UK-RTC

AbC-19™

RAPID TEST

Charting the course to a post-COVID world

Why quick, easy antibody tests will play an invaluable role in overcoming COVID-19

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Introduction

First identified in humans in the Wuhan province of China in late 2019, coronavirus disease 2019 (COVID-19) is thought to have originated in animals, crossing the species barrier to humans in open-air markets. The virus has spread rapidly around the globe, prompting governments to enact strict lock-down measures to hinder its spread, shuttering large parts of their economies in the process.

In the UK, around 1 in 16 people, or 6.2% of the population, has been infected[1]. The infection rate, which had been in decline until the end of June, has recently begun to pick up, with 4,200 new infections per day recorded at the end of July[2].

Large-scale testing is crucial if governments are to ease lock-downs and chart a return to normal life[3]. Diagnostic testing, to establish if an individual currently has the disease, is conducted using RT-PCR (reverse transcriptase polymerase chain reaction) to identify viral RNA in the nose and throat. This test confirms whether an individual has the disease at that moment. However, RT-PCR tests can occasionally be falsely negative, and do not provide any insight into prior exposure or immunity[4].

These questions can be answered by testing for the presence of antibodies in the blood. The immune system automatically produces a variety of antibodies (immunoglobin, or Ig) in response to viral infection. These appear, peak, and disappear from the blood at different rates.

SARS-CoV-2 infection provokes the production of different types of immunoglobin; IgA, IgM, and IgG. IgA forms part of the body's front-line defence against infection and is found most abundantly in tears, and in mucus in the nose and gut. IgM appears more quickly in the blood, around a week after infection, but tails off rapidly thereafter. IgG, on the other hand, takes longer to appear – around 10 days or two weeks. IgG forms the body's long-term response to infection and remain in the blood for several weeks afterward. The presence of SARS-CoV-2 specific IgG antibodies in the blood can therefore prove if a person has been infected by the virus in the past, even if they never developed symptoms[5].

For this reason, most antibody assays focus on IgG. However, the vast majority require specialist laboratories and expert personnel to process samples. Results may not be communicated to patients for 24 hours or more after testing.

The UK-RTC AbC-19 Rapid Test™ is a self-contained and highly accurate lateral flow IgG immunoassay for establishing if people have been infected by SARS-CoV-2 in the past. It delivers qualitative results within minutes, at the point of use. Deployed at scale across populations, the test can aid policymakers, healthcare systems, the scientific community, and the public at large by:

Clearly showing the extent of COVID-19 infection and its spread through communities (seroprevalence);

Enabling researchers to understand whether people are developing immunity;

Informing the development and evaluation of large-scale vaccine tests, and eventual mass immunisation campaigns.

This paper discusses some of the current science relating to infection and eventual immunity to COVID-19, stressing the vital role that rapid, accurate antibody testing can play in charting a path to population-level immunisation and a return to normal life.

> A sensitive and specific antibody assay could directly contribute to early identification and isolation of cases, address unknowns regarding the extent of infection to inform mathematical models, and support individual or population-level release from lock-down.
>
> National COVID Scientific Advisory Panel[6]

How the body responds to SARS-COV-2

Coronaviruses get their name from their distinctive crown-like shape, which comes from a distinctive spike protein dotting the virus membrane (figure 1). A wide variety of diseases from the common cold to Middle East Respiratory Syndrome (MERS), are coronaviruses, and new ones are discovered every year[7].

SARS-CoV-2 – the name given to the virus leading to COVID-19 – enters the body when people breathe in contaminated droplets in the air, often produced by coughing or sneezing. A spike (S) protein on the outside of the virus membrane binds to receptors on cells in the body called ACE2, commonly found on cells in the nose and throat.

The virus interacts with ACE2 to infect healthy cells and replicate itself, from where it spreads to other ACE2 receptors, starting in the lungs. Infection here can lead to severe respiratory problems – the leading cause of hospitalisation and death among COVID-19 patients.

There is scope for the virus to cause disruption beyond the respiratory system as ACE2 receptors – and therefore potential focal points for infection – are also found in the heart and kidneys[8].

