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Developing antibody tests for SARS-CoV-2

Laboratories and diagnostic companies are racing to produce antibody tests, a key part of the response to the COVID-19 pandemic. Anna Petherick reports.

In response to coronavirus disease 2019 (COVID-19), governments have instigated rules that constrain personal freedoms and hamstring their own economies, placing approximately 3 billion people under lockdown. Some have rolled out widespread testing for current infections, while others limited these tests to people who were hospitalised, at least during the early stages of their responses. As new controls begin to bite, the race to develop and approve a test with a different purpose—to assess not current viral infection, but immunity to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)—has heated up. Medical diagnostic companies are scrambling, and governments are looking to order these antibody tests by the millions.

The task now facing governments and national regulators is to balance urgency against the everyday sensitivity and specificity concerns that apply to any new medical diagnostic. A few technical questions still exist around optimising test design, primarily hinging on understanding how the viral coating triggers a healthy immune system's recognition and neutralisation of the virus. Yet, there is a palpable hurry to limit economic damage, to get people back to work, and to reopen borders—and those whose immunity can be demonstrated should be able to return to work, without risk. Some regulators, such as the US Food & Drug Administration (FDA), have already chosen to relax normal assessment criteria.

Demand could not be higher. "We really have been inundated with calls", says Dan Hanlon, director of international sales at CTK Biotech (San Diego, CA, USA), which has developed an antibody test. "The inquiries are coming from ministries of health, from various government institutions, from military contractors, from industry distributors that commonly work in this realm." Under normal circumstances, CTK Biotech would have to collect data for FDA approval of a novel diagnostic at three different sites, but under the emergency use authorisation issued by the agency in mid-March, this is no longer necessary. "Basically [the FDA authorisation] says, 'You can start selling right away and then send us your data, and if we don't like it, you have to take your product back'", says Sam Lewis, CTK Biotech's director of research and development. In Europe, a German company called Euroimmun has recently received certification that will allow their test to be sold within the EU. "Everybody is getting crazy at the moment with regard to antibody detection, especially for medical personnel, because it's very important to know if they already had the virus", says Konstanze Stiba, Euroimmun's antibody test product manager. Health worker shortages due to illness put further pressure on already strained health systems. If a test can show that a health worker has already had COVID-19, and is therefore probably immune, they can return to work without fear of infection. "That's something you can easily do with an antibody detection test and not with PCR—you can keep the health system stable."

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Antibody testing is multipurpose: it can verify that vaccines are working as intended during clinical trials, or be used in contact tracing weeks or longer after a suspected infection in an individual. Probably its most important current use, en masse, is to help inform public policy makers how many asymptomatic cases have occurred in a population. Antibodies reveal evidence of a previous infection any time from about a week after the infection occurred. "At the moment we are only estimating the number of people who have been infected. No one in the world has measured that properly yet", says Martin Hibberd, professor of emerging infectious diseases at the London School of Hygiene & Tropical Medicine, UK. "We think that children are infective but asymptomatic, for example, but we don't know enough about this—and that information matters for decisions about whether to close schools."

PCR tests detecting viral RNA, indicating current viral infection, are being used to diagnose cases of COVID-19 and are an essential part of contact tracing and testing. However, there are global supply challenges, with huge demand for the PCR primers, as well as for the positive controls needed to ensure the performance of individual machines. PCR tests for SARS-CoV-2 have been available since January, soon after the virus was identified. However, the technology behind antibody tests is fundamentally distinct and generally harder to get right. "If you have a sequence today, you have a PCR tomorrow", says Linfa Wang, director of Duke-NUS Medical School's programme in emerging infectious diseases, in Singapore. "Whether the sensitivity [of PCR] will be enough is another thing, but usually in the first round, it will give you data that you can use. Serology is different."

Antibody tests are different because they require some knowledge of the

proteins that form the viral coat—specifically, those proteins to which the immune system responds, triggering the production of antibodies that flag or neutralise the virus. Those sections of the viral protein coat must then be produced in the laboratory, using cell lines, for inclusion in an immunoassay (eg, ELISA) that detects whether antibodies are present. Such immunoassays will form the basis of home testing kits for people who think they have had COVID-19. But their development takes time. Expressing the protein in the right structure is often the most difficult step. In a non-native system, such as a bacterial cell, the complex protein structures can come out slightly deformed, enough to stop antibodies from recognising them as they would the original viral coat protein.

