https://doi.org/10.1038/s41551-022-00889-z

nature biomedical engineering

Simplified Cas13-based assays for the fast identification of SARS-CoV-2 and its variants

Jon Arizti-Sanz[1,2], A'Doriann Bradley[1], Yibin B. Zhang[1,3], Chloe K. Boehm[1,4], Catherine A. Freije[1], Michelle E. Grunberg[1,4], Tinna-Solveig F. Kosoko-Thoroddsen[1], Nicole L. Welch[1,5], Priya P. Pillai[1], Sreekar Mantena[1,3], Gaeun Kim[1,4], Jessica N. Uwanibe[6,7], Oluwagboadurami G. John[7], Philomena E. Eromon[6], Gregory Kocher[8], Robin Gross[8], Justin S. Lee[9], Lisa E. Hensley[8], Bronwyn L. MacInnis[1,10], Jeremy Johnson[1], Michael Springer[11,12], Christian T. Happi[6,7,10], Pardis C. Sabeti[1,3,10,13,14] and Cameron Myhrvold[4,14] ✉

The widespread transmission and evolution of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) call for rapid nucleic acid diagnostics that are easy to use outside of centralized clinical laboratories. Here we report the development and performance benchmarking of Cas13-based nucleic acid assays leveraging lyophilised reagents and fast sample inactivation at ambient temperature. The assays, which we named SHINEv.2 (for 'streamlined highlighting of infections to navigate epidemics, version 2'), simplify the previously reported RNA-extraction-free SHINEv.1 technology by eliminating heating steps and the need for cold storage of the reagents. SHINEv.2 detected SARS-CoV-2 in nasopharyngeal samples with 90.5% sensitivity and 100% specificity (benchmarked against the reverse transcription quantitative polymerase chain reaction) in less than 90 min, using lateral-flow technology and incubation in a heat block at 37 °C. SHINEv.2 also allows for the visual discrimination of the Alpha, Beta, Gamma, Delta and Omicron SARS-CoV-2 variants, and can be run without performance losses by using body heat. Accurate, easy-to-use and equipment-free nucleic acid assays could facilitate wider testing for SARS-CoV-2 and other pathogens in point-of-care and at-home settings.

Frequent and widespread testing is critical to prevent and respond to infectious disease outbreaks. For example, large-scale testing to track the prevalence and transmission of SARS-CoV-2 has been essential in managing the ongoing coronavirus disease 2019 (COVID-19) pandemic[1,2]. Ubiquitous and frequent diagnostic testing leads to the rapid identification of new cases and permits the swift treatment or isolation of infected individuals, thereby preventing further viral spread[3]. However, the gold standard for COVID-19 diagnosis—reverse transcription quantitative polymerase chain reaction (RT-qPCR)—remains suboptimal for orchestrating this response. RT-qPCR requires specialized equipment and expertise rarely found outside of centralized laboratories. In addition, an insufficient testing infrastructure coupled with reagent shortages and high testing demand have led to slow sample-to-answer times (often of several days) for RT-qPCR[4,5]. Alternative diagnostic technologies that enable rapid and decentralized testing are vital to respond to the current and future pandemics.

Lateral-flow antigen-capture tests and isothermal nucleic acid diagnostics with visual readouts represent promising alternatives for SARS-CoV-2 testing outside of centralized laboratories. The effectiveness of such tests in curbing the spread of SARS-CoV-2 has been demonstrated in multiple settings, ranging from nursing homes to whole countries[2,6]. Antigen-capture tests are quick and user-friendly, but their moderate sensitivity means they can miss potentially infectious individuals[7–9]. Isothermal nucleic acid amplification methods, such as loop-mediated isothermal amplification (LAMP), are more sensitive than antigen-capture tests and operate at a single temperature[10,11]. The deployment of LAMP-based tests has been facilitated by the use of auxiliary devices to eliminate the need for intensive nucleic acid purification and maintain a stable temperature during amplification[12]. However, these devices are often too costly for single use and are difficult to manufacture at the scale required for population-wide distribution, which limits their utility for frequent and widespread testing[13]. Hence, the continued development and deployment of user-friendly, sensitive and equipment-free diagnostics are key to enhancing the public health response to COVID-19.

CRISPR-based diagnostics (CRISPR-Dx) are promising technologies for SARS-CoV-2 testing with minimal equipment requirements. CRISPR-Dx usually combine isothermal nucleic acid amplification methods (often LAMP or recombinase polymerase amplification (RPA)) with an RNA-guided CRISPR-Cas nuclease

[1]Broad Institute of Massachusetts Institute of Technology (MIT) and Harvard, Cambridge, MA, USA. [2]Harvard-MIT Program in Health Sciences and Technology, Cambridge, MA, USA. [3]Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, USA. [4]Department of Molecular Biology, Princeton University, Princeton, NJ, USA. [5]Program in Virology, Harvard Medical School, Boston, MA, USA. [6]African Centre of Excellence for Genomics of Infectious Diseases (ACEGID), Redeemer's University, Ede, Osun State, Nigeria. [7]Department of Biological Sciences, College of Natural Sciences, Redeemer's University, Ede, Osun State, Nigeria. [8]Integrated Research Facility, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institute of Health, Frederick, MD, USA. [9]Biotechnology Cores Facility Branch, Division of Scientific Resources, National Center for Emerging and Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA. [10]Harvard T.H. Chan School of Public Health, Boston, MA, USA. [11]Department of Systems Biology, Harvard Medical School, Boston, MA, USA. [12]Laboratory of Systems Pharmacology, Harvard Medical School, Boston, MA, USA. [13]Howard Hughes Medical Institute, Chevy Chase, MD, USA. [14]These authors contributed equally: Pardis C. Sabeti, Cameron Myhrvold. ✉e-mail: cmyhrvol@princeton.edu