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Fast, portable tests come online to curb coronavirus pandemic

Testing kits delivered by courier and digital tools combine to battle the COVID-19 outbreak.

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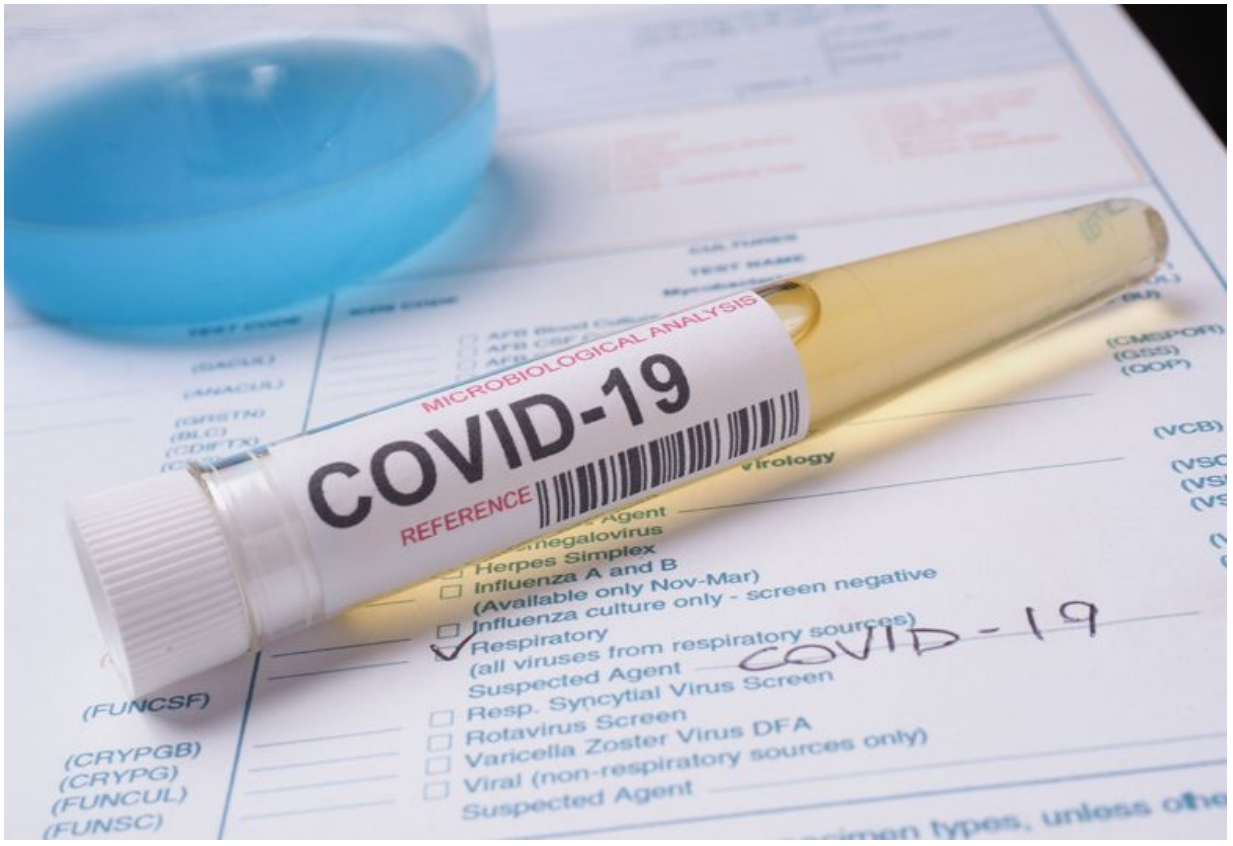
The rapid spread of COVID-19 across the world has exposed major gaps in the abilities of most countries to respond to a virulent new pathogen.