The human immune system is designed to continually adapt to new threats, and in the case of SARS-CoV-2, it takes 10 days to two weeks[9] for IgG antibodies specific to the virus to appear in the blood[10]. The antibody response chiefly targets the S protein . For any infection, IgG antibodies move around the body in the blood marking infected cells for destruction. The concentration of antibodies in the blood (antibody titre) declines over time.

The presence of antibodies specific to SARS-CoV-2 in the blood therefore proves that a person has been exposed to the virus in the past, irrespective of whether or not they developed symptoms. We examine the current science around infection, seroconversion and immunity later in this paper.

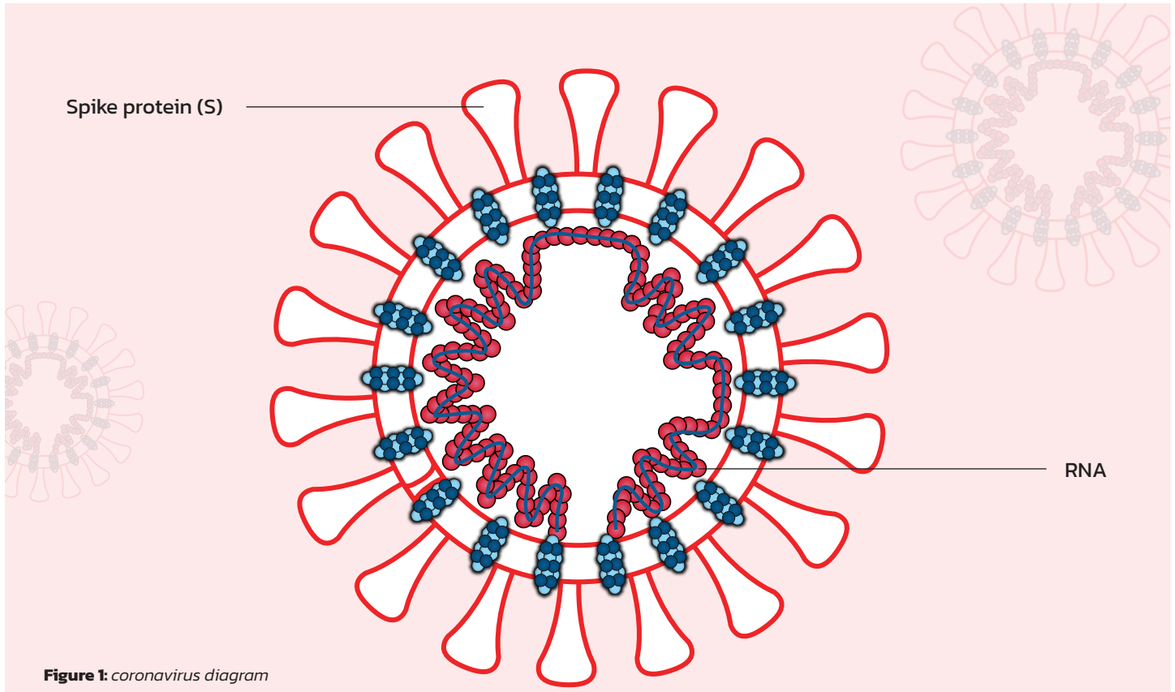
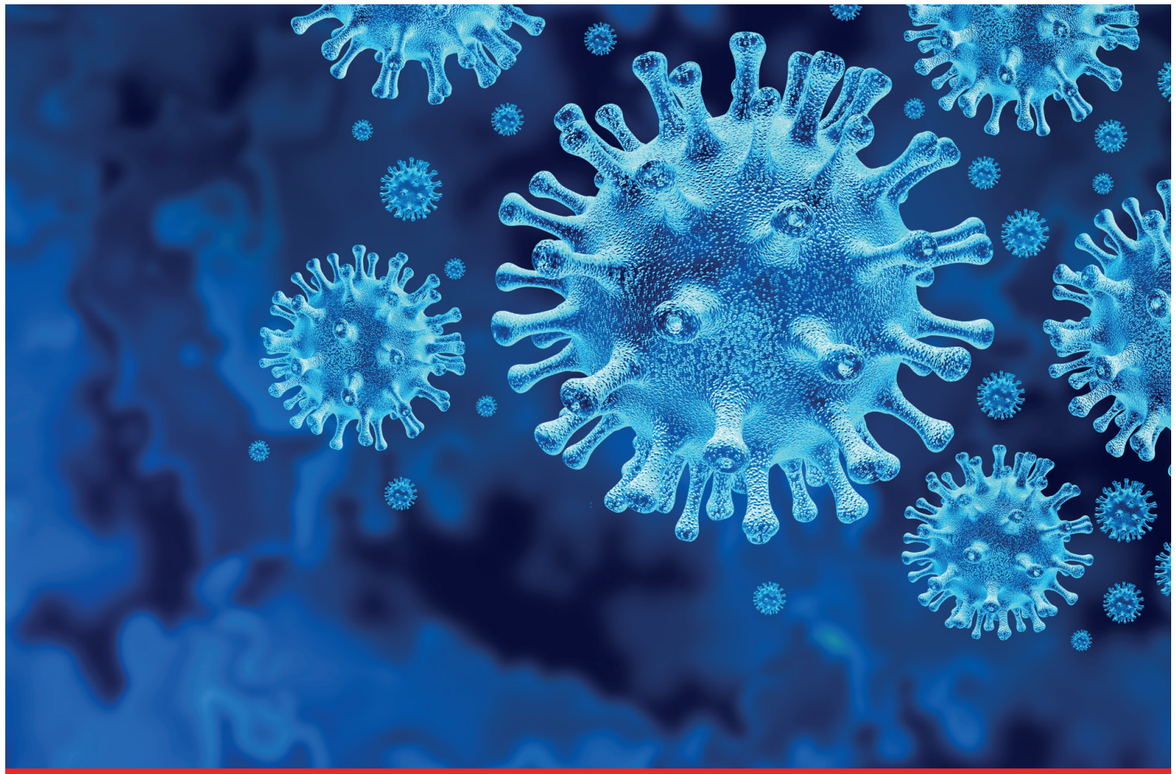


