XAVIER GALIANA/AFP VIA GETTY

Viral diagnostics often rely on a nasal-swab sample, and researchers are developing faster, simpler and cheaper methods of testing.

TESTING TIMES

Researchers are scrambling to find new ways to diagnose the coronavirus and churn out millions of tests a week — a key step in returning to relative normality. **By Giorgia Guglielmi**

The timing couldn't have been worse. In March, just as Thailand's coronavirus outbreak began to ramp up, three hospitals in Bangkok announced that they had suspended testing for the virus because they had run out of reagents. Thai researchers rushed to help the country's clinical laboratories meet the demand. Looking for affordable and easy-to-use tests, systems biologist Chayasith (Tao) Uttamapinant at the Vidyasirimedhi Institute of Science and Technology in Rayong reached out to an old acquaintance: CRISPR co-discoverer Feng Zhang, who had been developing an assay for the coronavirus inspired by the gene-editing technology.

Within days, Uttamapinant received starter kits from Zhang's lab at the Broad Institute of MIT and Harvard in Cambridge, Massachusetts, and tested them on samples from a hospital in Bangkok. "The kits are quite cheap and work well," says Uttamapinant, who hopes to get the test approved for clinical use by the end of the year. He has teamed up with biochemists in Thailand to produce the testing reagents locally, with Zhang on standby for support. "This effort to produce everything locally will have a lasting impact on infectious-disease monitoring and diagnosis in this part of the globe," says Uttamapinant.

Epidemiologists say mass testing for SARS-CoV-2 — requiring millions of tests per country per week — is the most practical way out of the current crisis. It allows officials to isolate those who test positive, limit the spread of disease and help to determine when it is safe to relax restrictions.

But countries are struggling to ramp up testing. One reason is that the standard test to detect SARS-CoV-2 — based on a mainstay lab technique called the reverse-transcription

polymerase chain reaction, or RT-PCR – requires trained personnel, specific chemical supplies and expensive instruments that take hours to provide results and are often available only in labs that provide routine, centralized services. This limits the number of tests that can be done, especially in developing countries. Even in wealthy regions such as the United States, providers have reported a severe shortage of test kits and required materials – from nose swabs to chemical reagents – because of supply-chain problems. Scaling up reliable tests quickly has proved challenging, too: early RT-PCR tests developed by the US Centers for Disease Control and Prevention malfunctioned, for example, leading to a series of delays.

Research groups around the world are now devising tests that go beyond PCR. Dozens of diagnostic methods are in development, all of which detect viral material but in different ways: some are tweaks for RT-PCR that make the test faster or easier to use; others use the gene-editing tool CRISPR to home in on genetic snippets of SARS-CoV-2; and some identify the virus using proteins that sit on its surface. Many of these tests, such as Zhang's, are being validated using clinical samples, and some are already in the clinic. In April, the US National Institutes of Health earmarked US$1.5 billion for coronavirus-test development, aiming to enable millions of tests per week by the end of this summer. "The sooner we can come up with a solution," Zhang says, "the sooner we can resume some form of normalcy."

The most promising way to perform large numbers of tests, says Mitchell O'Connell, a biochemist at the University of Rochester in New York, will be to use a mix of methods that rely on different instruments and supply chains so that a sudden worldwide demand won't deplete any key materials. "Any new technology that is able to expand the number of tests that we can do is good news," he says.

If those tests are ready soon, it would be good news for the current pandemic and for future outbreaks. Many of the assays in development could be readily adapted to an emerging pathogen once its genetic sequence is decoded, says Isabella Eckerle, a virologist at the University of Geneva in Switzerland. Eckerle says that, even though the ideal test doesn't yet exist – one that is accurate, rapid, inexpensive, and easy to use and scale up – "there are many things in the pipeline that could be useful."

Beyond PCR

Tests for the coronavirus fall into two broad categories: those that detect genetic material from the virus or molecules on its surface, which are used to diagnose whether a person has an active infection, and those that pick up on the presence of antibodies, revealing whether someone has been infected and has developed an immune response to the virus. Antibody tests have limited diagnostic use: if a person is tested early in the course of infection, when their immune response is still building up, the test might not detect antibodies. And because people with the coronavirus are most infectious at the onset of symptoms, tests for viral material are crucial to identify who should be in isolation. In the United States, viral diagnostic assays account for the majority of tests conducted.

