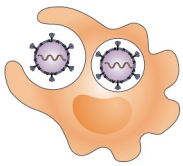
IN this section



Vaccine makers on alert for ADE
p773

Biotech news from around the globe
p775

Bacterial concrete self-repairs cracks
p776

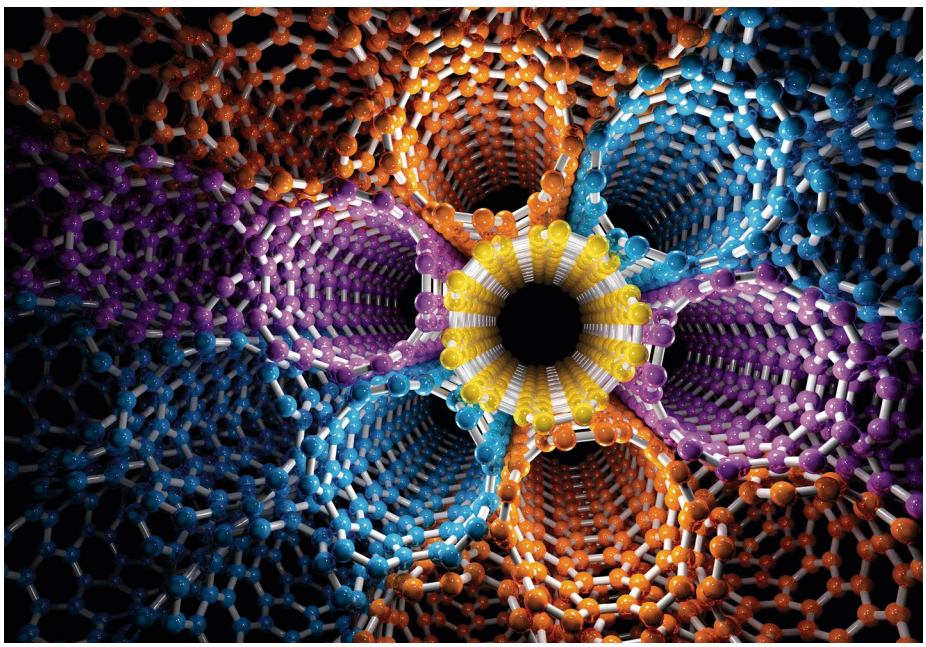
COVID-19 spurs wave of innovative diagnostics

Coronavirus is instigating new thinking in diagnostics, such as face masks that can detect viruses on a wearer's breath or paper-based microfluidics for pathogen identification in low-resource settings.

The FDA's historic approval of the first CRISPR-based diagnostic product, Sherlock Biosciences' one-hour test for SARS-CoV-2, and Mammoth Biosciences' recent deal with GlaxoSmithKline to develop a 20-minute CRISPR-based test for the same virus, highlights new thinking in pathogen identification technology. By brutally exposing the shortcomings of existing diagnostic technologies and clinical testing infrastructure, COVID-19 is supercharging R&D efforts to establish novel testing approaches that are faster, simpler, cheaper and more scalable than existing methods, and just as accurate.

Some radical ideas are in development: a face mask with embedded biosensors that can detect the presence of virus on the wearer's breath may sound like a science-fiction fantasy but could soon become a reality. Other devices under exploration use electronic or optical readouts to detect viruses within minutes from any type of sample, and yet others engineer sophisticated micro- and nanofluidic systems onto low-cost substrates, such as silicon or even paper. The idea is to bring laboratory-like precision to use at the bedside, at home or in low-resource settings. The continuing uncertainty about the trajectory of the pandemic — and whether or not a second wave of infection will erupt this summer — has imparted an extraordinary urgency to these initiatives (Table 1). Systems biology, nanobiotechnology, biophotonics and nanofluidic engineering are just some of the disciplines feeding into this broad effort.

To fund this work, the US Congress has more than doubled the annual budget of the US National Institute of Biomedical Imaging and Bioengineering (NIBIB), with an additional $500 million. At the same time, the Defense Advanced Research Projects Agency (DARPA) will fund two ambitious projects using CRISPR to deliver low-cost, high-volume point-of-care tests. The CARMEN-Cas13 platform, developed by Pardis Sabeti of Harvard University (who is a Sherlock Biosciences cofounder) and Paul Blainey of the Broad Institute, which DARPA previously funded, has paved the way. The multiplexed test, which is conducted in a mobile-phone-sized array containing 177,840 microwells, is an extension of the Sherlock assay. It was originally conceived to detect simultaneously 169 human viral pathogens for which at least ten genome sequences were available The group recently added CRISPR RNA (crRNA) sequences for detecting the 170th, SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). "That work broke open the door in terms of what is possible," says Renee Wegrzyn, program manager at DARPA.



