Ultra-Cheap, Sensitive DNA Nanoswitch Tech Developed for SARS-CoV-2 Detection

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NEW YORK – A research team at the University of Albany-State University of New York has pioneered a DNA nanoswitch technology to detect SARS-CoV-2 at the cost of a few pennies per reaction. The system can be easily reprogrammed to detect other viruses, and the group hopes to commercialize it through partnership with industry.

The work was carried out at the RNA Institute in the University at Albany, in the lab of Ken Halverson, which had previously been involved in the development of a nanoswitch-based test that was spun into a company called Confer Health. The new version is not related to that firm.

As described recently in *Science Advances*, the team developed a device with DNA nanoswitches that mechanically reconfigure in response to specific viruses. They tested it on strains of Zika virus and SARS-CoV-2.

The first author on the study, Lifeng Zhou, a post-doc in the Halvorsen lab, said that the DNA nanoswitches are made of long double stranded DNA, in the range of about 7,000 base pairs.

The researchers engineered the long double-stranded DNA with two single-stranded DNA probes on it, such that when the two probes detect the SARS-CoV-2 RNA, for example, the entire DNA nanoswitch will transform into a looped configuration which can be distinguished from unlooped ones, Zhou said in an email.

"After staining, the looped nanoswitches can be shown as a detection band on the gel electrophoresis image under UV light," he said.

In the study, the team showed it could detect SARS-CoV-2 RNA at a concentration as low as 200 copies per microliter, which is around the median concentration seen clinically. The entire test took about two hours, with one hour dedicated to a pre-amplification step, 40 minutes of nanoswitch incubation, and a 25-minute gel step.

There are several advantages to this type of DNA-based diagnostic platform, Zhou noted.

It can detect the viral RNA at clinical level concentrations without using any enzymes, which are currently critical in many other diagnostic methods. "Enzymes are expensive, always require cold-chain delivery system to maintain stability," he said, adding that they are also sensitive to contamination.

The method is also sensitive. The *Sci Advances* study showed the team could detect RNA of the Zika virus at about 100 copies per microliter, Zhou said, and the team is pushing for even higher sensitivity in its COVID assay.

The method has a high specificity, as well, "because detection is based on the accurate base pairing (A-U, G-C) between the sequences of nanoswitch probes and the viral RNA," Zhou said, providing the assay with the ability to distinguish RNA sequences that vary by a single nucleotide.

And, the nanoswitch devices can be "easily reprogrammed" for the detection of other new RNA viruses. "Once we know the sequence of the viral RNA, new DNA nanoswitches can be designed in a couple of hours by using our home-made software tool," Zhou said. The fabrication of a DNA nanoswitch takes less than two hours.

"The only major time limitation is the synthesis of new DNA oligoes which usually takes about one to three days for most companies," Zhou added.

The overall system is "simple and cheap," because the nanoswitch is constructed using DNA and the gel electrophoresis detection is a ubiquitous molecular biology technique. The team estimates cost of the DNA nanoswitches in each reaction to be $0.01.

"And the other reagents are very cheap, too," Zhou said, including the DNA oligos, such that "$1 of DNA materials can make more than 100 femtomole of DNA nanoswitches. For the design of new nanoswitches, we only need to replace two DNA oligos."

In the study, the authors also noted that they demonstrated the assay can be performed using a commercially available buffer-less gel cartridge, called the E-gel, and subsequently imaged on a small, and potentially portable, gel reader. Thermo Fisher Scientific's E-Gel Power Snap Electrophoresis System, which retails for less than $3,000, comes with a high-resolution image capture device, uses pre-cast agarose gels, and can process 8 to 16 samples per run.

The team also upped the sensitivity of the assay by using an isothermal preamplification step called nucleic acid sequence-based amplification, or NASBA.

Zhou said NASBA is a reliable technique that has been around for more than 20 years. The NASBA reaction is performed at 41 °C and can produce enough targeted RNA to be detected by the DNA nanoswitch in less than 1 hour.

In the study, the team demonstrated it could detect SARS-CoV-2 RNA in saliva in about two hours with using NASBA. "Currently, we are pushing forward to finish the detection in one hour," Zhou said.

Arun Richard Chandrasekaran, a research scientist in the Halvorsen lab, is spearheading the commercialization plans for the DNA nanoswitches. He had previously served as a senior scientist at Confer Health.

"We are currently focusing on the clinical detection aspects of the assay and are looking to partner with a company that will develop a diagnostic kit for field use and pursue [US Food and Drug Administration] approvals, Chandrasekaran said.

The COVID-19 outbreak has spurred the development of many diagnostics tests, some of which use DNA or DNA nanostructures, but Chandrasekaran said that the DNA "nanoswitches" are unique to the Halvorsen lab.

The primary funding for the lab's COVID-19 assay development comes from the National Science Foundation through a $177,774 Rapid Response Research (RAPID) award granted in May.

Chandrasekaran said that the group's long-term goal is to use the DNA nanoswitch assay "as a platform technology for detecting a variety of diagnostics markers such as nucleic acids and antigens."

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