Rapid and frequent testing

Upcoming inexpensive assays for the detection of SARS-CoV-2 RNA in less than one hour at points of care or at home should help suppress the COVID-19 pandemic.

In an Editorial this past April, we advocated that the world needs to see the mass deployment of rapid and frequent point-of-care testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in those likely to be exposed to the virus¹. In the May Editorial, we argued that testing (largely carried out in central laboratories by quantitative real-time polymerase chain reaction (PCR)) may be boosted by sufficiently sensitive and rapid assays for viral antigens and by CRISPR-based tests for viral RNA². Today, the world still largely relies on laboratory SARS-CoV-2 PCR testing to help control the pandemic. Despite [impressive scale-up efforts](#) in the past nine months, the cost and few-days turnaround time of centralized PCR workflows precludes their use for frequent mass testing.

Yet rapid assays for testing at the point of care and for self-testing at home are coming to the market. On 17 November 2020, the first at-home kit for coronavirus disease 2019 (COVID-19) self-testing was [authorized](#) for emergency use by the United States Food and Drug Administration (FDA), but only for those who are suspected of having COVID-19 by their healthcare provider. The test, which relies on loop-mediated isothermal amplification and on halochromic compounds, comes as an [integrated device](#) for single-use, and provides a qualitative result (positive, negative or invalid) within 30 minutes of inserting a self-collected sample in the form of a nasal swab. Other than nucleic acid assays, the majority of FDA-authorized rapid tests for the detection of COVID-19 infection are [antigen tests](#)³, which are cheaper and deliver a qualitative result in 10–30 minutes⁴. Most of these tests are lateral-flow immunoassays, and are less sensitive than PCR (they have a limit of detection that is two orders of magnitude lower). Hence, they detect infectiousness better than infection — that is, their sensitivity is comparable to PCR during days 5–12 after the onset of infection, when viral loads in the nasal and buccal cavities are higher and hence when the person is most contagious. Currently, the FDA authorizes the use of these tests only in symptomatic people.

Detecting infectiousness quickly should be a priority, and at-home tests ought to be optimal for this job. Why? The proportion

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of asymptomatic COVID-19 cases has been estimated to be in the range of 4% to 41%⁵, about 60% of all infections originate from pre-symptomatic cases⁶, and approximately 80% of the new cases have been traced back to 15% of recently infected individuals⁶. Hence, rapid and frequent testing, and the self-isolation of infected individuals as soon as they know they can be infectious, should cut most COVID-19 transmission chains. This would be achievable via rapid and inexpensive tests amenable to home use, and by tests that cannot be easily shipped or operated at home but are portable and widely available at points of care. Fast results and frequent testing (every few days) would minimize the chances of asymptomatic transmission⁷ and the impact of false negatives in tests that are less sensitive than those carried out by PCR machines in centralized laboratories; rapid and

repeated testing would also help confirm positive tests, which would be most useful in places and populations where COVID-19 prevalence is low (because of a higher fraction of false positives). Recent pilot mass testing in Slovakia and in Liverpool, UK, will help determine how well this strategy works⁸.

This issue includes four reports of fast and portable COVID-19 assays that could, with further development and optimization, complement the use of nucleic acid tests and antigen tests. In their [Article](#), Chayasith Uttamapinant, Navin Horthongkham and colleagues describe the results of the validation of a two-step CRISPR-based test (the SHERLOCK assay, which uses isothermal amplification of the viral RNA and Cas13a-mediated detection), with fluorescence and lateral-flow readouts, in a hospital in Thailand. The assay was

96% sensitive (and 100% sensitive for reactions with over 42 copies of viral RNA, corresponding to a cycle threshold of approximately 33.5 in PCR assays) and 100% specific, and gave quantitative results in 35–70 minutes. Improvements to a one-pot version of the assay⁹, which has been [authorized](#) by the FDA for emergency use in clinical laboratories for individuals suspected of COVID-19, could make it suitable for wider point-of-care settings. For example, amplification-free Cas13a-based assays, with fluorescence quantified by a smartphone camera, are being developed¹⁰.

Jeong Wook Lee, Gyoo Yeol Jung and colleagues report in their [Article](#) the design and performance, with clinical samples, of a one-pot isothermal assay for the detection of SARS-CoV-2 RNA in 30–50 minutes. The assay involves the hybridization and ligation of promoter and reporter RNA probes, followed by the transcription and amplification of an RNA aptamer that binds to a fluorescent dye, and achieved positive and negative predictive values of 95% and 100% when tested with 40 samples (with half of them positive for SARS-CoV-2).

The assay should be amenable to paper and lateral-flow formats.

Immunoassays can also be used to detect nucleic acids. In their [Article](#), Daming Wang, Xiaohui Wang, Yuguo Tang and colleagues report how they used a monoclonal antibody, labelled with fluorescence nanoparticles, that binds to hybrid double-stranded chains of RNA and DNA. Implemented in a lateral-flow format and using DNA probes binding to conserved regions of SARS-CoV-2 RNA, the assay provided quantitative results in less than 1 hour, and had a sensitivity of 100% and a specificity of 99% when tested with 734 samples (throat swabs or sputum) collected from three hospitals.

PCR assay times don't need to last one or two hours. Jinwoo Cheon, Hakho Lee, Jae-Hyun Lee and colleagues describe in their [Article](#) how they sped up the thermocycling step by using plasmonic heating through nanoparticles that are then magnetically cleared from the tested sample. The researchers designed an integrated, automated and portable device (pictured) that can test three samples within 17 minutes, and that correctly classified all

150 clinical samples tested (nasopharyngeal swabs or sputum; 75 of them positive for SARS-CoV-2).

Analytical accuracy, robustness, reagent availability, cost, speed, ease of use and portability largely determine whether, where and how a diagnostic assay will be deployed and used. Upcoming technologies should rapidly and consistently deliver in all of these.

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