POINT-OF-CARE DIAGNOSTICS

Streamlined detection of SARS-CoV-2 via Cas13

Assays leveraging the CRISPR-associated enzyme Cas13 and isothermal RNA amplification for the detection of viral RNA are being simplified for point-of-care use.

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Nearly half of the world's population lacks access to proper diagnostics[1]. This is a multifactorial problem that is magnified during pandemics, when limiting viral spread and the emergence of new variants of concern (VOCs) are paramount. Assays based on quantitative PCR with reverse transcription (RT–qPCR) remain the gold standard for nucleic acid testing, but they require central facilities, trained personnel and costly reagents[2]. Yet, routine surveillance and repeated testing for the detection of pathogens demands inexpensive, sensitive and specific point-of-care (POC) or at-home diagnostics[3].

Diagnostic tests based on CRISPR (short for clustered regularly interspaced short palindromic repeats) offer advantages in sensitivity, specificity and robustness, and their designs are amenable for POC or at-home use. Many CRISPR-based diagnostics (CRISPR-Dx) have used the CRISPR-associated (Cas) enzyme Cas9 (refs. [4,5]); yet, Cas13 (ref. [6]) and Cas12 (refs. [7,8]) can better detect a positive signal, owing to the collateral cleavage activity of these enzymes (that is, their indiscriminate cleavage of nucleic acids on enzyme activation is exploited to unquench nucleic-acid-bound fluorophores). However, CRISPR-Dx do not yet comply with the World Health Organization's ASSURED criteria (short for affordable, sensitive, specific, user-friendly, rapid, equipment-free and delivered) for POC or at-home tests. The main challenges relate to sample processing, assay sensitivity, the shelf life of the reagents, and the development of inexpensive and easy-to-use assays that can be produced at scale[9].

Two studies published in *Nature Biomedical Engineering* exemplify how some of these challenges can be addressed. On the one hand, Cameron Myhrvold and colleagues report simplified sample-processing and reagent-storage solutions for their previously reported[10] Cas13-based assay SHINE (short for streamlined highlighting of infections to navigate epidemics), and modified it to detect emerging VOCs[11]. On the other hand, Patrick Hsu, David Savage, Jennifer Doudna and collaborators describe a portable microfluidic device implementing an assay, which they named DISCoVER (short for diagnostics with coronavirus enzymatic reporting), that semi-automatically performs loop-mediated isothermal amplification and RNA transcription, Cas13-mediated detection and signal readout[12].

Myhrvold and co-authors' improved SHINE assay (SHINEv.2) is equipment free, and processes samples rapidly at ambient temperature. The authors found that a commercial lysis reagent (the 'FastAmp viral and cell solution', which is compatible with isothermal nucleic acid amplification and works at ambient temperature) is compatible with SHINE and effectively inactivates severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virions and nucleases through the addition of RNase inhibitors. In addition, they optimized the reagent-lyophilization process (by adding the excipients sucrose as a stabilizer and mannitol as a bulking agent, and by removing the destabilizing reagents poly(ethylene glycol) and potassium chloride) to maintain the performance of the assay in the absence of cold-chain infrastructure (long-distance transport of reagents to end users is often needed in low-resource settings). The lyophilized reagents for SHINEv.2 maintained their activity after five months at 4 °C, but only for one week when stored at room temperature. The authors also simplified the assay readout, performed in lateral-flow format via a paper strip, by optimizing the composition of a crowding polymer needed for nucleic acid amplification, to enhance flow and thus avoid the need for a dilution step. Moreover, the authors adapted SHINE to rapidly discriminate SARS-CoV-2 VOCs; however, the detection limit of the VOCs was lower than that for the original strain of the virus. Further improvements would be needed to ensure high confidence in the detection of VOCs from nasopharyngeal samples. Clinical validation against RT–qPCR and antigen-capture kits (Abbott's BinaxNow and Access Bio's CareStart) showed that SHINEv.2 was 50 times more sensitive than either antigen test, albeit with a slower turnaround time, and that the assay excelled at detecting samples with moderate viral loads (RT–qPCR cycle-thresholds of 25–30). Also, SHINEv.2 correctly identified all positive samples with titres above the analytical limit of detection of 200 copies of the virus per microlitre.