The gold-standard diagnostic test, which uses RT-PCR, works by searching a sample taken from cells or fluid in a person's nose or throat for a specific genetic sequence from SARS-CoV-2. If the viral sequence is found, the technique amplifies it to levels that can be detected (see 'Hunting for the virus: the gold standard'). First, the virus RNA is converted to DNA. Then, short designed DNA sequences known as primers perform several jobs. Some tag specific sections of the viral genetic code to help duplicate the sequence millions of times, using a process that requires repeated heating and cooling. This amplification makes it easy to detect even minuscule quantities of virus, down to just one molecule of RNA per microlitre. Other primers add labels to the amplified DNA strands. These labels release a fluorescent signal that is measured by a computer, flagging the presence of the virus. Standard RT-PCR tests for the coronavirus take between one and four hours and can be up to 100% accurate – although the accuracy of any diagnostic test depends on many factors, such as when in the course of infection a sample was taken.

> **"The ultimate goal is to take diagnostics directly to the consumers."**

Various approaches aim to reduce the time taken to get a test result, such as by amplifying the DNA at a constant temperature, which eliminates the need for multiple rounds of heating and cooling. Some of these are existing assays that are being customized to detect SARS-CoV-2. For example, US healthcare companies Cepheid and Abbott have developed coronavirus assays that run on toaster-sized hardware platforms and take less than one hour to perform. However, reagents and platforms can be expensive, and Abbott has warned that using a particular solution to dilute patients' samples can stop its device from detecting the virus.

Several other tests are based on a technique called loop-mediated isothermal amplification (LAMP), which also works at a constant temperature and has been used to identify viruses such as Zika. LAMP relies on two enzymes – one to convert the viral RNA to DNA, and another to copy DNA – as well as a set of four to six short primers designed to recognize different snippets of the viral genome. These fragments not only help to get the copying started, as in RT-PCR, but also allow newly copied DNA strands to form looped structures that can be amplified much more rapidly than in standard PCR (see 'Loop the loop'). It is less accurate, however, and only a few dozen samples can be run at a time.

Because the technique doesn't need special instruments, it can be used in the field and in regions that lack advanced equipment, including remote areas and refugee camps, says Vicent Pelechano, a genomics expert at the Karolinska Institute in Stockholm, who co-developed a LAMP-based assay for SARS-CoV-2. "All you need is a test tube containing the primers, a pipette, a hotplate and a pot of water," he says. A single test would cost about $1 – not counting labour.

In the lab, Pelechano and colleagues' LAMP-based test could detect as few as 10 copies of a SARS-CoV-2 genome in no longer than 40 minutes[1]. The researchers then tested the assay using samples from 248 people with confirmed coronavirus infection, and could detect the virus nearly 90% of the time[2]. Pelechano acknowledges that the test might turn out to be less accurate for some samples, such as those contaminated with blood.

But in some places, the trade-off in accuracy could be worth it. Low-income countries and war-torn areas don't have enough PCR machines to perform the standard diagnostic test for coronavirus, says Nabil Karah, a clinical microbiologist at Umeå University in Sweden. Karah is working with other scientists and with Pelechano's team to bring their LAMP-based test to Syria to increase local testing capacity.

Accelerating assays

In early March, as diagnostics struggled to keep up with the spread of coronavirus across the United States, chemical engineer Howard Salis felt compelled to help. To speed up testing, he decided to try a powerful sequencing approach that had revolutionized the pace of genomics research. About three weeks later, Salis's team of synthetic biologists at Pennsylvania State University in University Park came up with a way to test samples from nearly 20,000 people in one run (go.nature.com/2vzksvk).

