UK-RTC

AbC-19™

RAPID TEST

Charting the course to a post-COVID world

Why rapid, easy-to-use antibody tests will play an invaluable role in overcoming COVID-19

ABINGDON HEALTH

Innovating rapid testing to preserve and improve life

Leading the UK RTC

Contents

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.

To curb its spread, governments across the world enacted strict lockdown measures in the first half of 2020. However, restrictions were relaxed in many countries during July and August – leading to a "second wave" of infections during the closing months of 2020.

In the UK, the second wave had a pronounced effect on infections and mortality. According to the Office of National Statistics, the percentage of the population in England testing positive for SARS-CoV-2 grew thirteen-fold, from 0.07% in the first week of September 2020, to 0.96% at the end of November. Though the rate of infection appears to be slowing, the number of deaths related to coronavirus continues to rise[1].

Large-scale testing is crucial if governments are to ease lockdowns and chart a return to normal life[2]. Diagnostic testing, to establish if an individual currently has the SARS-CoV-2 virus, is conducted using RT-PCR (reverse transcriptase polymerase chain reaction) to identify viral RNA in the nose and throat. This confirms whether an individual has the virus at that moment. However, RT-PCR can occasionally provide false negative results, and these tests do not provide any insight into prior exposure or immunity[3].

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

The SARS-CoV-2 infection provokes the production of different types of immunoglobin: IgA, IgG, and IgM. While IgM generally are the first to appear in the blood after an infection, this is not always the case with SARS-CoV-2. Researchers have found that IgG are detectable in the blood at the same time – and sometimes even before – IgM[4]. Antibody testing for IgM alone is therefore of limited diagnostic utility.

In addition, it is generally understood that IgG antibodies form the body's long-term response to infection and remain in the blood for several months afterwards[5]. The presence of SARS-CoV-2 specific IgG antibodies in the blood can therefore identify if a person has been infected by the virus in the past, even if they never developed symptoms[6].

The UK-RTC AbC-19™ Rapid Test is a self-contained, highly accurate lateral flow immunoassay designed to give results within minutes at the point of use. The test is designed to confirm the presence of IgG antibodies to the full Spike protein of the SARS-CoV-2 virus.

Deployed at scale across populations, the test can aid policymakers, healthcare systems, the scientific community, and the public at large by:

Assisting health providers, researchers and governments in understanding the extent of the SARS-CoV-2 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[7]

How the body responds to SARS-COV-2

The SARS-CoV-2 virus comprises a single strand of RNA enveloped within four structural proteins: the nucleocapsid (N) protein which contains the RNA, the membrane (M) and envelope (E) proteins which form the cell membrane, and the Spike (S) protein, dotting the membrane (figure 1).

It is the Spike protein which gives coronaviruses their familiar name. A wide variety of diseases from the common cold to Middle East Respiratory Syndrome (MERS), are coronaviruses, and new ones are discovered every year[8].

SARS-CoV-2, the name given to the virus leading to the COVID-19 disease, enters the body when people breathe in contaminated droplets in the air, often produced by coughing or sneezing. The Spike protein binds to receptors on cells in the body called ACE2, commonly found on cells in the nose and throat.

Starting in the lungs, the virus interacts with ACE2 to infect healthy cells and replicate itself. 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 it spreads to ACE2 receptors elsewhere in the body, for example in the heart and kidneys[9].

The human immune response to SARS-CoV-2 involves complex interplay between binding antibodies and neutralising titres. The human immune system has evolved to continually adapt to new threats, and in the case of SARS-CoV-2, it takes 10 to 14 days for IgG antibodies specific to different areas of the virus to appear in the blood[10].

A subset of these IgG titres attacks the infection by preventing the virus from attaching to ACE2 receptors and reproducing itself. This neutralising activity peaks around 23 days after symptoms first appear[11]. Neutralising antibodies are of particular importance for immunity as they can block the virus from infiltrating and replicating within the cell[12].

IgG antibodies for both the Nucleocapsid and the Spike proteins are detectable in the blood after infection, meaning that antibody tests for either can identify whether someone has been infected with the SARS-CoV-2 virus.

Yet testing for antibodies binding to the Spike protein allows for broader conclusions to be drawn. Most people who become infected with SARS-CoV-2 develop neutralising antibodies specific to the Spike[13]. Thus, a positive test reading for Spike-specific IgG antibodies could therefore be predictive of neutralisation. At present, no such correlation has been proven with regard to antibodies to the Nucleocapsid protein[14].