Figure 1: *coronavirus diagram*



Trimeric S protein: a focal point in the fight against COVID-19

The SARS-CoV-2 S protein has become the object of intense scientific interest. It provides the docking system allowing virus RNA to enter and take over healthy cells. It is a trimer, meaning it is made up of three different protein molecules. Researchers have confirmed that the S trimer elicits the strongest immune response in the body. What is more, the extent to which it mutates over time may provide clues about re-infection and immunity[11].

However, spike trimers on SARS-CoV-2 share many features with other coronaviruses: around 50-60% of the material on the SARS-CoV-2 spike is identical to that of the common cold[12].

Oxford University's vaccine candidate, ChAdOx1 nCoV-19, is designed to train the human immune system to recognise and respond to the entire SARS-CoV-2 spike trimer. Based on a deactivated simian adenovirus, the candidate contains the full-length spike trimers from SARS-CoV-2[13]. Phase 1/2 trials published in July 2020 found the human immune system responds to the vaccine in the same way it would if faced with the harmful virus[14].

Approaches to antibody testing

Though COVID-19 is highly transmissible, its similarity to more benign coronaviruses, and the relatively low incidence of infection across the population, mean that any test must be highly accurate. The UK's Medicines & Healthcare products Regulatory Agency (MHRA) recommends that SARS-CoV-2 immunoassays should meet sensitivity and specificity thresholds of least 98% in order to be considered fit for purpose[15].

There are a variety of approaches to testing for SARS-CoV-2 antibodies. To date, lab-based assays such as ELISAs and CLIAs (See figure 2 below) have predominated. However, these depend on specialist facilities and equipment for samples to be analysed: anyone giving a sample for testing must wait until the analysis is complete for the results to be communicated to them.

Research into lateral flow immunoassays (LFIA), which can deliver results within minutes at the point of use, has therefore accelerated significantly.

> Rapid lateral flow immunoassay devices provide a quick, point-of-care approach to antibody testing.
>
> **National COVID Scientific Advisory Panel[16]**

Immunoassays explained

Serological assays (antibody tests) use a variety of different platforms. Though broadly comparable in terms of sensitivity and specificity, the equipment and workflow involved – therefore the time to results – varies considerably.