Diagnostic tests for COVID-19 could soon include carbon nanotubes. Credit: Forance / Alamy Stock Photo

All the test samples and detection reagents are pooled and introduced to the array in a single pipetting step, as a pair of color-coded, emulsified one-nanoliter droplets. Each discrete droplet contains a preamplified sample of viral RNA or detection reagents, including Cas13 (CRISPR-associated protein 13), crRNA sequences directed at a viral target, and a reporter RNA that generates a fluorescent signal when target recognition occurs. The large number of reaction wells allows every possible pairwise combination to occur several times over. Detection reactions start once the array is exposed to an electric field, which causes the droplets in each microwell to merge. The platform attained attomolar sensitivity for Zika virus.

Table 1 | Selected SARS-CoV-2 advanced diagnostics in development

Device	Developer	Analyte / Sample	Detection method	Platform	Status
CARMEN-Cas13a (Combinatorial Arrayed Reactions for Multiplexed Evaluation of Nucleic Acids)	Broad Institute, Harvard University	Amplified nucleic acid / Plasma, nasal or throat swabs	Color-coded droplets of samples are each paired randomly in arrayed microwells with color-coded droplets of CRISPR detection reagents, including quenched fluorescent RNA reporters; detection of target nucleic acid results in Cas13a activation, collateral cleavage of the reporter RNA species and the generation of a fluorescent signal, with a time to result of <8 hours	Microarray chip that contains 177,840 microwells and supports >4,500 statistically robust tests of crRNA:target pairs	Conceived for all 169 human viruses for which ≥10 genomes are available; crRNA specific for SARS-CoV-2 recently added
CRISPR-Chip	Cardea Bio	Unamplified nucleic acid / Buccal swab	Label-free electrical detection of a binding event between the target sequence and an immobilized, catalytically deactivated Cas9 enzyme complexed with a target-specific gRNA	gFET connected to portable digital reader	Demonstrated for detecting disease-associated mutations in purified whole-genome samples in <15 minutes; in development for SARS-CoV-2 detection
VIRRION (virus capture with rapid Raman spectroscopy detection and identification)	Pennsylvania State University	Whole virus / Nasopharyngeal swabs; exhaled breath version in development	Carbon-nanotube-based array for rapid size-based enrichment of viruses present in a sample coupled with label-free, non-destructive optical detection using Raman spectroscopy	Chip containing nitrogen-doped carbon nanotube arrays decorated with gold nanoparticles to enhance Raman spectroscopy signal	Validated with avian influenza A virus subtypes and with samples from humans with respiratory infections; in development for SARS-CoV-2 detection
CRISPR-Cas13-based electrochemical microfluidic sensor	University of Freiburg, Germany	Unamplified nucleic acid / Serum	CRISPR-Cas13-based detection of RNA that exploits non-specific collateral cleavage activity of Cas13 for post-recognition signal amplification through a reporter RNA species; uncleaved reporter RNA is recognized by antibodies bound to glucose oxidase, which produces hydrogen peroxide, which in turn is detected by current changes in an electrochemical cell	Dry film photoresist layers stacked on a polyimide substrate containing an electrochemical cell for measuring hydrogen peroxide produced in inverse proportion to the amount of target analyte in the sample	Demonstrated for detecting microRNAs at low concentration in serum; in development for SARS-CoV-2 detection
Convat optical biosensor	Catalan Institute of Nanoscience and Nanotechnology (Barcelona, Spain) and collaborators	Antigen in point-of-care test format and unamplified nucleic acid in multiplexed format / Nasal or saliva swabs	Bimodal waveguide interferometry; detects interference occurring between two modes of a single light wave as it interacts with an analyte bound to a sensing element, such as an antibody or a complementary nucleic acid strand	All instrumentation to be integrated into a portable 25 × 15 × 25 cm box under tablet control	Originally developed to detect nosocomial bacterial pathogens, now being adapted for SARS-CoV-2 detection in 30 minutes
Dual functional plasmonic photothermal biosensor	Swiss Federal Laboratories for Material Science and Technology, Swiss Federal Institute of Technology in Zurich (ETH Zürich)	Unamplified label-free nucleic acid / Bioaerosol	Optical detection in 6–10 minutes of viral RNA hybridization with complementary DNA sequences immobilized on gold nanoparticles, employing localized surface plasmon resonance and plasmonic photothermal heating	Glass surface supporting gold nanoislands functionalized with complementary DNA sequences	Demonstrated for SARS-CoV-2 detection using manual input of synthetic samples; version that detects virus in bioaerosols in development
FET biosensor	Korea Basic Science Institute (Cheongju)	Antigen requiring no sample pretreatment / Nasopharyngeal swabs	Label-free, real-time electrical detection of viral antigen binding graphene-based FET functionalized with antibody; 100 femtograms per milliliter limit of detection	gFET linked to a semiconductor analyzer	Demonstrated for SARS-CoV-2 detection

