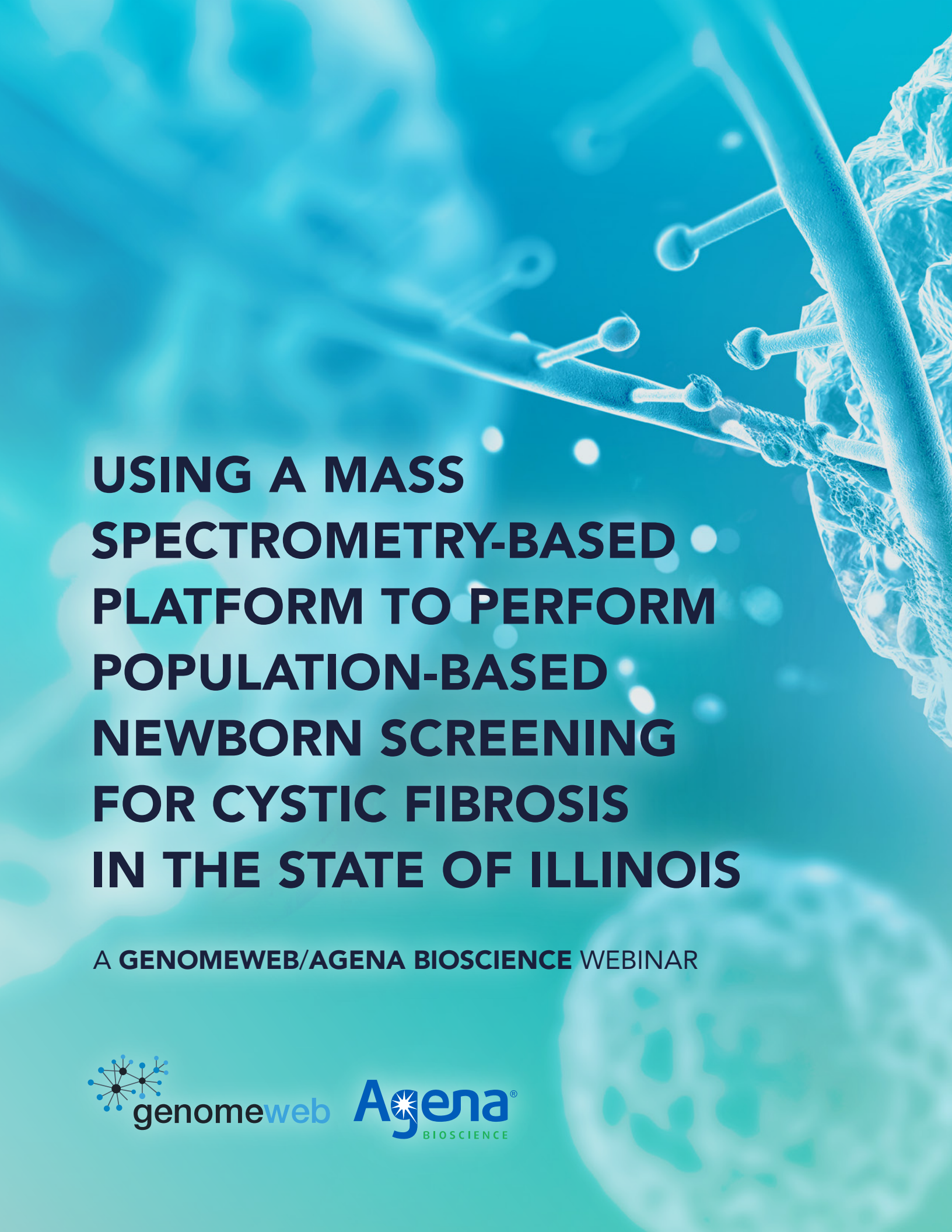

USING A MASS SPECTROMETRY-BASED PLATFORM TO PERFORM POPULATION-BASED NEWBORN SCREENING FOR CYSTIC FIBROSIS IN THE STATE OF ILLINOIS

A **GENOMEWEB/AGENA BIOSCIENCE** WEBINAR

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This white paper is based on a GenomeWebinar, sponsored by Agena Bioscience, in which Vineet Dhiman, a laboratory research scientist at the Illinois Department of Public Health (IDPH), discussed population-based newborn screening for cystic fibrosis (CF) at IDPH.

