sample splitting, so in principle we should be able to achieve lower limits of detection than these PCR arrays because it's a homogenous assay," he said.

The three-year SBIR project is set to run through June 2012. Akonni plans to have prototype instruments and assays ready in the spring of 2010, which it plans to share with its collaborators at the Wadsworth Center, Columbia University, Little Company of Mary Hospital, and the US Centers for Disease Control and Prevention.

"The rest of 2010 is going to be spent really optimizing the PCR array, understanding the boundary conditions and analytical performance characteristics of the test, and revising the instrument, the software, and the user interfaces to work well within a clinical workflow," Chandler said. The company then hopes to start collecting preliminary clinical data in late 2010 for a larger clinical trial and clearance with the US Food and Drug Administration planned for the following year.

The Phase II SBIR follows a one-year Phase I project under which Akonni demonstrated that it could purify influenza RNA from clinical samples, as well as the feasibility of the reverse-transcriptase PCR array with RNA targets.

The primary goal of the Phase II project is to commercialize the technology and get it ready for an FDA submission, Chandler said. "One goal is to complete the product development around the RNA sample-preparation kit — to be able to provide that for users. The second goal is to complete the development of the PCR array with the influenza model — targets, primers, probes, etc." In addition, Akonni plans to refine its instrumentation and software for reading the array.

The company expects the overall cost of the system to come in lower than that of current real-time PCR and microarray platforms due to the nature of its underlying microarray technology — a low-density array based on so-called gel drop technology. The three-dimensional drops — typically 100 microns in diameter by 20 microns in height — contain all probes and chemistry necessary for a reaction.

"Because they're three-dimensional, we can immobilize orders of magnitude more probe per element than 2D arrays or bead arrays, because they're immobilizing their probes on a flat surface," Chandler said. "That three-dimensional structure and higher probe immobilization capacity means that we can concentrate, capture, and collect more fluorescent signal per gel spot than a competitive microarray product."

That results in "much lower cost, inexpensive scanners and still achieves the same level of optical detection performance on the microarray as these more expensive scanners and glass-based or bead-based microarray products," he said.

The characteristics of the gel array technology also enable the company to integrate the PCR amplification with the array hybridization in a single tube without interference.

"The microarray doesn't interfere with the PCR because of the way that we manufacture the microarray," Chandler said. "Our probes are at very high concentration, but they're immobilized in such a way that those immobilized probes aren't going to interfere with stuff going on in the bulk solution over the top of the array."

Chandler noted that Akonni is considering also immobilizing the PCR primers in the array itself, "but that's not the general approach we're taking in the beginning."

Prepping for Commercialization

Akonni is working with several different partners on the Phase II project. For example, Kirsten St. George at the Wadsworth Center, the co-PI on the grant, is contributing drug-resistance markers "that we hope to incorporate into the product during this three-year project period," Chandler said. The Wadsworth team is also going to help out with assay development, "and they're going to help us design and implement some of the preclinical trials on patient specimens in preparation for the clinical trials."

Ian Lipkin's group at Columbia, meantime, has "the fundamental probe design database infrastructure to design new primers and probes in the event that our current selection runs into cross-reactivity problems in 2010 when we start applying it to clinical samples," he said.

While the company doesn't expect to see such cross-reactivity issues, Chandler noted that "there's always an opportunity for unanticipated amplification artifacts and cross-hybridization as you start generating higher order multiplexes."

If, over the next year or so, Akonni runs into difficulties that it can't resolve by tweaking amplification conditions or hybridization conditions, "then we're going to have to get into some probe redesign, and that's when we'll be working with Columbia on some of the probe and primer redesign issues."

Chandler noted that some aspects of the system are still unresolved. For example, the upper limit on multiplexing "hasn't really been established for this platform," though he said that the company is shooting for something in the range of tens of targets "because we think that the diagnostic community doesn't want, doesn't need, and can't pay for higher-order multiplexing."

As for the number of samples that can be processed, the PCR array "will probably be one sample per test, and the user should be able to batch process anywhere from eight to 16 samples at a setting."

Chandler said that Akonni will take a "tiered approach" to marketing the system that will first focus on CLIA high-complexity labs, reference laboratories, and major hospital clinics and research centers. He noted, however, that "the form factor, the portability, and the cost of the system suggest that we could start pushing this type of technology out to the next tier of clinics or laboratories."

The key to expanding into that market "will ultimately be simplifying the sample-preparation component of the test" so that these smaller labs can perform it, but "that was part of what the Phase I project was all about and we think we've got a solution that's almost simple enough to move into the next tier of laboratories because all it requires is a pipettor," he said.

While the company's first goal is to develop the system for influenza testing, it is also developing several other tests for the platform, including assays for encephalitis, methicillin-resistant *Staphylococcus aureus,* and tuberculosis, though Chandler noted that whether the company pursues FDA approval for these tests is "still to be determined."