Portable, rapid DNA test can detect Ebola and other pathogens

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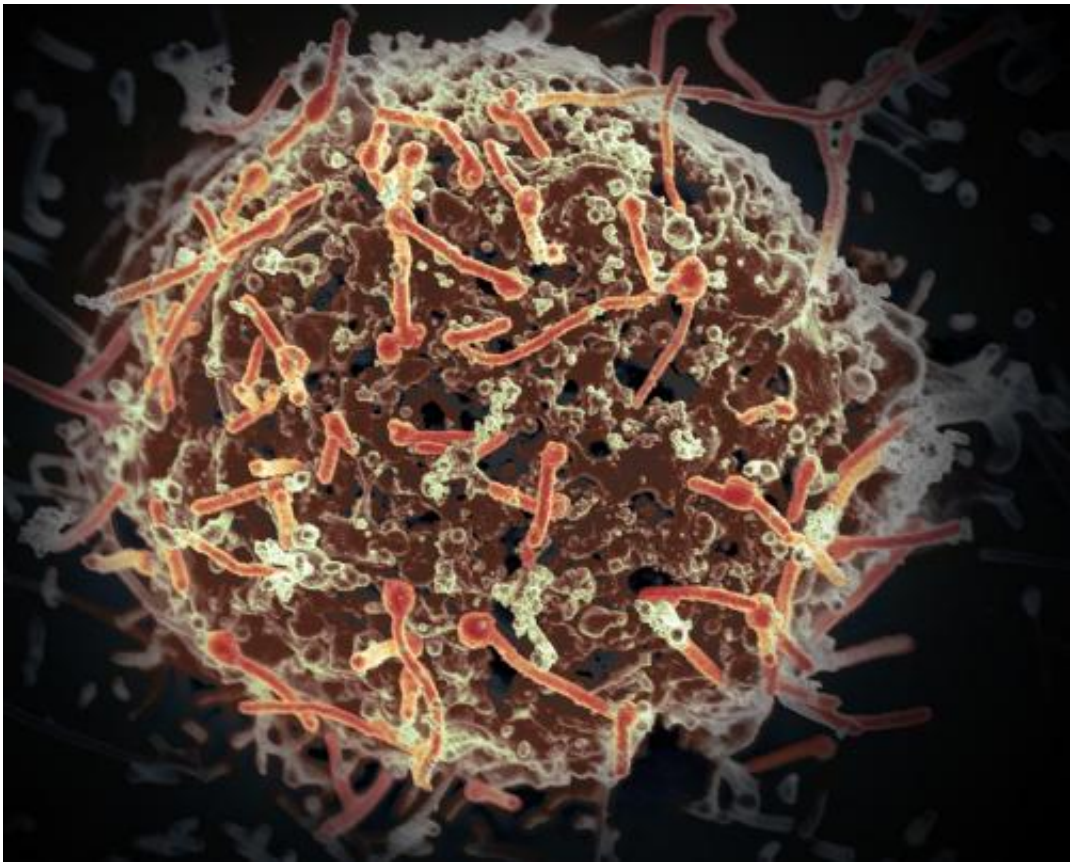

The Ebola virus, isolated in November 2014 from patient blood samples obtained in Mali. The virus was isolated on Vero cells in a BSL-4 suite at Rocky Mountain Laboratories. Credit: NIAID

Using technical advances not yet developed when the 2014 Ebola outbreak began, UC San Francisco-led scientists completed a proof-of-

principle study on a real-time blood test based on DNA sequencing that can be used to rapidly diagnose Ebola and other acute infections. The researchers said that the test can be used even where lab space and medical infrastructure are scarce.

Charles Chiu, MD, PhD, associate professor of laboratory medicine at UCSF, led a team that detected the genetic fingerprints of Ebola in stored blood samples from two African patients who had acute hemorrhagic fever, completing the diagnosis within five hours of opening the samples—the DNA sequencing itself took just 10 minutes.

Most commercially available or research-based genetic diagnostic tests target specific pathogens. But Chiu and UCSF colleagues have pioneered techniques that do not require suspected pathogens to be identified beforehand in order to detect their unique genetic fingerprints. This unbiased approach of analyzing all DNA in a clinical sample without knowing which species are present, which was used in the Ebola detection, is called "metagenomic" analysis.

To obtain such quick results the researchers developed new analysis and visualization software and used it on a laptop computer to leverage an emerging DNA-sequencing technology known as nanopore sequencing.

In the same set of experiments, published online in *Genome Medicine* on September 28, the researchers were able to detect Chikungunya virus, from a Puerto Rican outbreak, just as quickly in a blood sample from a donor with no symptoms, but who eventually reported having fever and joint pains. In another example of the technique's power, detection of hepatitis C virus in blood from an infected UCSF patient, present at a much lower concentration than the other viruses, took just 40 minutes from the start of sequencing.

"This point-of-care genomic technology will be particularly attractive in

the developing world, where critical resources, including reliable electric power, laboratory space, and computational server capacity, are often severely limited," Chiu said.

Many companies are developing nanopore technology, which distinguishes individual nucleic acids by the distinctive perturbations they create in electric currents as they individually pass through microscopic pores. Chiu's lab group was one of the first to pay $1,000 for access to an experimental DNA nanopore sequencer made by Oxford Nanopore Technologies, called the MinION. The device is small enough to fit in the palm of the hand and is powered by a USB connection to a laptop.

Last year, using a similar metagenomic approach to pathogen detection, Chiu teamed up with UCSF colleagues to solve a medical mystery that was highlighted in a *New England Journal of Medicine* case study. The researchers used their software and another DNA-sequencing technology to analyze all DNA in a spinal fluid sample, leading to the diagnosis of an unusual but treatable bacterial cause of encephalitis in a critically ill Wisconsin boy whose health had been worsening for months.

That earlier analysis took two days. The detection of Ebola in the new study was more rapid because nanopore sequencing yields data immediately and in real time, unlike the technology used in the Wisconsin case, which takes much longer to provide data for analysis.

Nanopore technology is new and still error-prone, Chiu said, but speed and accuracy are improving at a quick pace. With the time required for DNA sequencing, analysis and reporting now cut down to minutes, Chiu has set his sights on streamlining and automating the sample preparation step, which still requires several hours, for use in both clinical laboratory and field settings.

"To our knowledge, this is the first time that nanopore sequencing has been used for real-time metagenomic detection of pathogens in complex clinical samples in the setting of human infections," Chiu said. "Unbiased point-of-care testing for pathogens by rapid metagenomic sequencing has the potential to radically transform infectious disease diagnosis in both clinical and public health settings."

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