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FIRST CUT

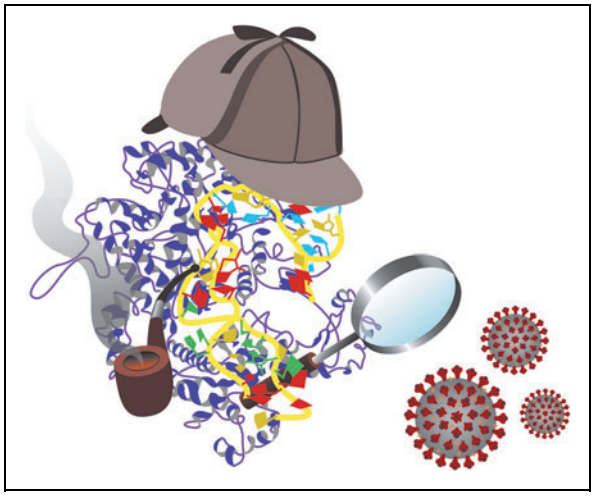
Adventure of the CRISPR Coronavirus Disease 2019 Diagnostic

Keith Robison

Sherlock Biosciences's CRISPR-Cas13 RNA diagnostics platform races to an urgent new application in the coronavirus disease 2019 pandemic.

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The coronavirus disease 2019 (COVID-19) pandemic has so far claimed more than 390,000 lives worldwide as of June 5th, 2020. While stringent public health measures have curtailed the virus's expansion, more than 6.7 million people across the globe have been infected, and too many nations are still experiencing exponential growth of cases. The requisite national lockdowns have had stark economic and social consequences, leading to intense pressure to reopen economies. Even with such measures, certain industries such as meat-packing have experienced workforce decimation due to close working conditions in such facilities enabling viral spread. Proposals for commerce reopening have been floated that rely on mass severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) testing coupled with intensive contact tracing and isolation of those infected to prevent further transmission.



(Credit: Mon Oo Yee.)

Such grand testing plans collide with continuing capacity shortfalls. The vast majority of tests for active COVID-19 have been quantitative reverse transcription polymerase chain reaction (qRT-PCR) assays for viral RNA. This well-established technology has enabled a high testing cadence, but it faces multiple challenges, impeding further expansion. Most qRT-PCR tests rely on RNA extraction from the sample, possibly with concentration of material to enhance sensitivity. These extraction reagents, in addition to the qRT-PCR reagents themselves, have been under ferocious supply-chain pressure as testing volumes set records. A special class of instruments, significantly more expensive and bulkier than generic thermocyclers, is required for qRT-PCR, as they must stimulate and measure fluorescence during thermocycled amplification.

These difficulties have heightened interest in alternative detection strategies, albeit with the proviso that alternatives be no less sensitive than qRT-PCR. Approaches using isothermal chemistries are particularly attractive, as they replace complex thermocyclers with simple heat blocks or water baths. Ideally, an extraction-free isothermal method would avoid any laboratory equipment, approaching the inexpensive simplicity of what is perhaps the pinnacle of *in vitro* diagnostics: the home pregnancy dipstick test.

On May 6, the U.S. Food and Drug Administration (FDA) granted an Emergency Use Authorization (EUA) to Sherlock Biosciences, a company co-founded by Feng Zhang, Pardis Sabeti, and Todd Golub among others, for the first CRISPR-based SARS-CoV-2 test. This is also only the second isothermal assay for this application to receive authorization.

Sherlock's assay targets two viral targets—the nonstructural ORF1ab and the highly expressed nucleocapsid ''N'' gene—as well as the human RNase P gene to control for the sample actually bearing RNA. Reverse

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transcription loop-mediated amplification (RT-LAMP) is used to amplify each target. LAMP relies on multiple primers that hybridize in the presence of the target so as to produce a loop structure that is amplified isothermally by a strand-displacing polymerase. This is the first LAMP assay to receive an EUA.

Lighting the LAMP

Once the target is amplified, the CRISPR angle kicks in. Among CRISPR effector enzymes, Cas9 has understandably received the most attention due to its early elucidation as an RNA-targeted DNase, the wide adoption for gene editing in research settings, and great excitement (as well as patent battles) around its use for therapeutic gene editing. Sherlock's assay relies on its less storied counterparts such as Cas13, which, upon recognizing their target, transform into high activity nonspecific RNases. This acts *in vivo* as a self-destruct mechanism, obliterating both host and viral RNA. In Sherlock's assay, supplied reporter RNAs are destroyed by the Cas enzymes, releasing fluorescent reporters. An advantage of CRISPR-based detection mechanism over others used for LAMP reactions is further specificity; nonspecific amplification products will lack guide RNA's target.

While Sherlock's founders have published point-of-care schemes based on such chemistry, the approved assay targets diagnostics laboratories. A separate spin column nucleic acid extraction kit is used to prepare samples, and RT-LAMP reagents are added to drive the 65°C amplification reactions for 40 min. Then, detection reagents are added, and a 10 min 37°C incubation is run on a fluorescence plate reader. Analytical sensitivity is similar to many qRT-PCR assays at 900 vg/mL for the N gene and 4,500 vg/mL for orf1ab.

Sherlock's EUA represents a relatively conservative step in terms of a new assay, still requiring a laboratory and trained technicians and an hour or more to run. It is also conservative in terms of sample types, targeting nasopharyngeal (NP) swabs and similar nasal samples used by most SARS-CoV-2 molecular assays. It also requires the separate addition of detection reagents—a step that both adds complexity and is an opportunity to spread amplified material. While the RT-LAMP and detection reagents are largely outside the strained qRT-PCR supply chain, the extraction kit used is featured in many other EUAs and so is in extremely high demand.

But the game is afoot. Sherlock's co-founders have deposited a preprint[1] describing a 15-to 20-min ''STOPCovid'' point-of-care assay, which substitutes a simple lysis buffer for an extraction kit, replaces plate reader readout with a visually assessed lateral flow dipstick, and would accept either NP swabs or saliva. In addition, Sabeti, Hung, Blainey, and colleagues have published a process called CARMEN that further expands these concepts using microfluidics to screen for a broad array of respiratory pathogens.[2] Presumably the FDA's acceptance of the initial Sherlock assay has primed regulators for favorably considering innovative workflows even more divergent than typical qRT-PCR protocols.

We should anticipate some bumps ahead. The isothermal viral RNA detection system previously granted an EUA is Abbott's ID NOW point-of-care system, which uses an unspecified amplification method to deliver results in ≤15 min. However, serious questions have arisen as to the clinical false-negative rate for ID NOW. Abbott has pushed back by claiming that observed discrepancies versus qRT-PCR are due to improper upstream sample handling. The mere appearance of issues may shift regulators to a more conservative stance or to requiring more intensive post-EUA validation of claimed assay sensitivity and specificity, which can be based on samples contrived in a laboratory and not from actual patients. Maintaining public confidence in testing systems should be a key priority, given the level of controversy, backbiting, and politicization that surrounds pandemic responses.

Sherlock's EUA represents a small but important step toward broader testing and the enhanced public safety that such testing enables. It also signals the entry of CRISPR technology into clinical testing. Worldwide crises often stimulate acceleration of technological advances. Let us hope that clinical diagnostic use of CRISPR isn't the only important advance spurred by the tragedy and devastation inflicted on humanity by COVID-19.

References

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