Diagnostic tests for COVID-19 help stave off the pandemic and buy time until more vaccines reach the population.

Pandemic pivot: How scientists answered the call for diagnostic tests

In medicine as well as in sports, the best defense is a good offense. When it comes to the current COVID-19 pandemic, the offense entails large-scale diagnostic testing that can rapidly, accurately, and reliably detect SARS-CoV-2. Although vaccines are currently being distributed, diagnostic tests–many of which are produced by companies that have already used similar technology to combat other diseases–remain key to containing the pandemic and preventing more deaths. **By Alan Dove**

At the turn of the 21st century, coronaviruses were among the most obscure subjects in virology–it's hard to draw much public interest in pathogens that cause little more than minor sniffles. Struggling for funding and toiling in obscurity, a handful of scientists nonetheless produced a substantial body of basic research on these RNA viruses. Then, in 2002, clusters of patients suddenly developed a novel form of severe acute respiratory syndrome (SARS), a life-threatening condition subsequently linked to a new coronavirus that had leapt from animals into humans. Several thousand cases and hundreds of deaths later, public health officials had contained the spread of SARS, but the field of coronavirology had been transformed forever.

After the SARS crisis, wildlife surveys and stepped-up surveillance revealed numerous coronaviruses in nature that could, in theory, spill into humans under the right circumstances. By 2019, those findings and the appearance of Middle East Respiratory Syndrome (MERS), another serious respiratory disease caused by a zoonotic coronavirus infection, made it clear that coronaviruses posed a serious threat to global health. Then SARS-CoV-2 emerged in 2020, and the global COVID-19 pandemic began. As the scale of the crisis became clear, scientists and pharmaceutical companies around the globe rallied to address it, often repurposing technologies developed to combat other diseases. 2020 was the year everyone became a coronavirologist.

Turning on the LAMP

For vaccine developers, the path ahead was as obvious as it was arduous. Even before the World Health Organization's declaration of a global pandemic on March 11, hundreds of academic and corporate scientists were already turning their existing tools and techniques toward developing SARS-CoV-2 vaccines. As that effort ground ahead, though, public health officials had a desperate need for diagnostic tests. Because many SARS-CoV-2 infections are asymptomatic or mild, tracking the virus's spread required fast, reliable testing, and existing resources weren't up to the task. Fortunately, reinforcements were on the way.

"Before the crisis, we didn't work at all on coronaviruses," says Franck Molina, director of the **Sys2Diag** program at CNRS in Montpellier, France. Molina's team was nevertheless in an ideal position to develop a new generation of SARS-CoV-2 tests. The lab's academic–corporate collaboration gives the team a multidisciplinary edge, encompassing everything from laboratory researchers to artificial intelligence and bioinformatics engineers. Having previously developed liquid biopsy tests for customized cancer therapies, Sys2Diag pivoted to measuring viral RNA instead of tumor biomarkers.

When the team's researchers began developing their new SARS-CoV-2 diagnostic system, established medical labs were already using sensitive, specific PCR tests. The key drawback to those tests is that they require sophisticated labs, trained personnel, and expensive thermal cyclers, all of which were in short supply. Molina and his colleagues focused on creating a much simpler, cheaper test using loop-mediated isothermal amplification (LAMP). "The machine [for LAMP] is just a heating machine, and we knew the properties of this technology," says Molina.

Besides using simpler equipment for DNA amplification, the investigators also streamlined everything from sample collection to results cont.>

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reporting as much as possible. The final iteration of the system, which received marketing approval in the United States and European Union in June 2020, yields robust results only 40 minutes after the patient spits into a tube. At the end of the process, a smartphone application reads the colorimetric result. "Then immediately you have the formatted readout of the test, positive or negative, and it automatically sends the information to a database," says Molina. Minimally trained technicians can conduct the tests almost anywhere.

SkillCell, a company spun off from Sys2Diag, now sells this testing system as EasyCOV. Molina says it's in use worldwide in airports, hospitals, clinics, and various industries that need to test their employees. In those settings, it competes with other rapid viral RNA testing products, such as **Abbott's** ID NOW COVID-19 system, which also uses isothermal amplification.

Immunity counts

While many biologists and life science companies changed focus to help fight the new virus, others simply continued their regular work as the pandemic came to them. That's what happened at PerkinElmer, one of the world's largest suppliers of laboratory and diagnostic reagents and equipment. "We have several thousand products in our catalog, and when the pandemic happened, this was immediately on our radar," says Iswariya Venkataraman, scientific affairs manager at **EUROIMMUN US**, a PerkinElmer subsidiary.

When the scope of the SARS-CoV-2 outbreak in China became clear in December 2019, EUROIMMUN began developing PCR tests to detect the virus, as well as an enzyme-linked immunosorbent assay (ELISA) for antibodies against it. "The priority for us was in [being] able to first diagnose infection, and then also understand the humoral immune response following this infection," says Venkataraman. While the immediate need to identify and quarantine infected individuals claimed much of the attention early in the pandemic, public health officials quickly realized the importance of antibody screening as well, to identify those who'd had the infection already.