Health officials have so far used PCR testing in specialist facilities, but home tests would enable quicker quarantine and access to care. Claudio Rampinini / Alamy Stock Photo

The extraordinary success of Singapore, Taiwan and Hong Kong in limiting the impact of the sudden acute respiratory syndrome coronavirus-2 (SARS-CoV-2) demonstrates that it is possible to mount an effective response to an outbreak by major investment in pandemic preparedness. Despite their proximity to China, these three regions have managed to keep case numbers and fatalities low. By learning from previous coronavirus outbreaks where these territories bore the brunt, they were able to rapidly deploy widespread testing, combine it with digital surveillance to trace individuals' movements, and impose strict quarantines in suspect cases, in addition to building large stockpiles of personal protective equipment.

footed in validating, implementing and distributing SARS-CoV-2 diagnostic tests and in establishing decentralized point-of-care (POC) testing. Now that picture is starting to change, with the first shipments of such tests (Table 1). What's more, the present crisis could be a defining moment for diagnostics based on CRISPR technology — the first diagnostic test based on CRISPR gene editing technology may only be weeks away. To stop the virus spreading, however, will take a massive effort to scale up the production of easy-to-use POC tests and then to deploy them widely.

So far, the frontline response to the SARS-CoV-2 outbreak has been polymerase chain reaction (PCR) testing. PCR is the gold standard for diagnosing an infectious agent, and it has the advantage that the primers needed for such tests can be produced with relative speed as soon as the viral sequence is known. The first quantitative reverse-transcriptase-based PCR (RT-PCR) tests for detecting SARS-CoV2 were designed and distributed in January by the World Health Organization (WHO), soon after the virus was identified. The test protocol is complex and expensive, however, and is mainly suited to large, centralized diagnostic laboratories. Tests typically take 4–6 hours to complete, but the logistical requirement to ship clinical samples means the turnaround time is 24 hours at best. In the United States, the US Centers for Disease Controls opted to develop its own test rather than adopt the WHO test. Foot-dragging by the US Food and Drug Administration in authorizing public health or hospital labs to run the tests, a glitch in one of the reagents used in the initial RT-PCR test developed and distributed by the Centers for Disease Controls in early February, and a shortage of RNA extraction reagents has further delayed the rollout of testing nationwide.

Despite these shortcomings, multiplex RT-PCR, which tests for multiple target sequences simultaneously, will remain the backbone of testing, particularly in centralized laboratories. Large providers such as Roche Diagnostics, Thermo Fisher Scientific, Qiagen (soon to be acquired by Thermo Fisher) and Quest Diagnostics are ramping up the capacity to perform such tests by rolling out automated SARS-CoV-2 testing systems and services.

POC tests are needed as well to accelerate clinical decision-making and to take some of the workload off centralized test laboratories. Rapid POC tests for use at community level were identified by a WHO expert group convened in Geneva last month as the first of eight research priorities. The Foundation for Innovative New Diagnostics (FIND), a Geneva-based not-for-profit that supports the development and delivery of diagnostics to low-income countries, is aiding that broad effort by inviting assay developers to submit their products for independent evaluation. It has received 220 submissions, which are being assessed, and qualifying manufacturers will be contacted by the end of this month. FIND has also compiled an extensive list of SARS-CoV-2 diagnostic developers, which is dominated by companies developing PCR-based nucleic acid detection tests or immunoassays. The Saw Swee Hock School of Public Health at the National University of Singapore is also closely tracking diagnostics development, in both commercial companies and academic labs.

