First Light Bio Developing Single-Molecule Counting Tech to Detect C. Diff. Toxins

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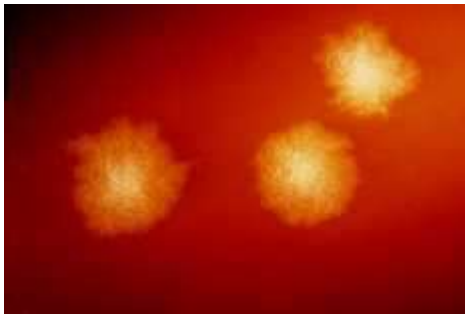

CDC/Wikimedia

C. difficile colonies after 48 hours on a blood agar plate

NEW YORK (360Dx) – First Light Biosciences is developing a molecule counting technology that detects *Clostridium difficile* toxin in stool samples with an eye on providing a more accurate testing option for clinicians struggling with the global growth of infections.

C. diff. is the most common cause of infectious diarrhea in patients in hospitals, and occurrences are common after antibiotic therapy. However, current testing methods — including enzyme immunoassay detection and nucleic acid amplification — lack the necessary combination of sensitivity and specificity to provide accurate detection of the toxin, according to infectious disease researchers and clinicians.

Earlier this month, at the American Society for Microbiology Microbe 2018 annual meeting in Atlanta, First Light researchers shared data in a poster presentation about the development of a sensitive *C. diff.* toxin B test on its MultiPath platform based on a digital imaging technology. The platform counts single target molecules in stool samples within 30 minutes and without sample preparation. The technology, First Light CEO Don Straus said in an interview, presents a potentially affordable solution to more accurately detect *C. diff.*

Its MultiPath *C. difficile* toxin B test leverages digital imaging to count target-specific magnetic and fluorescent particles that have been tethered together by toxin molecules. It uses a camera with a complementary metal-oxide semiconductor chip to perform imaging without needing magnification. Magnetic nanoparticles bind to a different part of the toxin from the fluorescent nanoparticles and, together, they are pulled through an opaque, aqueous liquid layer that lies beneath the sample. The liquid

layer is an important part of the design, Straus said, because it optically isolates the stool sample from the instrument and eliminates the need for sample preparation and wash steps.

The firm expects that an important patent for its technology will be issued this week, and it plans to apply to the US Food and Drug Administration for clearance of its platform and the *C. diff.* assay in the second half of 2019. If it is successful in developing a commercial platform and assay, the firm will target hospital labs with a benchtop analyzer with multiple potential applications, notably antimicrobial susceptibility testing from stool samples, in addition to detection of the C. *diff.* toxin, Straus said.

In its study, the group used clinical stool samples to estimate the limit of detection, precision, and dynamic range. They used a training set of 320 clinical stool samples from patients suspected of having *C. difficile* infection to select parameters to yield optimum accuracy relative to a cellular cytotoxicity neutralization assay (CCNA) reference test.

The MultiPath 30-minute test achieved a sensitivity of 97 percent and specificity of 98.3 percent, a significant improvement over existing tests, Straus said. In the same study, an enzyme immunoassay — a technology used by some hospitals for *C. diff.* testing — demonstrated a sensitivity of 77.8 percent and a specificity of 94.4 percent. A third assay — based on PCR-based nucleic acid amplification, which has achieved increasing adoption in hospitals for *C. diff.* detection — had a sensitivity of 98 percent and a specificity of 85.7 percent.

The group also tested a random subset of samples on a prototype of its automated MultiPath analyzer and consumable cartridge, and reported achieving a sensitivity of 95 percent and a specificity of 97 percent.

In May, the researchers described the use of the rapid immunoassay-based technology in the journal *Scientific Reports* and reported that the MultiPath test detected all *C. diff.* toxinotypes and ribotypes that it tested, including those most commonly occurring in the US and European Union. It showed no cross-reactivity with relevant bacterial species, and is robust to potential interfering signals commonly present in stool samples, they added.

Mark Wilcox, head of microbiology research and development at Leeds Teaching Hospitals National Health Service Trust in the UK, said in an interview that current tests for *C. difficile* gastrointestinal infections can be inaccurate.