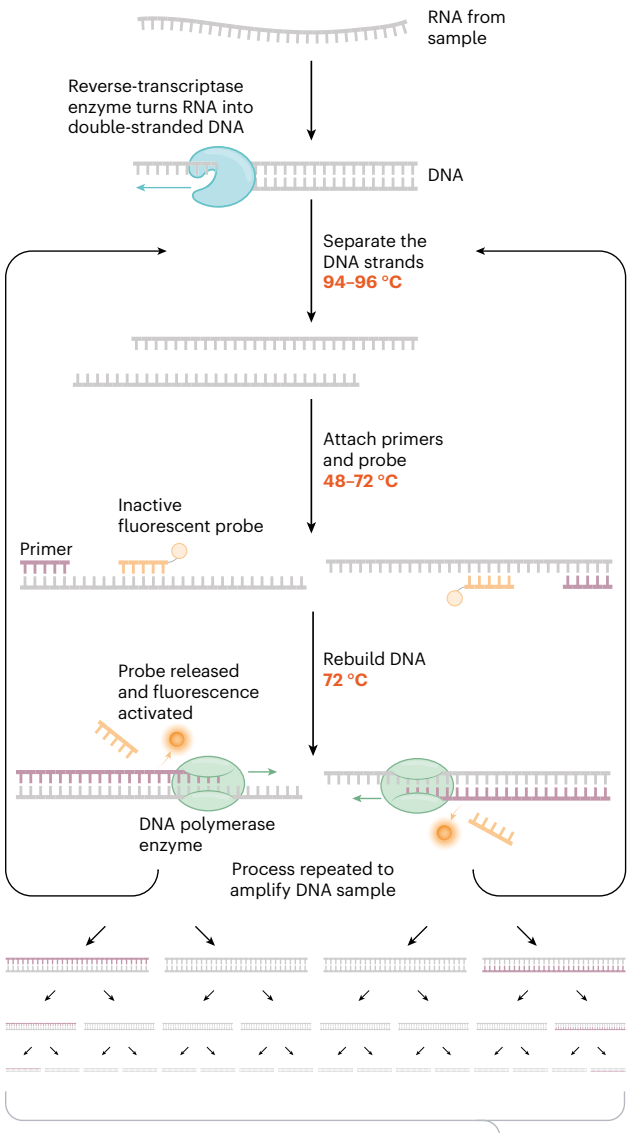
Their method adds individual 'molecular barcodes' to clinical samples before pooling them and using next-generation sequencing to decode them all at once. The barcodes then allow the researchers to identify which samples tested positive. Other teams have released details of similar mass-testing approaches, including the biotechnology start-up firm Octant in Emeryville, California (go.nature.com/3ggkodx), and researchers at the Broad Institute[3].

HUNTING FOR THE VIRUS

Widespread testing is considered the fastest way out of the current pandemic, but existing tests require specialist kit, skill and time. Researchers are developing several ways to speed up or simplify them. Most tests use nasal-swab samples; any viral RNA is amplified to a detectable level and its presence flagged.

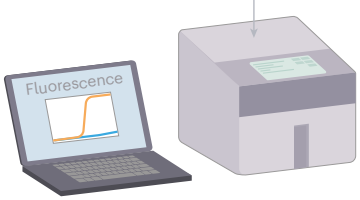
THE GOLD STANDARD

The most widely used method detects, amplifies and labels the viral RNA using matching pieces of sequence (primers), DNA-building enzymes and a stock of DNA 'letters'. The method, a laboratory mainstay called the reverse-transcriptase polymerase chain reaction (RT-PCR), requires time-consuming heating and cooling steps.



Testing positive

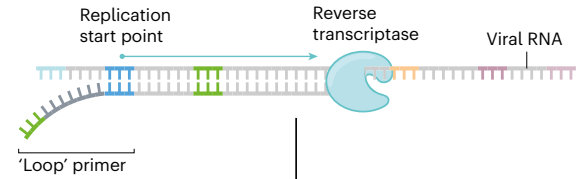
A computer measures the fluorescent signal, flagging the presence of virus. PCR tests can be up to 100% accurate, but they take 1–4 hours to perform and need specialized equipment.



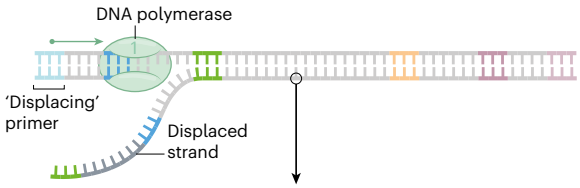
LOOP THE LOOP

Several tests in development use a set of virus-specific primers that both activate the copying process and amplify the RNA. The method, called loop-mediated isothermal amplification (LAMP), needs no repeated heating and cooling, and amplifies the viral sequence by coaxing it into loops of different shapes that mushroom very quickly.

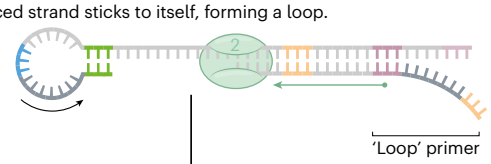
1. One primer prompts copying and provides an anchor for the loop.



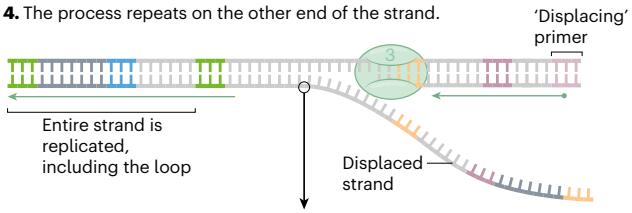
2. A second primer enables copying of the other strand, displacing the first.