Assay platform	Procedure	Equipment needed	Results Available
Enzyme Linked Immunosorbent Assay (ELISA)	Blood sample is mixed with chemicals in the laboratory which bind to the antibodies, changing colour based on their concentration in the blood serum.	Healthcare professional to take blood sample. Biosafety Level 2 laboratory. Specialised analysis equipment.	>24 hours. Results must be communicated separately to patients / HCPs
Chemiluminescence Immunoassay (CLIA) Electro-chemiluminescence Immunoassay (ECLIA)	As with ELISA, the sample is mixed with reagents which produce light if the anti-body is present.	Healthcare professional to take blood sample. Biosafety Level 2 laboratory. Specialised analysis equipment.	>24 hours. Results must be communicated separately to patients / HCPs
Lateral Flow Immunoassay (LFIA)	Blood sample is dropped onto a self-contained test kit.	Fingerprick blood sample. No other equipment needed.	Results within 20 minutes at the point of use.

Figure 2: *immunoassay platforms compared*

How lateral flow immunoassays work

The AbC-19 Rapid Test™ is an immunoassay designed to detect IgG antibodies, enclosed in a compact, disposable plastic cassette. The cassette has two windows, one for receiving the blood sample, the other for displaying the test result.

The cassette encloses a precision-manufactured combination of nitrocellulose membranes and other biochemical components in a strip which contains all of the chemicals needed for the test. The process is as follows:

01 **A fingerprick blood sample is taken from the patient using a lancet and pipette.**

02 **An absorbent pad on the assay receives the sample, preventing leakage and ensuring an even flow through the test chemicals.**

03 **Travelling along the strip by capillary action, the sample mixes with biochemicals including gold-labelled signal molecules (marker proteins) and antigen designed to mimic the spike protein of SARS-CoV-2.**

04 **If IgG antibodies to SARS-CoV-2 are present in the sample, they form a complex with the antigen and gold-labelled marker protein, and are captured at the test line producing a pink-purple line. If no IgG antibodies to SARS-CoV-2 are present, no line appears.**

05 **The sample continues along the strip to reach the control line which produces a pink-purple line to illustrate the test has been performed correctly.**

06 **The test results are clearly visible in the result window after 20 minutes.**