Immunoassays can provide historic information about viral exposure, as well as diagnostic evidence. They exploit antibody–antigen recognition, either by using monoclonal antibodies (mAbs) to detect viral antigens in clinical samples or by using cloned viral antigens to detect patient antibodies directed against the virus. The lateral flow assay format — essentially a dipstick encased in a cassette — contains the capture reagents (either an mAb directed at a viral antigen or a viral antigen that is recognized by patients' antibodies) immobilized at defined locations on a nitrocellulose membrane, as well as labelled detector mAbs that recognize the same target. A positive result, which is triggered by

Neither PCR nor immunoassay technologies are ideal, however. PCR tests are highly accurate and can be developed at speed, but they are complex to use and slow to deliver a result. Immunoassays are less accurate and take longer to develop, but they are easy to use and deliver results in 20–60 minutes. And because immunoassays detect patient antibodies to a pathogen, these inevitably must contend with the inherent variability of the polyclonal human antibody response. “That antibody response takes time to characterize,” says Ranga Sampath, chief scientific officer at FIND. Results obtained during validation testing, therefore, may not necessarily be replicated in clinical settings. “These tests tend not to be definitive — that’s the biggest challenge with immunoassays,” he says.

Even so, the speed and versatility of immunoassays make them invaluable tests; and efforts to produce them on a massive scale are beginning to ramp up. An-Suei Yang, research fellow at the Genomics Research Center, Academia Sinica, in Taipei, Taiwan, and colleagues claim to be first to develop a mAb against the nucleocapsid (N) protein of SARS-CoV-2, which could form the basis of a rapid antigen test. It has also generated additional antibodies that recognize both SARS-CoV-2 and the original SARS-CoV N proteins or SARS-CoV N protein only. None of the mAbs binds the nucleocapsid protein of other human coronavirus strains. “If successfully manufactured and validated, the rapid immune-based test kit could detect coronavirus antigen in only 20 minutes without instrumentation and trained personnel, with the cost and utility of a rapid influenza LFIA [lateral flow immunoassay] test,” he says. Yang’s group accomplished the feat in just 19 days, by working with artificial-intelligence models of antibody–antigen interactions, which they used to generate artificial antibody libraries. The predicted antibody DNA can be chemically synthesized and the resulting mAbs can be expressed on phage display systems in bacteria. Avoiding immunizing animals to obtain monoclonal antibodies, which then have to be sorted for binding activity — the traditional method — shaves about two months off the usual time frame. The group is now working on a prototype LFIA device but also wants to license the antibody panel to third parties.

For its immunoassay, Halifax, Nova Scotia-based Sona Nanotech has chosen the S1 domain of the SARS-CoV-2 spike protein as the basis of an antigen-based LFIA it is developing in cooperation with GE Healthcare Life Sciences. The spike (S) protein facilitates viral entry to airway epithelial cells by binding angiotensin-converting enzyme 2 (ACE2) expressed on their surface. “We anticipate making a research-use-only product available in the next six to eight weeks,” says CEO Darren Rowles. Beyond that, the company also plans to develop a dual product for the next northern hemisphere flu season, which would combine SARS-CoV-2 and influenza virus detection in a single test.

Tests to detect polyclonal antibodies against SARS-CoV-2 in patients are quicker to develop than tests to detect the virus itself, as they only incorporate immobilized recombinant viral antigen (which is much easier to generate than mAbs against the virus). They also offer similar levels of accuracy to mAb-based tests.

Berlin-based Pharmact has already started shipping a 20-minute immunoassay containing three SARS-CoV-2 antigens: the N protein and the S1 and S2 domains of the S protein. It is designed to detect any patient antibody that recognizes these protein structures. It aims to scale production to one million tests during the month of April. As is typical for such a test, it detects both immunoglobulin (Ig) M and IgG antibodies, which are released during the initial and later stages of an infection, respectively. It has conducted validation studies that compared its test with PCR, using samples from 114 infected patients and 126 uninfected controls. The test scored highly in terms of specificity. “We had a true negative rate of 100% — zero false positives,” says Gunther Burgard, medical director at Pharmact. Its sensitivity was lower,

false negative rate of 13%, Burgard says.