"The goal of newborn screening is for early detection of conditions that have early and timely interventions [which] can lead to the elimination or reduction of associated mortality, morbidity, and disabilities," said Dhiman.

With the goal of improving newborn screening outcomes, IDPH has been seeking to bolster its screening efforts ever since it started screening newborns for phenylketonuria some 60 years ago. Since then, the agency has added screens for hypothyroidism; amino, organic, and fatty acid disorders; and many other conditions. In total, IDPH currently screens for more than 40 disorders.

SPEAKER:

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SPEAKER:

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Cystic fibrosis was added to the agency's repertoire in 2008. CF is a genetic disorder that mainly affects the respiratory and digestive systems, resulting in a variety of symptoms, including difficulty breathing, infertility, and poor growth in children. CF is the most common life-threatening autosomal recessive disorder in the United States, with an incidence rate of about one in 4,000, according to recent publications.

The disease is caused by mutations in the CF transmembrane conductance regulator gene, known as *CFTR*. First sequenced fully in 1989, *CFTR* is a large gene, measuring 250 kb and containing 27 exons. To date, more than 2,000 mutations have been identified within this gene. However, only about 10-15 percent (about 200 to 300) of these mutations have been confirmed to cause disease. These mutations occur differentially across races and allele frequencies.

The earliest clinical presentation of CF occurs with meconium ileus at birth. "If there is a mutation and it causes a defect in the *CFTR* gene, this causes various fluids to become sticky and thick. Instead of acting as lubricants, these fluids plug up ducts and passageways. This occurs especially in the lungs and pancreas, and it [is a] source of digestive and breathing issues," Dhiman said. "In patients with CF, the thick mucus blocks the pancreatic ducts. This prevents trypsinogen from reaching the small intestine, to be converted into trypsin. As a result, you're getting inefficient breakdown of food, as well as blockage of various foods in these areas and organs."

Beyond the Status Quo

Currently, chloride sweat testing is the gold standard for clinical detection of CF. The sweat test is typically performed at the hospital or clinic at least 48 hours after a baby's birth. "Any baby with chloride sweat levels above 60 mmol/L is typically seen as positive. Unfortunately, this is not always clear-cut," Dhiman said. What's more, clinicians sometimes do not collect enough sweat from the baby, and, in those instances, the result is apt to read "quantity not sufficient."

Newborn screening for CF was first implemented in 1985 in Colorado and was fully implemented in all 50 states by 2010. A study published in the *Journal of Pediatrics* in 2005 shows that babies screened for CF via newborn screening experienced better overall growth compared to those diagnosed after clinical presentation.

"Obviously, with newborn screening, you are getting earlier detection and, again, earlier detection means earlier treatment," Dhiman said.

A Two-Part Process for CF Screening

IDPH currently employs a two-tiered CF screen. First, the blood is assessed for immunoreactive trypsinogen (IRT) levels via an IRT DNA CF screening algorithm.

Just assessing IRT levels, however, is not enough. "IRT can produce a lot of false positives, because it can be inaccurate if, for example, the blood is collected less than 24 hours post birth. For example, during the birthing process, there can be some slight damage to the pancreas of the baby, which can create elevated IRT levels," Dhiman noted. As a result, any screen using blood that was collected before 24 hours is typically ruled unsatisfactory for IRT screening.

For babies who were screened more than 24 hours after birth, if the IRT level is "not within the daily top four percent ... we report that as normal for CF screen. However, if it falls in the top four percent, an IRT repeat for confirmation is conducted. If that repeat is still elevated, it is reflexed for *CFTR* mutation analysis," Dhiman said.

