Cutting-Edge Infectious Disease Diagnostics with CRISPR

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Three recent Science articles (Chen et al., 2018; Gootenberg et al., 2018; Myhrvold et al., 2018) describe the use of CRISPR-Cas technology to develop point-of-care diagnostics that directly detect viruses from clinical samples. These tests could radically transform approaches to diagnosing infectious diseases at the bedside and in the field.

SARS/MERS coronavirus, 2009 pandemic H1N1 influenza, Ebola virus (EBOV), and Zika virus (ZIKV)—every couple of years, a new virus emerges from the hinterland and threatens to devastate the population. During each of these epidemics, we were woefully unprepared to meet the urgent need for field-deployable diagnostic tests to guide surveillance, treatment, and bioccontainment efforts. The challenge faced by clinicians in accurately diagnosing infections extends not only to outbreaks in the field but also to routine patient care in community hospitals and clinics. Individual molecular testing for the vast majority of pathogens is typically not available except as send-out assays to highly specialized reference laboratories. Up until now, point-of-care diagnostic platforms for rapid, flexible, and accurate detection of pathogens directly from clinical samples did not exist.

In three papers published in Science (Chen et al., 2018; Gootenberg et al., 2018; Myhrvold et al., 2018), two groups report the use of CRISPR-Cas-based technology for the development of molecular diagnostic assays, with a focus on infectious diseases. The CRISPR-Cas system in bacteria has evolved as a mechanism to fight off viruses by cleaving DNA or RNA sequences from the invading phage (Hille et al., 2018). This technology, now widely repurposed for gene-editing applications, involves families of enzymes that are able to precisely cleave double-stranded DNA (dsDNA) or single-stranded RNA (ssRNA) at sequences complementary to a guide RNA. For purposes of detection, the groups here leverage the “collateral activity” of either the Cas13 enzyme in indiscriminately cleaving ssRNA (Gootenberg et al., 2018; Myhrvold et al., 2018) or the Cas12a enzyme in cleaving single-stranded DNA (ssDNA) (Chen et al., 2018). Once the guide RNA is activated by a complementary sequence, cleavage of a labeled ssRNA or ssDNA probe generates a signal that can provide a fluorescent readout in a number of portable formats, including the use of disposable paper strips (Figure 1).

The CRISPR-Cas13 (SHERLOCK)-based (Gootenberg et al., 2018; Myhrvold et al., 2018) and CRISPR-Cas12a (DETECTR)-based (Chen et al., 2018) assays described in these papers carry several advantages over traditional microbiological diagnostics such as nucleic acid amplification testing (e.g., polymerase chain reaction [PCR]) or antigen-based testing (e.g., immunostrips) (Drancourt et al., 2016). Nucleic acid amplification tests such as PCR are generally considered to be the most sensitive and specific tests available. However, these tests typically incorporate multiple steps to run the assay, including an upfront nucleic acid extraction step, and require dedicated instrumentation. In contrast, both groups demonstrate here that CRISPR-Cas-based assays can be run directly on primary clinical samples as a single reaction and performed using minimal or no equipment. The assays are also amenable to the use of lyophilized reagents, foregoing the need for maintenance of a cold chain, and thus are attractive for use in the field. However, the CRISPR-Cas assays are also shown to exhibit high sensitivity and specificity of detection, achieving a performance comparable to that of PCR. Interestingly, this may be due to the fact that an isothermal amplification is still required as part of the CRISPR-Cas-based assay protocol to generate detectable fluorescent signal. At the same time, these assays are similar to antigen-based tests with respect to ease of use and speed, with a sample-to-answer turnaround time of 1–2 hr.

In the paper by Myhrvold et al., work deriving from the collaboration of two prominent investigators at the Broad Institute (Feng Zhang and Pardis Sabeti), the authors illustrate several applications for the SHERLOCK assay, including (1) ZIKV and dengue virus (DENV) detection directly from bodily fluids such as saliva and urine in < 2 hr, (2) specific discrimination among the four different DENV serotypes or identification of region-specific strains of ZIKV from the 2014–2016 pandemic, and (3) detection of single-nucleotide polymorphisms such as a recent ZIKV mutation, S139N, linked to cases of microcephaly in affected infants (Yuan et al., 2017). The last two examples are of particular relevance to public health epidemiology. However, the clinical utility of the assay can also be potentially extrapolated to individual patients, such as the determination of a particular hepatitis C virus (HCV) genotype to guide the choice of antiviral therapy (AASLD/IDSA HCV Guidance Panel, 2015).