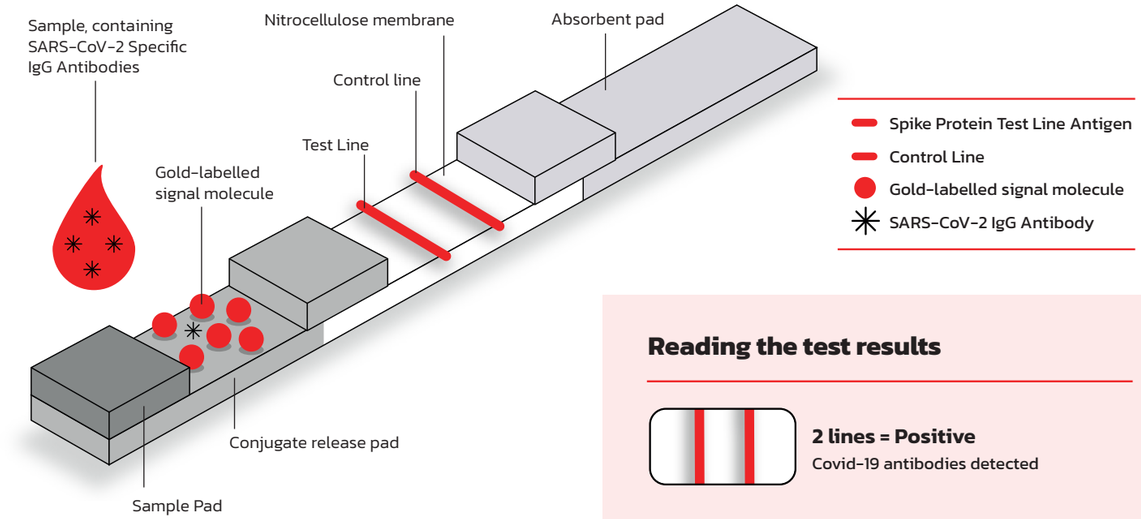


Figure 3: *schematic of AbC-19 lateral flow immunoassay*

Reading the test results

2 lines = Positive
Covid-19 antibodies detected

1 C line = Negative
Test performed correctly and no antibodies present

UK-RTC AbC-19 Rapid Test™

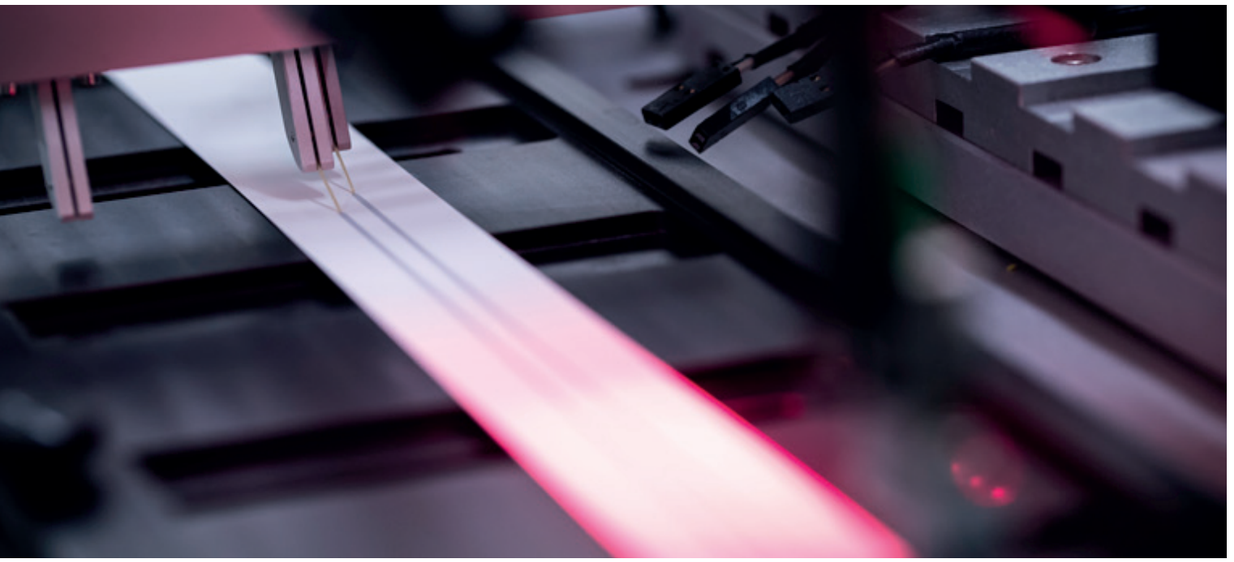
a class-leading lateral flow SARS-CoV-2 immunoassay

Given their speed and ease of use, research has progressed rapidly into highly sensitive LFIAs, suitable for large-scale antibody testing for antibodies to SARS-CoV-2. The UK Rapid Testing Consortium (UK-RTC) has spearheaded this work, developing a new assay, the AbC-19 Rapid Test™, which boasts sensitivity and specificity comparable with laboratory-based platforms (figure 4).

Through Oxford University's involvement in UK-RTC, the research teams at Abingdon Health developed the AbC-19 Rapid Test™ have had access to the same antigens used for developing the vaccine candidate ChAdOx1. The assay is therefore designed to look for precisely the antibodies which the vaccine encourages the body to produce. When tested for sensitivity and specificity with human blood samples, the AbC-19 Rapid Test™ performed strongly, closely rivalling well-established ELISA and CLIA based alternatives, as we outline in figure 4.

> "Our research and development teams have been working two shifts a day, seven days a week, to develop the test. We have deployed nearly fifteen times the number of people that would be on a typical project to deliver this test as quickly as possible."
>
> **Chris Yates, CEO, Abingdon Health**[17]

UK-RTC AbC-19 Rapid Test™ shows sensitivity of **98.03%** and specificity of **99.56%**



Validation results of AbC-19 Rapid Test™

Validation of three different production scale assay batches included testing of patient samples was carried out at Ulster University and Abingdon Health laboratories. This evaluation showed the UK-RTC AbC-19 Rapid Test™ to have sensitivity of 98.03% (95% confidence level 95.03%-99.46%) and specificity of 99.56% (95% confidence interval 98.4%-99.95%).