Continued

Table 1 | Selected SARS-CoV-2 advanced diagnostics in development (Continued)

Device	Developer	Analyte / Sample	Detection method	Platform	Status
FemtoSpot COVID-19 Rapid Detection Test	Nano DiagnosiX	Antiviral immunoglobulin G and M antibodies / One drop of untreated blood	Patient-operated serological test that uses electronic amplification to detect antibodies or disease biomarkers at low concentrations	Change in conductivity of a nanoribbon-based FET	Originally developed for detection of cardiac biomarkers; COVID-19 test in development
COVID-19 biosensor	University of Utah	Viral antigen / Saliva	Rapid one-minute test using surface-immobilized oligonucleotide aptamers to bind viral antigen	Change in electrical resistance	Originally developed for Zika virus detection; COVID-19 test in development
One-step COVID-19 test	Northwestern University, Stemloop	Viral nucleic acid / Nasal or saliva swab; environmental samples	Uses primer-free CRISPR isothermal amplification for one-pot amplification and detection of nucleic acid at ambient temperature with attomolar sensitivity	Fluorescence read-out in less than one hour	Originally developed for detecting environmental pollutants; COVID-19 test in development

Sources: PubMed, organization websites, NSF.gov, European Commission

The next wave in diagnostics will necessarily emerge from the intersection of physical and engineering sciences and biology. The NIBIB is open to proposals that reinvent every step in a diagnostic test, from alternative sampling strategies — such as saliva or exhaled breath — to novel analytical approaches and chemistries, and integration with mobile devices. "Fundamentally, RADx is designed to get out some winners that come as a complete package," says Bruce Tromberg, NIBIB director. In contrast, the 90-odd diagnostic tests for SARS-CoV-2 that have already received FDA Emergency Use Authorizations represent incremental additions to standard technologies. "They're largely iterative — there's nothing breakthrough," he says.

RADx winners will be supported all the way through clinical validation and manufacturing scale-up. The aim is to have millions of tests shipping by the fall. "We now have a strong evolutionary pressure to drive innovation and advances," Tromberg says. The NIBIB is open both to new technologies, such as CRISPR, and to existing technologies reconfigured for use in different contexts. "CRISPR is cool, because it's CRISPR," Tromberg says. "It's also cool to take a real-time PCR lab instrument, hit it with a shrink gun and have a modular, microfluidic device with laser-based detection," he says. "None of the technologies we have are fixed."

At the moment, diagnostics fall into one of two camps: those based on polymerase chain reaction (PCR) to recognize nucleic acids, which are mostly performed in centralized labs, and those that detect antibodies using immunoassays on devices. There are drawbacks to both. Even the most advanced CRISPR-based tests — those from Sherlock and Mammoth — still rely on nucleic acid extraction and amplification and have been limited to laboratory settings initially. By contrast, biosensor technologies for SARS-CoV-2 that couple molecular recognition to electrical or optical detection are free from these constraints. They generally do not require extensive sample preparation or analyte amplification, so they will enable rapid in situ detection of viruses in, for example, air handling units or on surfaces in hospital rooms. "We have so many potential measurement demands in the ongoing pandemic," says Blake Johnson, an assistant professor at Virginia Tech. Biosensors typically rely on the physicochemical properties of their substrate — such as their electrical or optical characteristics — to detect the binding of an analyte to an immobilized recognition element and on their transduction properties to convert the resulting signal into a measurable output. "These transducers can be highly sensitive to the detection of the molecule," he says.

Cardea Bio employs graphene, an allotrope of carbon that forms a hexagonal lattice consisting of a single atomic layer, as the transducer in its chip-based implementation of CRISPR. "Graphene is super, super sensitive to interactions with charged molecules," says Cardea Bio cofounder and CSO Kiana Aran. Her group functionalized a graphene field-effect transistor (gFET) — a standard electronic signaling component — by immobilizing on its surface a catalytically deactivated Cas9 enzyme and an appropriate guide RNA (gRNA). When the target sequence is present in a test, it modulates the device's electrical characteristics and generates an electrical signal. The group originally developed the chip to detect genetic mutations that cause Duchenne muscular dystrophy. "With minimal optimization, we were detecting single point mutations," Aran says. They did so at a concentration of 1.7 femtomolar. The company, which is collaborating with Lithuanian CRISPR pioneer Virginijus Šikšnys, is now adapting the technology for SARS-CoV-2 detection — but only in the context of multianalyte detection, says CEO Michael Heltzen.