Integrating decentralized testing with epidemiological surveillance would further strengthen the hand of public health authorities battling the SARS-CoV-2 outbreak. The Seattle Flu Study, led by the University of Washington, is adapting to the present crisis an innovative approach to tracking seasonal flu outbreaks on a city-wide scale. Participants who develop respiratory symptoms can electronically request a nasal swab kit, which is delivered by courier. Recruitment, consent and data collection are all performed electronically, but the protocol relies on centralized laboratory testing rather than a POC test. In Finland, a study showed that it was possible to obtain real-time epidemiological surveillance by integrating automated POC testing with laboratory information systems. The POC system, mariPOC Respi, developed by Turku, Finland-based ArcDia, now transmits data through the cloud. It currently covers eleven respiratory pathogens, including ten viruses (among them coronavirus OC43, one of three human coronaviruses that cause only a mild illness) and one bacterial species. The company now plans to include SARS-CoV-2 in their panel within a couple of months. The mariPOC Respi system is not a high-throughput device: it relies on a benchtop analyzer, which can process about one hundred samples per day, but reagent costs are about one-third of those associated with PCR testing. It is pitched at community settings, such as large primary care centers. Although it has a 50% market share in Finland, uptake elsewhere has been limited so far. “Core labs prefer multiplex PCR,” says Aleksi Soini, senior vice president, strategy and business development, at ArcDia. The heads of these facilities are the opinion leaders in diagnostics — and are reluctant to relinquish their power to decentralized testing regimes. “It’s a structural issue,” Soini says. Scanwell Health is gearing up to launch an even more decentralized test — a home-delivered immunoassay and an accompanying smartphone app, which will allow users to share the result with a doctor or nurse practitioner. It has obtained US rights to Tangshan, China-based Innovita Biological Technology’s antibody-based immunoassay (Table 1).

PCR is, of course, based on science that is now several decades old. Mammoth Biosciences and Sherlock Biosciences, companies cofounded by CRISPR pioneers Jennifer Doudna and Feng Zhang, respectively, both aim to match its accuracy while achieving genuine POC testing speeds by employing the specificity and sensitivity of CRISPR–Cas editing to develop rapid POC SARS-CoV-2 tests. Mammoth scientists, along with collaborators from the University of California, San Francisco, and the California Department of Public Health, recently reported on the design and performance of its SARS-CoV-2 DETECTR, a lateral flow assay that takes 30 minutes to perform — or about 45 minutes including an RNA extraction step. The test employs simultaneous reverse transcription of the viral RNA and loop-mediated amplification, a simplified nucleic acid amplification technique that uses PCR-style primers but that does not require repeated cycles of heating and cooling. The amplified nucleic acid is then incubated with a guide RNA (gRNA) molecule targeting sequences in the envelope (E) and N genes of SARS-CoV-2 and the lbCas12a enzyme, which, after gRNA-mediated target-site recognition, cleaves nearby single-stranded DNA (ssDNA) indiscriminately. This allows the addition of ssDNA-based reporter molecules that confirm the presence of viral RNA.

According to initial validation testing against RT-PCR using samples from 6 patients infected with SARS-CoV-2, from 12 patients infected with seasonal influenza or the three coronavirus strains that cause mild symptoms (OC43, HKU1 and NL63), and from 5 healthy volunteers, the test had a positive prediction value of 100% and a negative prediction value of 91.7%. “We’re right in the middle of additional validation,” says Mammoth cofounder and CEO Trevor Martin. The company aims to ship the test as quickly as possible. “In an ideal scenario, it’s weeks, not months,” he says. It was purposely designed with simplified reagents to allow easy manufacturing. “There’s nothing too exotic in what you’re

Sherlock Biosciences is also working on several SARS-CoV-2 initiatives. “It’s one thing to have a rapidly deployed technology,” says Sherlock CEO and cofounder Rahul Dhanda, but “to get an assay to market requires a lot more than getting the assay working.” The company aims to tap into partners to gain access to their regulatory expertise and manufacturing and distribution muscle. It has formed an alliance with Cepheid to develop CRISPR-based tests for a range of infectious diseases, including COVID-19, which will run on the latter firm’s GeneXpert automated molecular diagnostic systems. The relationship was already under discussion before the present crisis broke, but it was an obvious step to include SARS-CoV-2. Given the urgency of the present crisis, Sherlock is also investigating whether it can bring a simpler test to market in a shorter time frame. “We started to engage with kit manufacturers to determine whether or not we could assemble a kit,” Dhanda says. “There is the acute response and the comprehensive response we all need to be thinking of.”