A variety of results are possible with *CFTR* mutation analysis. If a mutation is detected in a sample, but IRT levels are below 170 ng/mL (indicating low risk), the result is reported as CF normal. "However, if we do not see a mutation, but we see ultra-high levels of IRT, those specimens are still reported as [a] CF-positive screen," Dhiman said.

"Any specimen with an observed mutation in the CFDNA screen is automatically sent for repeat confirmatory testing. Then, if we do see that again on final confirmatory testing, we'll [report] that out as screen positive. We will notify the hospital or the pediatrician, typically recommending referral to a genetic counselor and diagnostic testing for CF."

Pushing the CF Screening Envelope

IDPH is looking to improve outcomes by taking screening beyond established requirements. Instead of merely screening for the 23 mutations recommended by the American College of Medical Genetics and Genomics, IDPH is using a panel from Agena Bioscience that tests for an additional 51 mutations as well, making it a better screen for the mutations that are likely to be present in the patient population.

The goal at IDPH is to automate the assay. "We want to make sure we remove as much human error as possible," Dhiman said.

As such, IDPH utilizes the EpMotion 5075 Automated Handler, which is a liquid handler that performs all the PCR and the alkaline phosphatase treatment. In addition, IDPH leverages two Agena MassArray Systems. Each of these instruments can run two plates with a total of 29 specimens simultaneously.

"The Agena cystic fibrosis assay is set up … to test the 74 mutations on their panel [by breaking] that up into three different PCR reactions. This makes it possible to assay for all 74 mutations, across each specimen," Dhiman said. The software then analyzes the assay to determine if each sample is presenting as a homozygous phylotype, homozygous mutant, or heterozygous mutant.

While the Agena Bioscience assay efficiently analyzes mutations, there are potential challenges with data interpretation. A prime example can be observed with the delta F508 mutation, where the mutational status of both the delta F508 and F508C mutations needs to be interpreted to determine whether there is a true homozygous mutation on both alleles. The interpretive software can also sometimes fail to detect low-signal mutations. And finally, amplification failure can create stumbling blocks of repeated mutational no-calls across all specimens in each run, requiring repeat testing to resolve the issue. "Oftentimes, a clear peak is present, but it's too weak for the baseline interpretive software to call. What we'll have to do is manually look at every single specimen, make sure that the peak is present, and manually [make the call]. As much as you want to automate the entire process, there is some manual interpretation that is required, for some of these mutations," Dhiman said.

When More is Better

This expanded screening makes it possible to more fully detect CF in minority populations, according to Robin Everts, senior manager of Scientific Affairs at Agena Bioscience.

"When you look at the 23 recommended mutations, they do really well for people from Caucasian descent, [identifying] about 98 percent [of those affected by CF]. However, in other ethnicities, such as Asians, you might only find about 49 percent of carriers or of people affected by CF," Everts said.

The goal for health care organizations should be to screen for an optimal number of mutations. While 23 mutations might not be enough to identify CF in the patient population, according to Everts, it's not practical to

"do a whole-genome sequencing, where you find everything under the sun, including a lot of variants of unknown significance. It's best to do something in between. That is where Agena comes into play, because we can cover from 23 [mutations] up to a couple of hundred [mutations]," and can, therefore, more efficiently and effectively screen for CF.

When a health care organization in Ontario created a population-specific panel with the assay, leaders found it to be "a very flexible and cost-effective alternative to their previous genotyping assay. … They found more mutations because they could get a broader panel and they had a high detection of different variants. … On their previous platform, they were spending about $43,000 for a given number of samples. When they ran these on MassArray, they actually only spent $17,000," Everts said.

While having the ability to screen for the optimal number of mutations is key, when purchasing an assay kit, organizations also should consider open versus closed platforms, ease of deployment, validation, workflow and personnel, reagent and running costs, and reimbursement, he added.

"[The Agena assay] is very cost-effective. It has a very efficient workflow; it's a single-day workflow from sample to results. It is customizable, as I show, able to run not only *CFTR*, but also other applications such as oncology — tumor and liquid biopsy screening — on the same plate/chip. Agena has a strong presence in sample integrity, pharmacogenetics, and other hereditary diseases as well," Everts concluded.