"*C. difficile* toxin enzyme immunoassays often lack clinical sensitivity, and although nucleic acid amplification tests have excellent clinical sensitivity, they can have diminished clinical specificity due to their inability to distinguish patients with *C. diff.* infection from patients that are carriers of *C. diff.* organisms" not generating infections.

C. diff. diagnosis is undergoing a transformation, said Wilcox, who was not involved in the First Light researchers' study, but is an advisor to the company and its competitors and is the lead on *C. difficile* infection at Public Health England, a national health agency.

Over the past decade, PCR tests "have made inroads" in *C. diff.* testing, particularly in the US, he said, but while they have significantly improved the sensitivity of *C. diff.* testing, it has come at "an enormous cost to specificity."

The result is an occurrence of false positives indicating that people have *C. diff.* infection when they don't.

The problem of *C. diff.* false positives is seen frequently in older people in hospitals who are taking antibiotics, Wilcox said, adding that between 10 and 20 percent of the patients are *C. diff.* positive. However, when physicians see that these patients have diarrhea, have taken an antibiotic, and test positive using a PCR test, they frequently conclude that the patient has *C. diff.* infection, he said.

This is a problem for hospitals, especially in the US where quality-of-care requirements are increasingly being tied to reimbursements, and hospitals receive reimbursement penalties when they are seen to have high levels of infections, including *C. diff.* infections, Straus said. That has sensitized hospital executives to the need to reduce *C. diff.* infections and to look into false positives of nucleic acid tests, he said.

The most accurate way of determining whether someone has the infection is to look for the toxin, Wilcox noted, but because the current diagnostic method that detects the toxin — the enzyme immunoassay technology — lacks sensitivity, "new detection methods are needed."

Wilcox and his colleagues noted in a multicenter study published in the *Lancet Infectious Diseases* in 2014 that a wide variety of testing strategies for *C. diff.* infection are used across Europe. An estimated 40,000 inpatients with *C. diff.* infection are potentially undiagnosed every year in 482 European hospitals, they said, primarily because of suboptimal laboratory diagnostic methods and the absence of clinical suspicion.

A few companies are looking to generate more sensitivity assays while maintaining high specificity, and First Light is among them, Wilcox said. The early data presented by the company ast ASM Microbe "looked impressive," and if additional clinical trial data confirms the early findings, "we will be able to use a highly sensitive toxin-based method for detecting patients who may have *C. Diff.* infection, and at the same time have high specificity so that we won't encounter this problem of false positives," he said.

The target customer for First Light's platform and assay are hospital labs. With the benchtop, automated platform, clinicians can do 20 tests simultaneously, a level of throughput necessary in a hospital for this type of infectious disease testing, Straus said.

It is too soon to set pricing, he said, but the instrument and assay in use will be "significantly less expensive than nucleic acid tests" and available at a price comparable to that of lower sensitivity enzyme immunoassays.

For *C. diff.* testing, he said, the firm's closest competitors are Singulex and Quanterix.

In a poster presented at the Anaerobe Society of the Americas in Nashville, Tennessee, in 2016, researchers from BioMérieux concluded that it is possible to develop a duplex assay for the simultaneous detection of both toxins A and B using the Quanterix Simoa technology. "For the next steps, the importance of each toxin, A and B, in diagnosis and pathology will be first defined [and] assessed by using the two assays separately," the researchers wrote.

In April, Stanford University investigators presented the results of a study using the investigational Singulex Clarity *C. diff* toxin A/B assay at the European Congress of Clinical Microbiology and Infectious Diseases in Madrid. “Data show that this new, rapid, standalone immunoassay addresses…shortcomings by detecting nearly all cell cytotoxicity neutralization assay-positive samples," Niaz Banaei, associate professor of pathology and medicine at the Stanford University Medical Center and the primary investigator of the study, said in a statement.

First Light noted that its MultiPath platform has the ability to perform a variety of tests, including pathogen identification, toxin and biomarker detection, and rapid phenotypic antimicrobial susceptibility testing on the same random-access platform.

The technology can be applied to rapidly determine differential growth patterns of selected pathogens in the presence of antibiotics, making it an ideal tool to calculate susceptibility of multidrug resistant bacterial infections, Straus said.

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