Given the uncertainties attached to the trajectory of the present pandemic, it is unclear whether CRISPR technology will make a major contribution to global efforts to track the progress of SARS-CoV-2. But the need for such a technology appears pressing. “This has exposed a big gap in the molecular diagnostics market,” says Martin. It is just one of the many gaps in most countries’ levels of preparedness for a viral pandemic.

TABLE 1 SELECTED COMMERCIAL RAPID TESTS FOR SARS-COV-2

Developer	Test	Description	Status
Guangzhou Wondfo Biotech (Guangzhou, China)	Wondfo SARS-CoV-2 antibody test	Lateral flow 15-minute immunoassay that detects IgM and IgG antibodies directed against SARS-CoV-2	National Medical Products Administration EUA in China; CE mark in Europe
Innovita Biological Technology	SARS-CoV-2 antibody assay	Lateral flow 15-minute immunoassay that detects IgM and IgG antibodies directed against SARS-CoV-2	National Medical Products Administration EUA in China
Jiangsu Medomics Medical Technologies (Nanjing, China)	SARS-CoV-2 rapid combined IgM/IgG antibody test kit	Lateral flow 15-minute immunoassay that detects IgM and IgG antibodies directed against SARS-CoV-2	Shipping
Mammoth Biosciences	SARS-CoV-2 DETECTR	30-minute lateral flow assay	In validation studies
Pharmact (Berlin)	SARS-COV-2 Rapid Test	POC 20-minute test for detecting SARS-CoV-2 exposure through identification of IgG and IgM antibodies	CE-marked and shipping
Snibe Diagnostic (Shenzhen, China)	MAGLUMI 2019-nCoV IgM/IgG kit	Automated central laboratory rapid test that runs on MAGLUMI chemiluminescence immunoassay system	CE mark received 19 February 2020

Sherlock Biosciences, Cepheid	Rapid CRISPR-based tests for SARS-CoV-2 and other pathogens	Combines SHERLOCK Cas12 and Cas13 enzymes for nucleic acid detection with Cepheid's GeneXpert test-processing instruments	Intended as proof of concept for a broad product development alliance in infectious disease
Zhejiang Orient Gene Biotech (Zhejiang, China)	COVID-19 IgG/IgM Rapid Test	Solid-phase immunochromatographic assay	Aytu Bioscience has sublicensed US distribution rights from L.B. Resources (Hong Kong) and plans to obtain EUA; already has CE mark
Biomerica	Rapid POC IgM/IgG antibody test	$10 lateral flow immunoassay	Commenced shipping samples; seeking FDA EUA approval
Caspr Biotech	Ultrasensitive, rapid, and portable coronavirus SARS-CoV-2 sequence detection	Based on CRISPR-Cas12	Proof of principle evaluation
Sugentech (Daejeon, South Korea)	SGTi-flex COVID-19 IgM/IgG	Ten-minute lateral flow immunoassay that detects IgM and IgG antibodies directed against SARS-CoV-2	CE Mark
Cepheid	Xpert Xpress SARS-CoV-2	Rapid PCR test that runs on GenXpert benchtop system – delivers result in two hours from sample collection to delivery of result	Received FDA emergency use authorization
Xiamen AmonMed Biotechnology (Fujian, China),	COVID-19 IgM/IgG test kit (Colloidal gold)	Ten-minute lateral flow immunoassay that detects IgM and IgG antibodies directed against SARS-CoV-2	CE Mark

EUA, emergency use approval. Sources: Company websites, PubMed.

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