Despite overcoming limitations in sample processing, shelf-life storage and readout, SHINEv.2 is not ready for POC use. The assay involves two liquid-handling steps: the rehydration of the lyophilized reagents, and the transfer of 10% (v/v) of the lysed sample into the detection mix. An ideal diagnostic test for POC use would not require liquid handling, and would provide a one-step sample-in–answer-out solution. This may be achieved by developing universal chemistry that combines sample lysis, nucleic acid amplification, Cas13-mediated detection and assay readout in a single-step assay, or by developing a portable closed-form device that performs all liquid-handling steps automatically and that is resistant to contamination (Fig. 1).

That is, in fact, the approach taken by Hsu and co-authors with the DISCoVER assay. The device consists of three microfluidic chambers (hosting the temperature-controlled reactions leading to nucleic acid amplification and detection, and to signal acquisition), a heater surrounding the amplification chamber to maintain it at 65 °C, a thermoelectric heater and cooler to maintain the detection chamber at 25 °C, air-displacement pumps, valves and custom fluorescence detectors. A lysed saliva sample is loaded into the first chamber, where the amplification-and-transcription reactions run for 30 min. Then a valve opens, metering 4 µl of reaction products to the Cas13-based detection mix (this takes approximately 5 min). The detection mix then enters the fluorescence-detection chamber, where the signal is acquired.

The DISCoVER assay uses two simple steps for extraction-free sample processing: heat denaturation at 75 °C, followed by chemical reduction deploying the