By early May 2020, EUROIMMUN had gained approval for its rapid qualitative test to detect antibodies against the SARS-CoV-2 spike glycoprotein. The test uses a purified preparation of the S1 domain of the spike, generally considered the most specific antigenic target on the virus, to bind immunoglobulin G (IgG) antibodies in a standard ELISA procedure familiar to any medical testing lab. IgG arises late in an infection and is considered a good long-term marker of antibody-based humoral immunity. "For IgG antibodies, the median onset is about day 14 or 15 [after infection]," says Venkataraman.

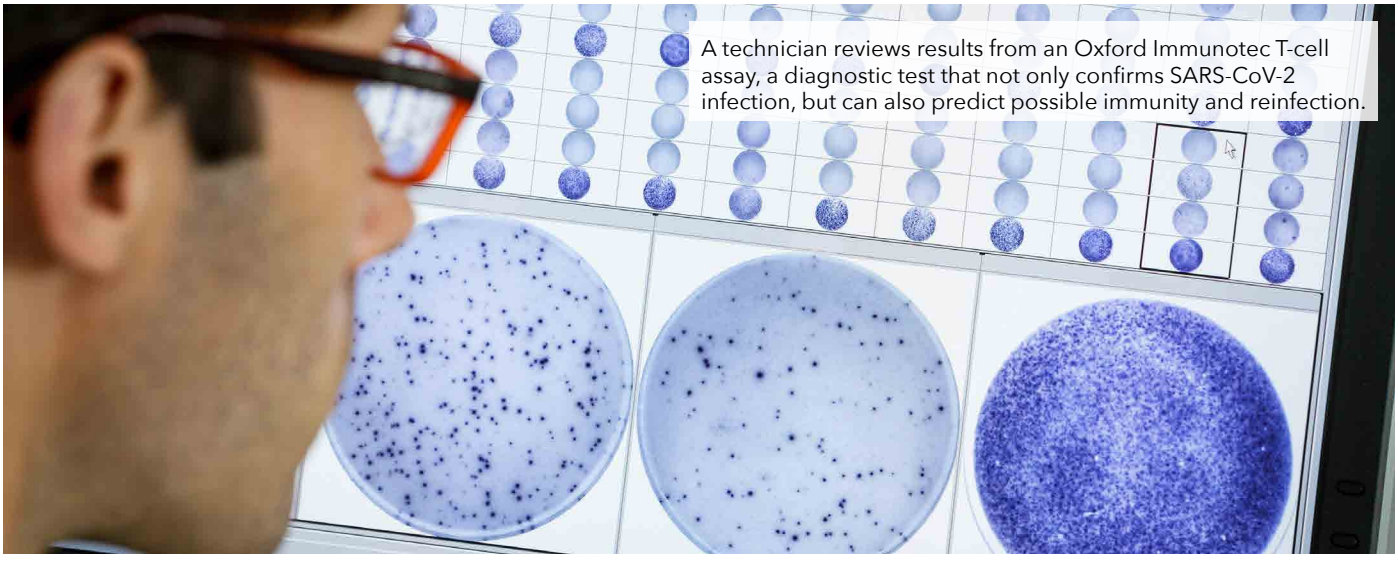
As the pandemic continued, qualitative testing revealed wide variation in individual immune responses against the virus, with some people testing negative for antibodies within weeks of infection, while others remained positive for months. At the same time, vaccine developers were gearing up for clinical trials and needed to know not only that their products elicited antibody responses, but how strong those responses were. EUROIMMUN answered this need by calibrating their now-established qualitative test. "It's exactly the same antigen target, just with the addition of calibrators to make it quantitative," says Venkataraman. The company released the new test, called QuantiVac, in December 2020.

QuantiVac is now marketed as a quantitative test in Europe and a semiquantitative test in the United States. The distinction is due to the U.S. Food and Drug Administration (FDA) requirement that quantitative tests be based on established international reference standards, which don't yet exist for SARS-CoV-2 antibodies. "When there is an international standard available, we will calibrate our assays to that," says Venkataraman.

The QuantiVac kit includes a set of six reference samples representing a range of antibody concentrations. "With that, you're able to plot a standard curve, and once you have a standard curve in place, you're able to determine the unknown concentration in your sample," says Venkataraman, adding that vaccine developers and immunologists should find the quantitative data useful in detailing patients' immune responses.

Antibody can see the difference

Other diagnostics-focused companies have followed a similar path, with similar timelines. "It was around February [2020] when it became clear that the pandemic was evolving, and then we had discussions cont.>



A technician reviews results from an Oxford Immunotec T-cell assay, a diagnostic test that not only confirms SARS-CoV-2 infection, but can also predict possible immunity and reinfection.