These metrics were calculated based on analyses of 450 negative samples, taken from individuals before September 2019, and positive samples taken from 203 patients who either had COVID-19 symptoms or a positive COVID-19 PCR result[18].

			Sensitivity		Specificity	
Test name	**Manufacturer**	**Type**	No of samples	Overall Sens	No of negative samples	Overall Spec
Euroimmun Anti-SARS-CoV-2 ELISA (IgG) serology assay	Euroimmun	ELISA	73	94.4%	1344	99.60%
SARS-CoV-2 IgG	Abbott Laboratories	CLIA	88	100.00%	1070	99.60%
Roche Elecsys AntiSARS-CoV-2 serology assay	Roche	ECLIA	29	100.00%	5272	99.81%
AbC-19 Rapid Test™	UK-RTC (Abingdon Health)	LFIA	203	98.03%	450	99.56%

Figure 4: *comparison of leading SARS-CoV-2 immunoassays*[19]

UK-RTC AbC-19 Rapid Test™

a class-leading lateral flow SARS-CoV-2 immunoassay

Interference

A range of substances commonly found in the blood, and known to affect immunoassay readings, were tested using the AbC-19 Rapid Test™ for positive and negative interference. No false positives or false negatives were recorded at the concentrations stated in figure 5.

Substance	Upper limit of normal serum levels mg/dL	Level Tested mg/dL
Unconjugated Bilirubin	2	40
Cholesterol (total)	<200	400
Triglyceride	200	1500
IgG	1400	4,200
IgM	250	750
Haemoglobin	17.5	1000
Biotin	0.117	0.351
Acetaminophen (paracetamol)	5.2	15.6
Acetylsalicylic acid (aspirin)	1	3
Ibuprofen	7.3	22
Caffeine	3.6	11

Figure 5: *even at high concentrations, common sources of interference do not affect test outcomes*

Cross reactivity

Known positive serum samples from other viral infections were tested as follows (the value in square brackets refers to the number of samples tested):

- Seasonal Coronavirus (HCoV- NL63 [x5] and HCoV-229E [x5])
- Influenza A [x5]
- H5N1 Influenza [x1]
- Influenza B [x6]
- Respiratory Syncytial Virus (RSV) [x6]
- Haemophilus Influenzae type b [x5]
- Bordetella Pertussis [x1]

In all cases, no cross reactivity was observed, with all tests demonstrating a negative result on the AbC-19 Rapid Test™.

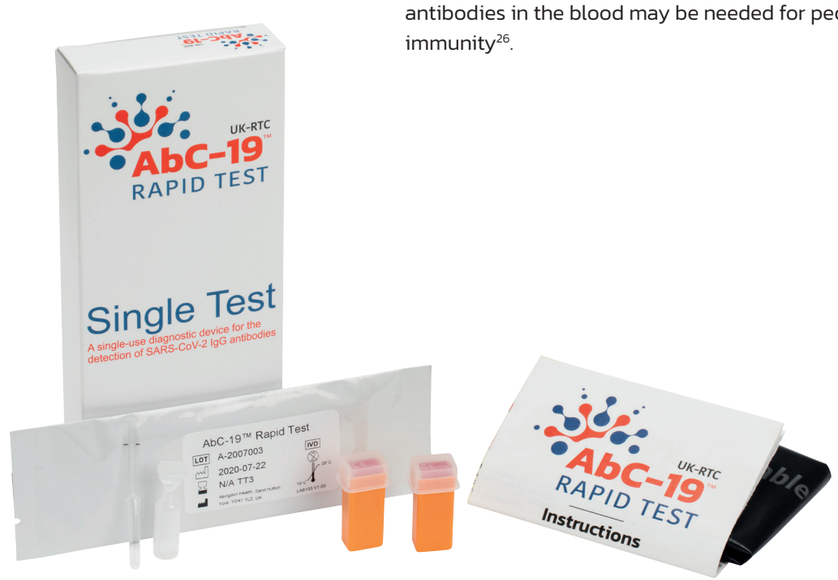
Understanding the link between antibodies and immunity

People with SARS-CoV-2 infection display an IgG antibody response 10 days to two weeks after initial infection. However, considerable uncertainty surrounds the extent to which the presence of antibodies actually protects people from reinfection in the long run. For example, people are regularly reinfected by the common cold, another coronavirus.