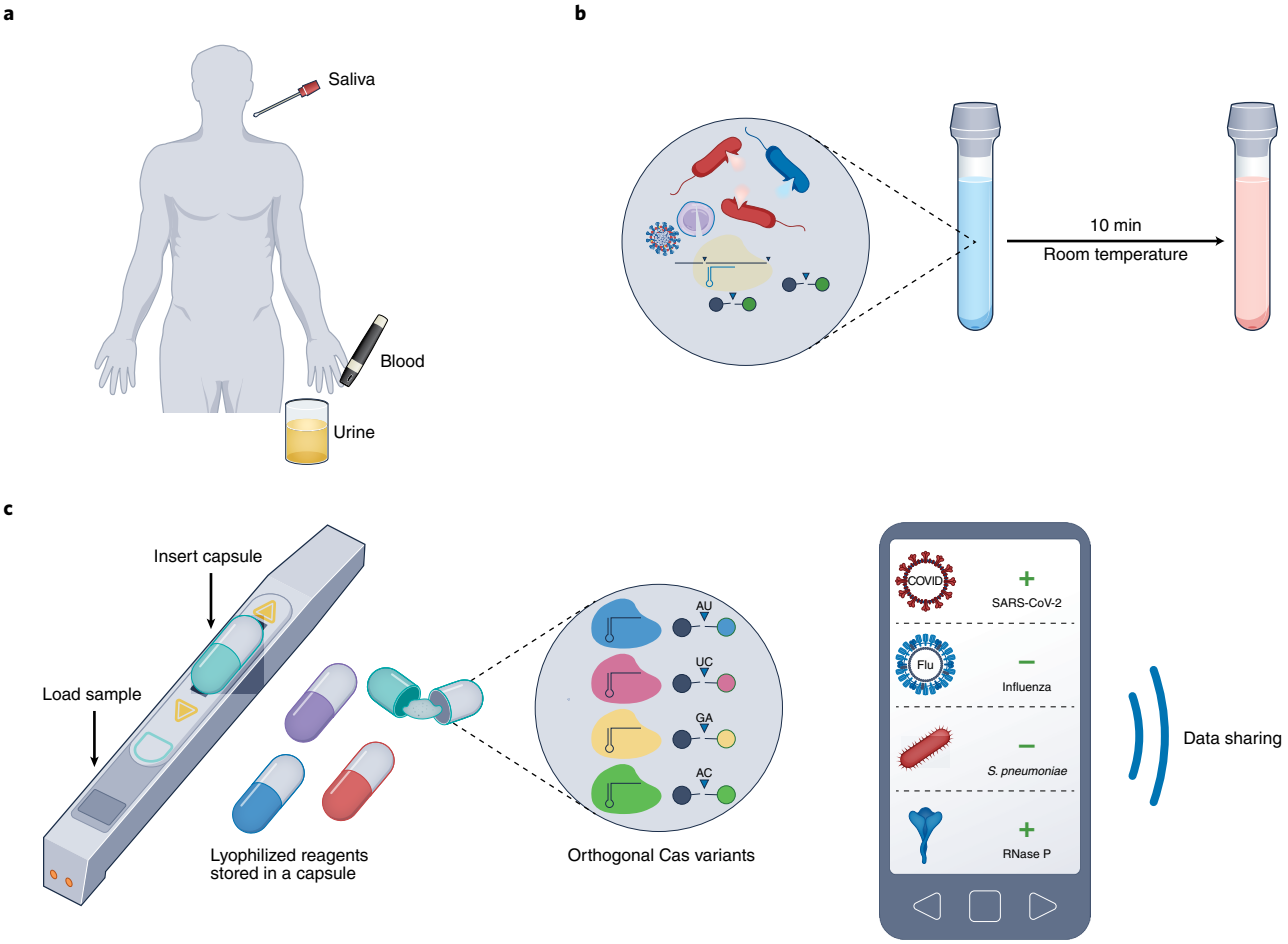

Fig. 1 | Simplifying CRISPR-based diagnostics for POC use. a, Sample collection should ideally be minimally invasive (such as blood samples via fingerpicking) or non-invasive (saliva or urine, in particular), so that samples can be acquired without trained personnel. **b**,**c**, The collected samples would then be loaded directly into a single tube with universal chemistry that would combine lysis, amplification and detection (**b**), or into a fully automated device that would take the sample, process it and carry out multiplexed nucleic acid amplification and detection, yielding easily interpreted signals that can be shared with public-health systems (**c**). The device would take as input a capsule with lyophilized reagents. Each capsule would test for one or multiple pathogens and biomarkers, and include an internal control.

shelf-stable reducing agent TCEP (tris(2-carboxyethyl)phosphine) paired with the ion chelator EDTA (ethylenediaminetetraacetic acid). TCEP–EDTA can interfere with the amplification and Cas13-mediated reactions, yet low concentrations of it protected the RNA and did not interfere with downstream reactions.

After optimizing the reaction chemistries, Hsu and co-authors tested the sensitivity and specificity of the DISCoVER assay using serial dilutions of viral stocks and synthetic RNA samples. The assay had an analytical limit of detection of about 40 RNA copies per microlitre, and it was 100% specific when tested with saliva samples spiked with different viruses. Using 20 saliva samples spiked with 40–200 RNA copies per microlitre and 5 negative samples, the microfluidic device detected the viral RNA in all 20 samples with an average sample-to-answer time of 78 min. A larger validation study involving samples with a broad range of cycle-threshold values will be needed to suitably determine the sensitivity, specificity and applicability of the DISCoVER assay for POC use.

POC diagnostic kits require an internal control so that the kit's proper performance can be verified. Hsu and co-authors used primers for the SARS-CoV-2 *N* gene and for the RNase *P* gene as an internal control, and divided the amplified reaction mixture into two detection chambers. Such multiplexed detection could be expanded by taking advantage of the varying collateral cleavage activity of Cas13 and Cas12 enzymes[13] with diverse cleavage motifs to produce different output colours for the optical detection of multiple viruses and to implement multiple internal-control sequences.

The DISCoVER assay requires two manual steps for sample processing. A fully automated device that combines the highest sensitivity reported so far for CRISPR-Dx[14], the lyophilization and sample processing of SHINEv.2, and one-pot chemistry via a thermostable Cas13 enzyme[15] may enable POC applications in low-resource settings and enhance access to these diagnostic tests. The sensitivity of CRISPR-Dx may not reach that of RT–qPCR technology, yet assays that make frequent testing easier and that have short turnaround times are more useful for screening and for other public-health needs[3]. Further advances in sample processing, in reaction chemistries for multiplexed detection and in device engineering are expected to facilitate the eventual broader use of CRISPR-Dx. ❐

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Competing interests

M.M. has filed a patent on the use of thermostable Cas13 for CRISPR diagnostic applications. A.G. declares no competing interests.