Can Dincer and colleagues at the University of Freiburg in Germany have also implemented amplification-free CRISPR-based nucleic-acid recognition on a chip. This method exploits the non-specific collateral cleavage reaction that occurs after Cas13a binds its target sequence On binding its target, Cas13a mediates the cleavage of a labeled reporter RNA sequence; its absence results in no cleavage. The labeled reporter RNA is linked to an antibody, which is in turn coupled to glucose peroxidase. When glucose is introduced into the microfluidic electrochemical cell, this results in hydrogen peroxide production, which offers an indirect measure of the amount of target RNA present. "The detection itself is electrical," says Dincer. "Our transduction is electrochemical."

The physical scale of individual virus particles offers an alternative approach to analyzing samples without the need for preparatory steps. A group led by Mauricio Terrones at Pennsylvania State University has combined nanoscale filtration with Raman spectroscopy for virus capture and identification from clinical samples. It employs arrays of carbon nanotubes, which Terrones likens to a tightly packed forest, for size-based capture of viral

particles. "If the size of the virus is of the order of the spaces between the trees, it will be trapped," he says. Virus enrichment takes a matter of minutes, and the sample is then analyzed by Raman spectroscopy, a non-destructive technique that builds a footprint of a given structure by probing its interactions with photons from an incident laser. New isolates are compared, using a machine learning algorithm, with existing profiles stored on a remote database. The group has identified, with up to 90% accuracy, several respiratory viruses and has more recently also identified several animal coronaviruses as it awaits access to a biosafety level 3 (BSL3) facility to conduct experiments with SARS-CoV-2. Its sensitivity is several hundred times greater than that of PCR, says Terrones. "We're trying to improve the signal-to-noise ratio, but it looks like it will work for COVID-19," he says. Its system, called VIRRION, can be deployed in handheld units, which could be used in airports and busy train stations, for example. "You can test people in almost any place," he says. Discussions with prospective manufacturing partners are underway.

Even if developers of next-generation diagnostics succeed in overcoming numerous technological barriers, they will also need to overcome market inertia. The present crisis has resulted in a massive scale-up of the incumbent technology, centralized PCR testing, as countries struggle to keep pace with the spread of the virus. "What I see is that the current paradigm is only being reinforced," says Nicolas Vergauwe, CEO of Leuven, Belgium-based miDiagnostics. In its present form, however, the technology has proven inadequate to the task because of the complexity of the PCR testing and its slow turnaround time. "It's based on technology from the nineties, essentially," says Vergauwe. The current generation of instruments relies on mechanical components for fluid handling and needs access to an external power source for heating and other operations. MiDiagnostics, a spin-out from IMEC, a Leuven-based nanoelectronics research center, is developing a silicon chip with complex nanofluidics that relies on capillary forces to run multianalyte diagnostic workflows and that has an embedded heater for controlling reaction temperatures. "It requires very little power and does not require any heater," he says.

Paper substrates represent an even simpler option for low-resource settings. Jim Collins of the Massachusetts Institute of Technology is working on paper-based microfluidics tests, which would support at-home detection of either viral nucleic acid, through CRISPR recognition, or of antiviral antibodies. Collins and co-workers previously devised a portable lab-in-a-box system that not only is low cost, as it relies on paper, but is also independent of the PCR reagent supply chain. The system uses toehold switch sensors — programmable hairpin RNA structures that prevent translation of a reporter gene unless they detect complementary nucleic acid sequences — to analyze samples on a freeze-dried, paper-based protein expression system. The approach does require nucleic acid amplification, but it relies on an isothermal RNA amplification method instead of PCR. Former lab member Keith Pardee, now at the University of Toronto, participated in a recently completed field trial in Brazil, Colombia and Ecuador, which clinically validated the platform for Zika, dengue and chikungunya viruses, and he is extending the approach to SARS-CoV-2 detection. Collins, another Sherlock cofounder, now favors CRISPR-based detection, given the success of the Sherlock test in delivering results with "excellent sensitivity" in less than an hour. Porting that same concept to a similar paper-based substrate is challenging, he says, but the group is making progress. "Within the next few weeks our goal is to have proof of concept and a pre-prototype, if not prototype, demoed in our academic lab," he says.

Collins is also pursuing concept of a biosensing face mask, although this work is not as advanced, he says. Can Dincer is scoping out this area too, having last year published work from a collaboration with Firat Güder at Imperial College London on a paper-based electrochemical system for sensing hydrogen peroxide in human breath. "To detect COVID-19, we can use, for example, antibodies against the viral surface proteins that are immobilized on the electrode surface," he says.

The big trade-off in diagnostics at present is between accuracy and throughput on the one hand and rapidity and simplicity on the other. Slashing the turnaround times of highly accurate molecular diagnostics and boosting the reliability of rapid tests are both important goals. Inevitably, only a fraction of the current diagnostic pipeline will result in successful products. But rapid adoption of innovations that can strengthen the present system will be crucial as countries start to reopen their economies. Equally essential is sustained R&D funding for new diagnostic technologies. Chronic underinvestment in the field has been tragically fatal for many — and ruinous for many more. ❐

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