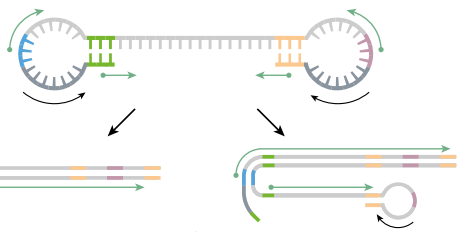
3. The displaced strand sticks to itself, forming a loop.



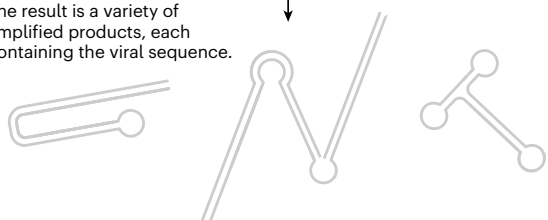
4. The process repeats on the other end of the strand.



5. Displaced strand forms a dumb-bell structure with multiple replication start points.

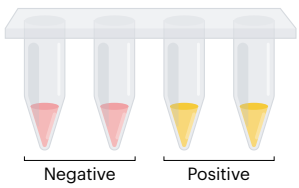


The result is a variety of amplified products, each containing the viral sequence.



Testing positive

As the DNA amplifies, it makes the sample acidic. A pH-sensitive dye flags the change. The test requires only basic equipment and can be done outside a lab.



GRAPHICS: NIK SPENCER/*NATURE*

Because DNA sequencers can read out hundreds of millions of DNA snippets at once, researchers estimate that sequencing-based tests could be used to analyse up to 100,000 samples in one run. By contrast, a standard PCR machine can test just dozens or hundreds of samples at the same time. But these sequencing tests take time – at least 12 hours – and require specialized equipment in centralized facilities. Getting millions of samples delivered to those facilities isn't trivial.

Another way researchers are trying to bring testing to the masses is to devise assays that could be used in temporary testing facilities, drive-through testing centres and even in people's homes.

At least two teams are taking advantage of the gene-editing technology CRISPR to power such tests. For example, researchers led by Zhang have developed a coronavirus assay that can be run in a single test tube in about an hour[4]. But it still requires heating the sample to about 65 °C, and it's not as sensitive as a PCR-based assay. "That's okay, because it's much easier to use," Zhang says. When tested multiple times on samples from 12 people infected with coronavirus, the assay detected the virus on nearly every occasion.

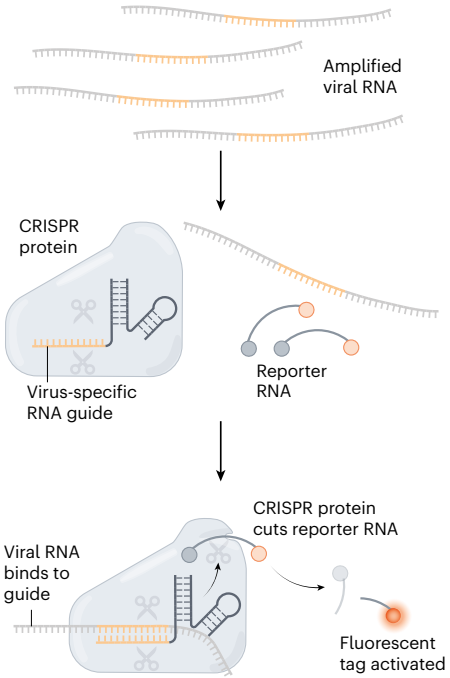
The test builds on an approach that Zhang co-developed in 2017, called SHERLOCK[5], which relies on the ability of the CRISPR machinery to home in on specific genetic sequences. Researchers program a guide molecule to latch on to a particular stretch of the SARS-CoV-2 genome. If the guide molecule finds a match, a CRISPR enzyme generates a signal that can be detected either as a fluorescent glow or as a dark band on a paper dipstick (see 'Cut and detect'). On 6 May, the US Food and Drug Administration (FDA) authorized a SHERLOCK coronavirus assay for emergency use. The test is made by biotechnology firm Sherlock BioSciences in Cambridge, Massachusetts (of which Zhang is a co-founder), and the company has partnered with a manufacturer to mass-produce the kits.