In the Chen et al. paper from the laboratory of Jennifer Doudna at UC Berkeley, the authors use the DETECTR assay to detect “high-risk” human papillomavirus...
types 16 and 18, associated with invasive genital tumors, with 96% overall accuracy. This successful demonstration extends the potential application of CRISPR-Cas technologies from infectious diseases to other fields. Rapid detection of single-nucleotide polymorphisms from circulating tumor cells in blood, for instance, may usher in a new era of point-of-care diagnostics for early cancer. A CRISPR-Cas-based assay can also be leveraged for use as both a companion diagnostic and treatment. For example, the Broad Institute group describes the repair of a mutation in the APC gene that has been linked to familial adenomatous polyposis, an inherited condition greatly increasing the risk of colon and rectal cancers in affected individuals. Using SHERLOCK, they simultaneously genotyped and edited out the APC gene from a human embryonic kidney (HEK293) cell line containing the insert. 

So, is this technology ready for prime time? At first glance, a CRISPR-Cas-based assay appears to have all of the characteristics desired in a point-of-care diagnostic: (1) implementation as a single reaction; (2) compatibility with lyophilized reagents; (3) under 2-hr turnaround time; (3) design flexibility, with generation of a new assay from scratch in under a week; (4) portability without the need for electricity or expensive instrumentation; (5) detection directly from clinical samples; and (6) a colorimetric fluorescent readout. Such an assay would have been tremendously helpful, for instance, if rapidly deployed and available for diagnostic testing early in the course of the West African EBOV or ZIKV epidemics.

However, it is unlikely that a CRISPR-Cas approach by itself will completely solve the challenges of point-of-care diagnostic testing. Fundamentally, the assay is still a targeted detection test using specific primers and probes and is thus subject to the same limitations. Deploying the test for emerging outbreak investigation may not be useful if the cause of an outbreak is not known. For example, cryptic transmission and circulation of ZIKV had been ongoing in Brazil for more than a year prior to its initial identification in May of 2015 (Faria et al., 2017), in part because ZIKV had never been seen in the Americas and so had not been considered as a candidate outbreak pathogen a priori. In addition, the high specificity of CRISPR/Cas cleavage, although of value for a targeted diagnostic, can be challenging with RNA viruses such as ZIKV and EBOV that rapidly mutate. Indeed, mutations in the EBOV genome likely resulted in decreased sensitivity and performance of existing PCR assays in detection of the Makona strain that caused the 2014 EBOV outbreak in West Africa (Sozhamannan et al., 2015).

The field of infectious disease diagnostics is also rapidly moving toward clinical syndromic-based multiplexing for detection, such as the use of targeted panels for diagnosing neurological, respiratory, and gastrointestinal infections (Ramanan et al., 2017). Highly multiplexed or even fully untargeted methods (e.g., metagenomic next-generation sequencing) (Schlaberg et al., 2017; Somasekar et al., 2017) may be preferable for diagnosis of unknown acute febrile illnesses that may be caused by a variety of different pathogens. For instance, a significant proportion of patients during the 2014 West Africa EBOV outbreak had other infections, such as Lassa fever or malaria, or were co-infected. Of note, these diagnostic methods can be complementary. A CRISPR-Cas-based assay may be used to initially screen for antibiotic resistance, for example, with reflex sequencing to characterize the specific genes and mechanisms involved.

Other foreseeable challenges to routine implementation of CRISPR-Cas-based assays include performance, cost, and regulatory issues. Since a target amplification
step is still incorporated as part of the assay, there remains the possibility of cross-contamination and/or contamination from the laboratory, reagents, or environment. In addition, the “real-life” performance and accuracy of CRISPR-Cas-based diagnostic testing is not yet known, especially outside of the confines of a controlled laboratory environment. It also remains to be seen whether CRISPR-Cas-based assays can be standardized for high-throughput diagnostic testing and how they will be viewed by regulatory agencies such as the U.S. Food and Drug Administration. There may be issues regarding availability and cost of CRISPR-Cas-based testing to the developing world, especially given that many of the intended-use cases for the technology have been patented. Finally, a key consideration is how amenable the approach is to multiplexing. In the current papers, multiplexing of only one or a few targets in parallel was demonstrated, and it is unknown whether there is an upper limit to the number of targets that can be successfully incorporated in the assay. Nevertheless, despite these concerns, CRISPR-Cas-based technology has now provided us a powerful new tool in our perpetual fight against infectious diseases.

REFERENCES