One way scientists are looking to understand the immune response to coronaviruses is to measure how fast antibodies disappear from the bloodstream over time. However, the 'acid test' for the immune response involves intentionally "rechallenging" subjects with the same virus for a second time, and monitoring their response.

Rechallenge studies are important for shining a light on the immune system's ability to "recall" an antigen, even when the relevant antibody titres have almost completely disappeared from the blood.

Among patients of SARS-CoV-1, which emerged in Asia in 2003, researchers detected antibody binding titres in patients up to two years after initial infection, though considerable variation in the rate of decline over that time was noted[22].



A separate SARS-CoV-1 study examined how effectively immune cells destroyed the virus upon reinfection. After testing blood serum from 19 recovered SARS-CoV-1 patients, researchers found evidence of virus neutralisation in 89% of cases 36 months after first infection. However, the extent of observed neutralisation activity declined, from 96% of virus cells at month three, to 48% at month 36[23].

A study in 1990 rechallenged a group of 15 volunteers with coronavirus 229E at 12 months. Their antibody titres peaked around two weeks after first infection, declining slowly thereafter. Though detectable at one year, antibody titres were only slightly higher than baseline. When exposed to the virus for a second time, several volunteers became infected, but none fell ill[24].

A rechallenge study of two rhesus macaque monkeys involved reinfection with SARS-CoV-2 at 28 days. Anti-spike antibodies were detected in the serum, and neither of the animals became infected[25].

Though this last study suggests that immediate reinfection with SARS-CoV-2 may not be possible, too many questions remain unanswered to formulate health policy clearly. For example, there are no clear data on how long SARS-CoV-2 antibodies remain in the body, or what concentration of antibodies in the blood may be needed for people to retain immunity[26].

Towards therapies and immunisation

Following the publication of positive safety and immunogenicity trial data for Oxford University's vaccine candidate[27], ChAdOx1 nCoV-19, stage three trials are under way, that will test its ability to provide immunity from infection.

The trials will involve 8,000 volunteers in the UK. In addition, up to 5,000 people in Brazil and around 2,000 in South Africa – both countries which have experienced high coronavirus transmission rates – will take part[28].

Antibody plasma as medicine

Separately, trials are under way where antibody plasma from patients who have recovered from SARS-CoV-2 is used as a medicine for people who are very ill. Researchers in the USA infused 25 patients with severe symptoms with so-called convalescent plasma, and 19 of them showed improvements[29].

In the UK, NHS Blood and Transplant is running larger-scale trials to establish the safety and effectiveness of convalescent plasma in patients who are very ill as a result of SARS-CoV-2 infection. The trials are being conducted across over 170 hospitals[30].

> "The AbC-19 Rapid Test™ and the Oxford University vaccine candidate both target the same antibody response to SARS-CoV-2. The assay could help establish whether people's immune systems are responding to the vaccine in the right way, and, ultimately, could be predictive of a protective immune response."
>
> **Professor Lawrence Young**
> **Virologist, University of Warwick Medical School**

The UK Rapid Test Consortium

The UK Rapid Test Consortium (UK-RTC) was founded in response to a UK Government call for businesses and universities to work together on a new antibody test to be rolled out nationally. Led by Abingdon Health, its members include Oxford University, BBI Solutions, CIGA Healthcare and Omega Diagnostics[20].

Oxford University's involvement is significant: a vaccine candidate under development at Oxford's Jenner Institute has had positive results at phase 1/2 trials[21]. UK-RTC researchers developing the AbC-19 Rapid Test™ had access to the SARS-CoV-2 antigens used by the Oxford research teams, ensuring both the assay and the vaccine target the same antibody response.

Conclusion

It is hard to overstate the practical and policy benefits of rapid, accurate point of use testing. Results are qualitative (i.e. positive or negative) and unequivocal. No workflow is needed for storing, transporting, and analysing samples, nor are protocols required for communicating the results to patients.

With a large number of vaccine candidates nearing readiness for phase 3 (efficacity) trials, LFIA technology, such as the AbC-19 Rapid Test™, can play an instrumental role in assessing initial immune responses, and in determining where follow-up or booster campaigns may be needed.