IMAGE: COURTESY OF OXFORD IMMUNOTEC

Featured participants

Abbott
www.diagnostics.abbott/us/en/home

EUROIMMUN US
www.euroimmun.com

Oxford Immunotec
www.oxfordimmunotec.com/north-america

Roche Diagnostics
diagnostics.roche.com

SkillCell
www.skillcell-alcen.com

Sys2Diag
www.alcediag-alcen.com/alcediag/sys2diag

on what kind of role an antibody assay may play in the management of the pandemic," says Beatus Ofenloch-Haehnle, head of immunoassay development and system integration at **Roche Diagnostics** in Penzberg, Germany. By the second week of March, he and his colleagues were working on adapting established Roche assays to detect anti-SARS-CoV-2 antibodies. The resulting qualitative assay for pandemic virus exposure launched in Europe and the United States 6 weeks later.

Unlike the EUROIMMUN assay, Roche's qualitative test identifies antibody responses against the SARS-CoV-2 nucleocapsid protein instead of the spike glycoprotein. Rather than a standard ELISA, the test uses Roche's proprietary Elecsys electrochemiluminescence immunoassay. In this technique, two different antigen preparations, each bound to a different conjugate, bind to the antibodies in the sample and attach them to magnetic beads and luminescent markers. A magnet then precipitates the antibody-bound beads, and an applied voltage causes the precipitated antibody sandwiches to glow.

The entire test runs on Roche's well-established cobas automation platform. "We have twenty years of experience in developing all kinds of immunoassays [on it]," says Ofenloch-Haehnle. Because Elecsys hardware is already used for other assays in more than 40,000 diagnostic and research labs around the world, the new SARS-CoV-2 exposure test was easy for users to adopt.

High levels of automation and decades of user-interface refinements mean that the cobas-driven Elecsys assay is also easy for new entrants to the field to pick up. Indeed, Ofenloch-Haehnle says many labs have bought cobas systems for the first time just to run the new SARS-CoV-2 test. Using a 12-microliter blood sample, the entire process takes 18 minutes. The largest cobas systems can run 300 tests an hour, requiring the technician to do little more than load and unload samples.

Because it detects the viral nucleocapsid, the Roche qualitative test is useful for identifying individuals who've been infected with the virus. All the vaccines currently in late-stage clinical development, however, are designed to stimulate immune responses only against the external spike glycoprotein. The Elecsys qualitative SARS-CoV-2 test doesn't detect those antibodies. "So, we decided to develop a second assay, and this time a quantitative assay, because you need quantitation to assess antibody levels and come up with potential thresholds for revaccination or immunity," says Ofenloch-Haehnle.

The second assay, released in the United States and Europe in September 2020, uses the same Elecsys platform to provide quantitative measurements of anti-spike IgG antibodies. The two assays could also be useful together in some settings. "While nucleocapsid is solely from natural infection, having the [anti-spike assay], one could see in vaccinated individuals whether some natural infection was going on and also differentiate between the response to the vaccine and the natural infection," explains Ofenloch-Haehnle.

Time for T

While antibody tests have been crucial for monitoring the scope of the pandemic and tracking vaccine responses, they measure only one side of the immune response to SARS-CoV-2. Besides acquiring humoral immunity with antibodies, people who contract the virus also develop cellular immunity mediated by killer T cells. "T cells are very relevant in how your body responds to SARS-CoV-2," says Peter Wrighton-Smith, chief executive officer of **Oxford Immunotec** in Abingdon, United Kingdom.

T-cell responses are more difficult to measure, though, and both research and clinical laboratories have struggled to determine exactly how important cellular immunity is in COVID-19. "One of the problems we have in the academic literature is that the findings are ... all using different methods," says Wrighton-Smith. To address that, his company began producing standardized kits for research labs to test SARS-CoV-2 T-cell responses. Released in May 2020, those kits then became a centerpiece of a study the company undertook with Public Health England, analyzing T-cell responses in health care workers and first responders.

"We tested people at the beginning, and then we followed them up for months afterwards to see who was getting SARS-CoV-2," says Wrighton-Smith. The results revealed that T cell–mediated immunity was a common feature in many infections, including some that didn't produce robust antibody responses. Critically, a robust T-cell response correlated strongly with immunity from reinfection; people who failed to develop such responses were significantly more likely to get reinfected. "T cells are probably more important than antibodies in determining who has immunity," says Wrighton-Smith.

To measure cellular immunity in clinical settings, Oxford Immunotec turned to T-Spot, the platform they've been developing for the past 18 years. With this system, technicians isolate T cells from a blood sample, count and distribute them into multiwell plates, and then perform an assay to measure the number of cells that react to the test antigen. The company's first product, T-SPOT.*TB*, is already in use worldwide for tracking cellular responses to tuberculosis. "By discerning whether you have these cells, we're able to confirm whether you currently have the infection or have recently seen it, and by counting how strong the response is, we may also be able to tell ... the extent of your immunity," says Wrighton-Smith.

The T-SPOT *Discovery* SARS-CoV-2 test can be used as either a qualitative or quantitative test, and the company expects to get it licensed by early 2021. "We're working feverishly to get that to happen, [because] we have a technology that we think will be really important in the next phase of the pandemic," says Wrighton-Smith. Like others working on pandemic-related technologies, he emphasizes that while the arrival of effective vaccines promises to bring the virus under control, the need for new innovations–both for this pandemic and future ones–will persist.

Alan Dove is a science writer and editor based in Massachusetts.