There are also questions about which antigens (proteins) are best for this purpose. Some diagnostic developers are cagey about giving away too many details, although the viral spike protein is universally perceived as the obvious candidate. "All viral proteins will elicit antibody responses to some extent", says Berend-Jan Bosch, a coronavirus specialist at Utrecht University in the Netherlands. "But the spike protein is the main antigen that elicits neutralising antibodies, as this protein is the sole protein on the viral surface that is responsible for entry into the host cell." Researching the spike protein also presents avenues to the development of therapeutics for COVID-19, and Bosch and his team have now created a human monoclonal antibody that neutralises SARS-CoV-2 in vitro. In parallel, he is working with Marion Koopmans of Erasmus Medical Centre (Netherlands) to develop antibody tests.

Which part of the spike protein to use is less obvious, however. A team at New York's Icahn School of Medicine at Mount Sinai (NY, USA), has published details of antibody tests that use either the whole spike protein, modified slightly to improve its stability during mass production in cell lines, or only the receptor-binding domain. Others, such as Peng Zhou, who leads the bat virus infection and immunity group at the Wuhan Institute of Virology in China, and was part of the team that sequenced SARS-CoV-2's genetic code in January, have used the nucleocapsid protein and the spike protein. Zhou's antibody test is one of at least ten antibody tests that have already been deployed in hospitals across China. "[The] nucleocapsid protein is the most abundant viral protein, which means it's easy to detect. But we also chose spike protein because it's very specific", he says. "Actually, for coronavirus, the most divergent protein is [the] spike protein."

> **"… another four coronaviruses cause the common cold, and ensuring there is no cross-reactivity to these is essential."**

There is a lot hanging on the uniqueness of the spike protein. In terms of the specificity of serological tests in which it is used, the more unique it is, the lower the odds of cross-reactivity with other coronaviruses—false positives resulting from immunity to other coronaviruses. The most similar of these is severe acute respiratory syndrome coronavirus (SARS-CoV), which led to the SARS outbreak of 2002. But another four coronaviruses cause the common cold, and ensuring there is no cross-reactivity to these is essential. "If you line up the amino acids of the spike proteins of SARS and the COVID-19 virus, there's a 75% identity", says Lewis. Hibberd reckons the overall figure for common cold-causing coronaviruses is probably about 50–60%, but the potential for cross-reactivity really depends on whether the new tests select sections of the spike protein that are particularly distinct across coronaviruses. Even though SARS cases were recorded in only a handful of countries, many antibody test developers—Euroimmun, Koopmans, and Wang among them—are working to demonstrate the absence of cross-reactivity of the new tests with SARS-CoV or other coronaviruses.

Because, in Bosch's words, the spike protein is the sole viral protein responsible for entry into the host cell, its stability as SARS-CoV-2 mutates is important for understanding whether re-infection with a novel strain is likely. Wang says that the spike protein is highly conserved. Virologists generally agree that media reports of reinfection with SARS-CoV-2 are most likely due to erroneous PCR tests. Hibberd argues that once people produce antibodies against a particular coronavirus, they probably have immunity for life. Indeed, Wang's laboratory has investigated how long immunity against SARS-CoV and Middle East respiratory syndrome coronavirus lasts. "17 years later, a SARS survivor still has neutralising antibodies against SARS—we found that not only were the antibodies there, but they could still neutralise the SARS virus."

This is reassuring news for governments that intend to deploy antibody tests to establish which health-care workers are immune, and to get their populaces back to work as soon as possible. Nonetheless, scaling up production quickly enough to meet the informational needs of public policy makers is no mean feat. Behind the scenes, experts worry that, despite some big recent promises, national authorities have not attempted anything like this before. Medical diagnostic companies might be better positioned to meet demand, but they too are stretched. "We've tried to notify our [other] customers that we're focusing on the crisis, which allows scale-up to increase output", says Hanlon. Other contracts, except urgent HIV and malaria diagnostics, have been downgraded in production schedules. "This really is the main focus product now."

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