Moreover, in regions where demand for antibody testing is high, laboratory capacity is limited, and communications links are weak, LFIAs could play a vital role in tracking seroprevalence across entire populations.

Real-world data, in the form of large-scale antibody testing, has emerged as a key driver of both scientific research and health policy during the COVID-19 pandemic. However, the early stages of the pandemic shone an uncomfortable light on the limits of laboratory testing capacity.

The UK-RTC AbC-19 Rapid Test™ LFIA represents the state of the art in antibody assay technology. It exhibits class-leading accuracy, in comparison not only with other LFIA assays, but also with those based on ELISA, CLIA and ECLIA platforms.

As research continues into vaccines and potential treatments for SARS-CoV-2, quick, easy antibody tests suitable for use in the field will prove invaluable in helping governments, researchers and healthcare systems chart a course to a post-COVID-19 world.

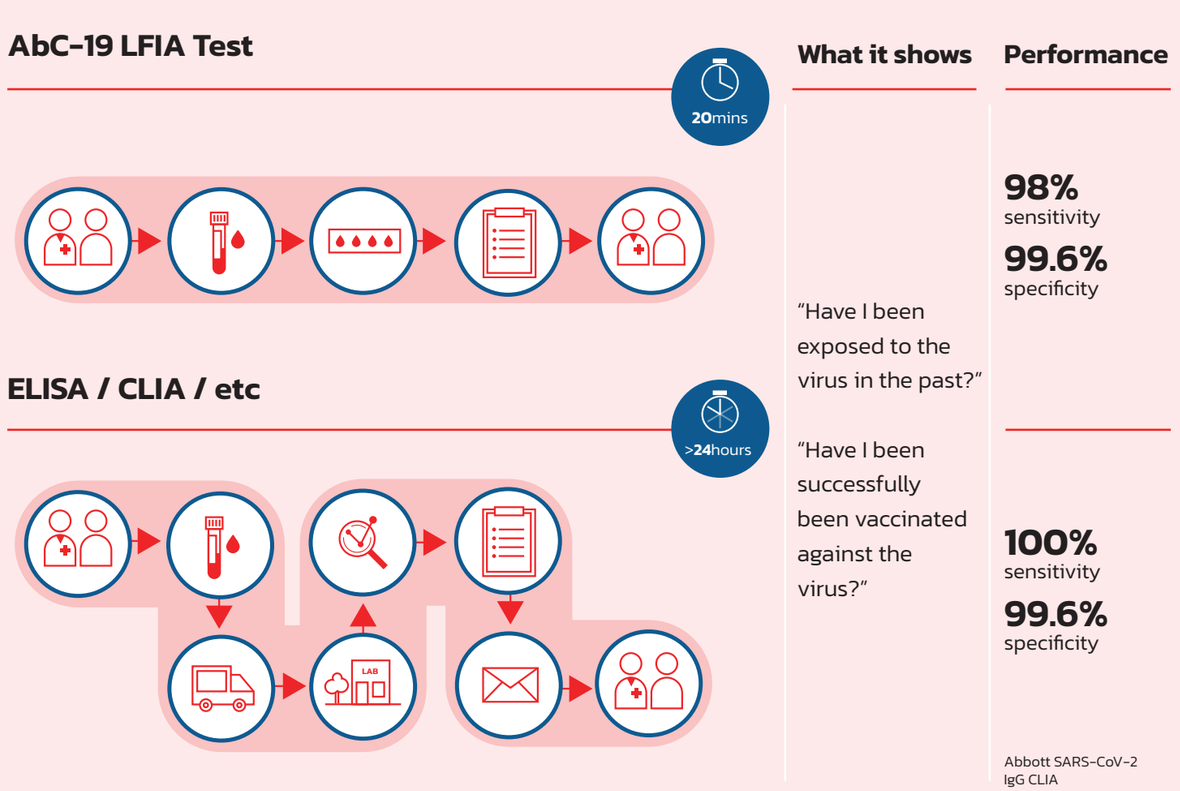


Figure 6: *AbC-19 LFIA workflow is simpler, quicker and comparable to lab-based assays*

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