Synthetic Biology Takes on Zika

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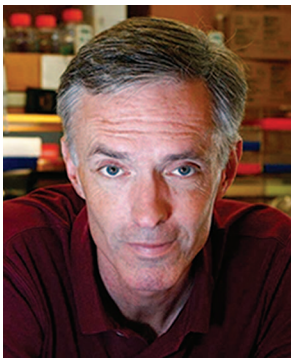
By the time public health officials implement infrastructure to deal with an outbreak, the threat has often passed. This could change with innovation on a new diagnostic work flow and platform designed by Jim Collins, a professor at the Massachusetts Institute of Technology (MIT).

Many innovators develop a novel assay and stop there. When Collins, a design thinker, explores a new novel assay, he likes to think about how the whole process could improve.

Collins' technology uses synthetic biology to design sequence-specific sensors and develop an environment-proof protein expression platform that can rapidly deployed to the field. The journal spoke with Collins about his application toward a field-ready Zika diagnostic.

What Is the Innovation?



Jim Collins

Zika is a flavivirus—an RNA virus family that also includes famous members such as dengue, yellow fever, and West Nile. Which means a simple antibody test to detect Zika would show cross reactivity with others in its family. Collins and colleagues strove to develop a test that would eliminate such cross reactivity—plus, one that does not carry the cost and complexity of PCR.

The workhorse of the system is the toehold switch, a programmable RNA sensor *(1)* that translates a repressed mRNA (a color sensor, for example) when a linear strand of trigger RNA binds. The trigger RNA finds the complementary single-stranded toehold sequence and opens up the ribosome binding site (RBS)[3] and start codon within its hairpin structure. The strong RBS recruits a ribosome to initiate translation at the start codon and translate a repressed LacZ enzyme to cause a color change.

But a toehold switch is of no use without knowledge of the sequence of trigger RNA to detect. This is easy to find for known RNA viruses, but what about in the setting of an emerging outbreak? Given the sequence of the pathogen, an algorithm finds potential regions of the genome to use as trigger RNA. The chosen regions do not have cross talk with human RNA or, in the case of Zika, related viruses such as dengue.

It takes 4 days to design and synthesize the sensors, 7 h to screen potential trigger RNA sequences, and 1 day to manufacture the kits, which involves embedding into paper, and the more unusual step of freeze-drying—a move that eliminates the need for cold storage.

The paper diagnostics are stable at room temperature for a year.

How Does It Work?

Kimberly Hamad-Schifferli

For the Zika diagnostic specifically *(2)*, the first step is to release the RNA for detection through a simple boiling step at 95 °C for 2 min. Because Zika viral loads in saliva and serum are low—in the femtomole range—it is necessary to subsequently amplify material. The team chose to use nucleic acid sequence-based amplification (NASBA), an amplification technique that has components compatible with freeze-drying and has been proven to work in the field. It typically requires an initial heating step followed by isothermal amplification, but Collins found that it works just as well without it.

So with RNA available for detection, the next step is NASBA isothermal amplification at 41 °C for 2 hours. The NASBA reaction in water then rehydrates the freeze-dried components on the paper to detect the trigger RNA sequences with the toehold switches. The paper reactions are housed in an acrylic chip that fits in

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Received November 16, 2016; accepted November 22, 2016.

[3] Nonstandard abbreviations: RBS, ribosome binding site; NASBA, nucleic acid sequence-based amplification.

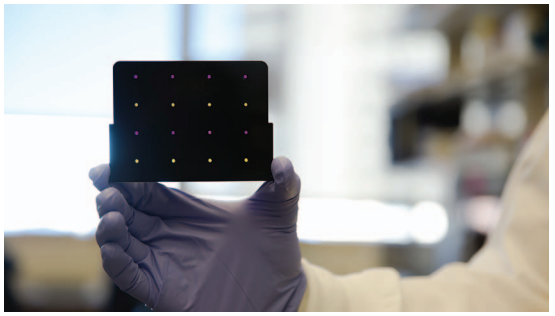

Fig. 1. A black cartridge containing a paper-based diagnostic for detecting the Zika virus is held up by a researcher at Harvard's Wyss Institute.

Areas that have turned purple indicate samples infected with Zika, while yellow areas indicate samples that are free of the virus. ©Wyss Institute at Harvard University. Reproduced with permission.

an electronic reader, between a 570-nm light source and electronic sensors. A change in color from yellow to purple signifies the presence of Zika virus (Fig. 1). The team is also able to distinguish between different strains of the virus.

Postamplification, the experts saw detection in reactions initiated "with as little as 3fM of trigger RNA."

Where Can This Fit in the Laboratory?

"The sensitivity of the test is high enough for clinical ranges," wrote Kimberly Hamad-Schifferli in an email to *Clinical Chemistry*. Hamad-Schifferli is an associate professor at the University of Massachusetts Boston and a visiting scientist at MIT; she specializes in rapid diagnostics and was involved in the current study. "However, I think the most challenging question is whether it can reach those sensitivity values in the field—often patient samples are not as well behaved as laboratory solutions." It's a challenge, she says, faced by any paper diagnostic.

We'll soon find out: early this fall, the team received funding for a field trial in South America *(3)*.

Beyond Zika, Collins says, "I would love to see this as a platform that leads to millions of diagnostic tests across the world."

As this test can be designed to target regions specific to any given genome, the authors believe it has potential for a variety of diseases and public health situations, keeping in mind the tool's flexibility in adapting to different symptoms, modes of transmission, and time from infection to disease, says Hamad-Schifferli.

Moreover, this proof of concept design speaks to just how quickly teams might be able to address an outbreak—it took Collins' group just 6 weeks to bring this method to life. With this team's creative engineering immediately on the case, emerging diseases might seem just a little less scary.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures or Potential Conflicts of Interest: *No authors declared any potential conflicts of interest.*

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DOI: 10.1373/clinchem.2016.269464