Mammoth Biosciences, a diagnostics company co-founded by CRISPR pioneer Jennifer Doudna of the University of California, Berkeley, is also seeking an emergency-use authorization for its CRISPR-based coronavirus test[6], says Mammoth's chief technology officer and co-founder Janice Chen. The test is based on a previous result showing that the technology can detect human papillomavirus[7]. The company, based in San Francisco, California, is now trying to make the test simple and cheap enough for anyone to use at home, Chen says. "The ultimate goal is to take diagnostics directly to the consumers – PCR has not been able to go there," she says.

Guozhen Liu, a bioengineer at the University of New South Wales in Sydney, Australia, says that technologies such as CRISPR could be "a game changer" in the current pandemic.

CUT AND DETECT

Some tests in development use the precision of the gene-editing technique CRISPR to detect viral material. First, any viral RNA is amplified, for example using LAMP (see 'Loop the loop'). Then a CRISPR-associated protein is added along with some free strands of 'reporter' RNA. If viral genetic material is found, the CRISPR protein cuts a reporter RNA, generating a fluorescent signal.



Testing positive

Fluorescence can be detected by a computer, or the reporter RNA can be coupled with a molecule that produces a band on a dipstick, like a pregnancy test.

Thanks to their ability to quickly and precisely identify genetic snippets, these approaches "can find a needle in a haystack", Liu says. They use different reagents from RT-PCR-based assays – useful when there are shortages of chemical supplies for standard tests – and they can be designed to target any pathogen. For example, a team led by computational biologist Pardis Sabeti at the Broad Institute created rubber 'chips' about the size of a smartphone that can search 1,000 samples for a single virus, or 5 samples for a panel of 169 viruses that are known to infect humans[8].

Surface screening

A different approach for faster and cheaper diagnostic tests would be to look for molecules that sit on the surface of the virus, rather than trying to detect the virus's genome. Such a test would contain an antibody tailored to bind to a specific protein, or antigen – similar to the technology that enables home pregnancy tests. These assays, which are inexpensive to produce and simple to conduct, are already used to detect influenza infections. But antigen tests don't contain an amplifying step in the same way that tests for viral material do, so they are less sensitive.

On 8 May, the FDA granted its first emergency-use authorization for a coronavirus antigen test that targets the nucleocapsid protein on the virus's surface. The Taiwan FDA is evaluating a similar assay that could provide results within 20 minutes, says computational biologist An-Suei Yang of Academia Sinica in Taipei, who developed the test. Yang's team used artificial intelligence to identify antibodies that could bind to proteins on the coronavirus surface. Yang says the researchers have not yet tested it on coronavirus samples from infected people.

Even once a test is working beautifully in the lab, it still faces an arduous journey to mass usage. The first challenge is to verify performance, because quality can vary. "It's a Wild West out there for assay development," says Catharina Boehme, chief executive officer of the Foundation for Innovative New Diagnostics (FIND), a non-profit group in Geneva that is collaborating with the World Health Organization and the University Hospitals of Geneva to assess hundreds of SARS-CoV-2 testing options. Most RT-PCR-based tests that FIND has evaluated perform just as well as the gold standard does, whereas antigen tests have so far fallen short of expectations, Boehme says.

Another hurdle is scaling up the assays for mass production. Given this constraint, Boehme thinks it is unrealistic that all the new tests will be deployed before the end of the year – although a small number might be. But once they are available, they could work alongside the gold standard to push countries closer to the target of millions of tests per week – and prepare the world for the next pandemic.

Even during this one, Boehme says, researchers mustn't neglect the development of tests for other viruses that cause respiratory symptoms, and monitoring for conditions such as diabetes, which can worsen the outlook for people with COVID-19. "We have to go beyond testing just for the coronavirus," she says.

Giorgia Guglielmi is a freelance science journalist in Basel, Switzerland.

1. Yu, L. *et al.* Preprint at medRxiv https://doi.org/10.1101/2020.02.20.20025874 (2020).
2. Yu, L. *et al. Clin. Chem.* https://doi.org/10.1093/clinchem/hvaa102 (2020).
3. Schmid-Burgk, J. L. *et al.* Preprint at bioRxiv https://doi.org/10.1101/2020.04.06.025635 (2020)
4. Joung, J. *et al.* Preprint at medRxiv https://doi.org/10.1101/2020.05.04.20091231 (2020).
5. Gootenberg, J. S. *et al. Science* **356**, 438–442 (2017).
6. Broughton, J. P. *et al. Nature Biotechnol.* **38**, 870–874 (2020).
7. Chen, J. S. *et al. Science* **360**, 436–439 (2018).
8. Ackerman, C. M. *et al. Nature* **582**, 277–282 (2020).