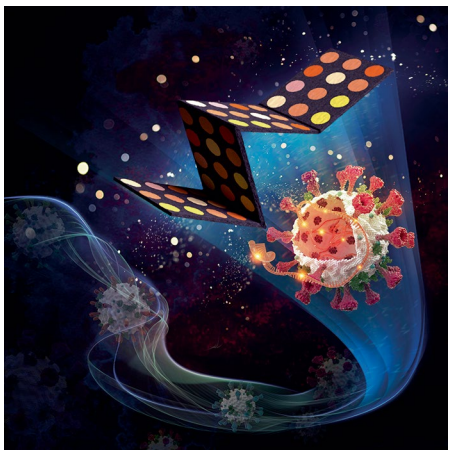
Straightforward, inexpensive and sensitive

Designing viral diagnostic tests for point-of-care use involves many trade-offs. Yet ease of use, low cost and accuracy shouldn't be compromised.

An ideal point-of-care test to check for infection (or for contagiousness) should be accurate, affordable and accessible. Accuracy involves high sensitivity and specificity for the infectious agents being tested for, and accessibility implies that the test is rapid, easy to use and available where it is most needed. These are the three main attributes of the ASSURED criteria, which were established by the World Health Organization special programme for research and training in tropical diseases in 2003 to help drive widespread access to point-of-care diagnostics. ASSURED stands for affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and deliverable to end-users. Two additional opportune criteria — real-time connectivity and ease of specimen collection — and the acronym REASSURED were proposed in 2019 (ref. [1]).

The realization of an ideal nearly always comes with substantial trade-offs. Accurate tests, in particular nucleic acid tests using gold-standard polymerase chain reaction (PCR) technology for nucleic acid amplification, involve expensive benchtop instrumentation, are not immediately or widely available and are run by trained experts. Accessible and affordable tests, such as over-the-counter tests for the detection of antigens, may be relatively inexpensive and easy to use, but are not as sensitive and may not be robust. And rapid tests for the detection of antibodies can be insufficiently specific.

The relevant trade-offs and how they inform the design and implementation of a point-of-care test depend on the intended use of the test and the conditions in which it is used. For example, for respiratory infections, an insufficiently sensitive yet cheap test may be sufficient to rapidly check for infectiousness (but not for an active infection), and can be supplemented with a more accurate and expensive confirmatory test. An assay that can simultaneously test for multiple viruses or viral strains may be more complex to operate and less affordable, yet suitable in the context of a hospital setting or a doctor's visit. And an easy-to-use and accurate test that requires equipment or reagents that need to be stored in refrigerators may not be sufficiently accessible for use in low-resource settings.



Credit: Ting Zhang and Chengyong Wu, Sichuan University

Yet, regardless of use settings, the design of diagnostic tests for truly widespread point-of-care use should not compromise on user-friendliness, affordability or accuracy. Tests that are slower at providing the result yet hardware-wise require only basic equipment are more likely to be useful and easier to adopt across healthcare and public-health settings, even if the tests are not as robust (as long as they implement an internal control), than fast and robust tests that are not as accurate or affordable or that require user training. Indeed, the utility of at-home lateral-flow antigen tests to determine when an individual infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) should self-isolate was unappreciated for too long[2]. Four papers included in this issue of *Nature Biomedical Engineering* provide solutions to some of the design and engineering challenges that stand in the way of adapting and simplifying assays to make them amenable to point-of-care use.

In one Article, Cameron Myhrvold and collaborators report how they adapted their previously published[3] Cas13-based assay for the detection of SARS-CoV-2 to make the assay easier to use and deploy. The assay does not need the extraction of RNA from a nasal sample, involves lyophilized reagents and the fast inactivation of ribonucleases at ambient temperature, can be run using body heat to catalyse the reactions, and the result can be read out on a strip of paper; hence, heating or cold-storage equipment and optical lenses and detectors are not strictly needed, making the assay fairly inexpensive. Moreover, the assay is substantially more accurate (100% specific and 90.5% sensitive when benchmarked against gold-standard PCR) than similarly rapid commercial antigen tests, and can be readily adapted for the discrimination of viral variants of concern. However, despite these capabilities and optimizations, the test does not meet the ASSURED criteria. As explained in an accompanying News and Views article by Ahmed Ghouneimy and Magdy Mahfouz, the assay involves manual liquid handling and five simple steps, and thus would need additional design optimizations to make it easier for an untrained person to run.

In another Article, Patrick Hsu, David Savage, Jennifer Doudna and colleagues also report a sensitive and rapid Cas13-based assay designed for point-of-care use that can detect multiple variants of SARS-CoV-2 in saliva samples and that doesn't need the extraction of RNA from the sample (but requires that it be denatured at high temperature). The assay incorporates an internal control, and leverages a microfluidic cartridge (single use, and driven by gravity) that automatically performs liquid-handling operations. Still, processing the sample before introducing it into the device involves two manual steps. Also, the need for custom equipment (the cartridge, and its housing device incorporating a compact fluorescence reader) is an obstacle to the affordability and deployability of the assay.

A third Article, authored by Jinghong Li, Weimin Li, Ruijie Deng and co-authors, reports a test that dispenses of nucleic acid amplification and that colorimetrically detects multiple specific RNAs simultaneously (as the authors show for various circulating strains of SARS-CoV-2) by leveraging strand-displacement reactions (via a DNA-toehold exchange probe) and the enzymatic amplification of the detection event (via the release of Ag(I) ions, which hydrolyse urea and change the pH and colour of the solution). The assay is fast (the result is obtained in 30 min) and sensitive, and the reaction steps are implemented on inexpensive origami paper (pictured) and run by folding it. As highlighted by Kaiyue Wu and Alexander Green in an accompanying News and Views article,

the assay combines the specificity of nucleic acid tests with assay times and costs that approach those of lateral-flow tests. However, the assay may not be adequately sensitive to detect infections early, and the actual execution of the paper-folding steps may not be sufficiently robust for widespread use.

The ASSURED criteria do not directly consider the point-of-care advantages of assays that integrate multiple tests. Donald Ingber, James Collins and colleagues argue, in an Article also included in this issue, that a multiplexed electrochemical assay on a chip may help to quantify the rates of SARS-CoV-2 reinfections and the waning of the protection levels provided by vaccination. The article describes the integration of simultaneous nucleic acid and antibody tests for SARS-CoV-2 RNA in saliva and for immunoglobulins in blood plasma. The assay runs within two hours and has single-molecule sensitivity, and the device extracts, concentrates and amplifies SARS-CoV-2 RNA from unprocessed saliva. To make it affordable and more accessible, the lab-on-a-chip would need to be reusable and robust, and fully integrate the electronics, pumping and readout systems.

Tests that meet all or most of the ASSURED criteria exist for the detection of antibody or antigen biomarkers of HIV, malaria, syphilis, tuberculosis and a few other infectious pathogens[1]. Inexpensive and accurate tests amenable to at-home use that detect infection by endemic respiratory viruses should follow. Yet tests that warn of exposure to the viruses[2] in real time and without user intervention, and thus before actual infection, would be truly reassuring. ❐

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