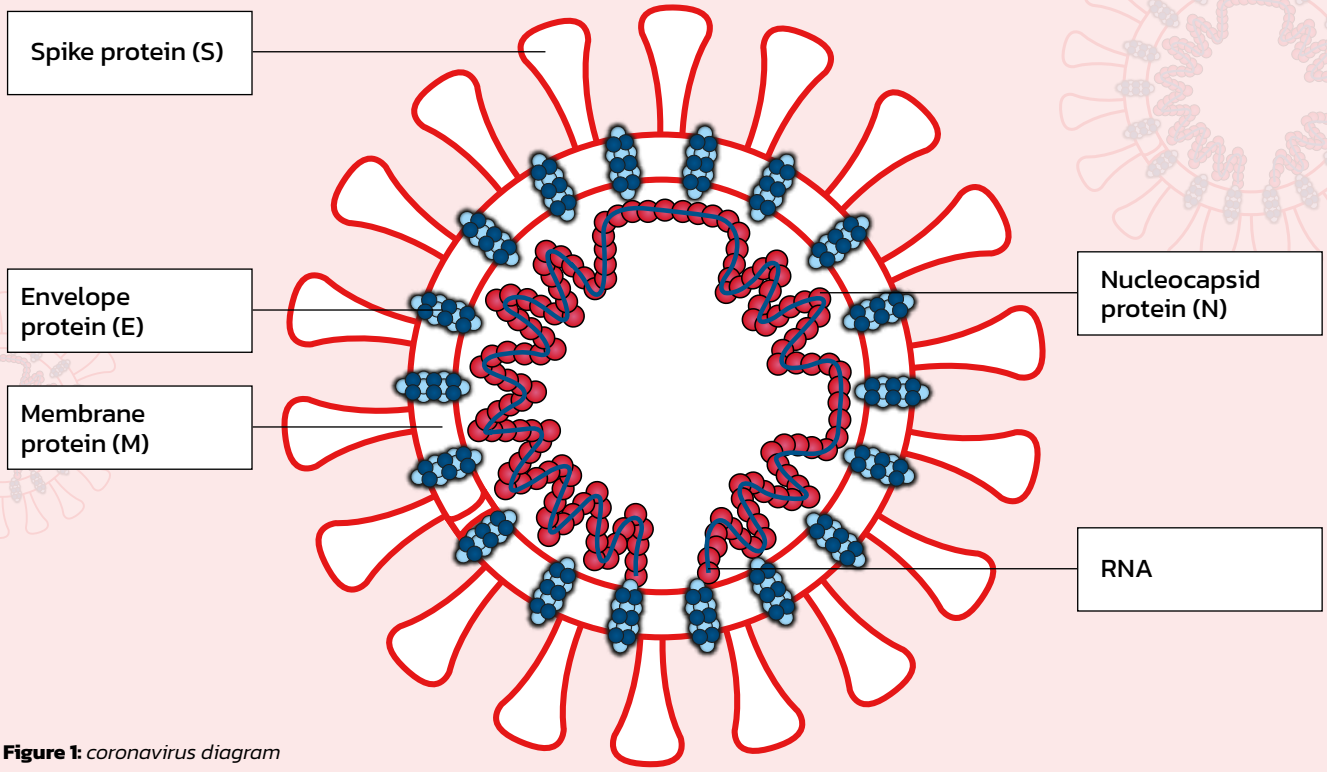

Figure 1: *coronavirus diagram*

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

The SARS-CoV-2 Spike 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 Spike 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[15].

These factors help to explain why the Spike trimer has emerged as the focal point for vaccine development. Of the 180 vaccine candidates currently under development around the world, the vast majority are designed to elicit Spike trimer antibodies one way or another.

The three leading vaccine candidates at time of writing (December 2020), Pfizer / BioNTech's BNT162b2, Oxford University / AstraZeneca's ChAdOx1, and Moderna's mRNA-1273, all express full-length Spike trimer proteins.

BNT162b2 and mRNA-1273 encode the Spike trimer within a modified RNA sequence. The RNA stimulates the harmless expression of the SARS-CoV-2 Spike protein inside the body, and thus the production of antibodies[16]. ChAdOx1 differs in that it uses an engineered, deactivated simian adenovirus as a vector to present fully formed SARS-CoV-2 Spike proteins to the immune system[17].

All three candidates are currently undergoing accelerated regulatory approval in multiple jurisdictions. The UK government granted approval to BNT162b2 in early December 2020[18].

Approaches to antibody testing

There are a variety of approaches to testing for SARS-CoV-2 antibodies. To date, lab-based assays such as ELISAs and CLIAs (see box) 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[19]

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 pro-duce 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*

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 SARS-CoV-2 antibodies. The UK Rapid Testing Consortium (UK-RTC) has spearheaded this work, developing a new assay, the AbC-19™ Rapid Test.

The research teams that developed the AbC-19™ Rapid Test 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 well, closely rivalling well-established ELISA and CLIA based alternatives including those that specifically identify the Spike protein (figure 5).

How the AbC-19™ Rapid Test works

AbC-19™ Rapid Test is an immunoassay designed to detect IgG antibodies to the Spike protein, 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 the chemicals needed for the test. The process is as follows:

01

A finger-prick blood sample is taken from the patient using a lancet and pipette.

02

A sample pad on the assay receives the sample and ensures 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).

04

If IgG antibodies to the SARS-CoV-2 Spike protein are present in the sample, they are captured at the test line by immobilized antigens, producing a pink-red line. If these specific antibodies are not present, no test line appears.

05

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

06

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

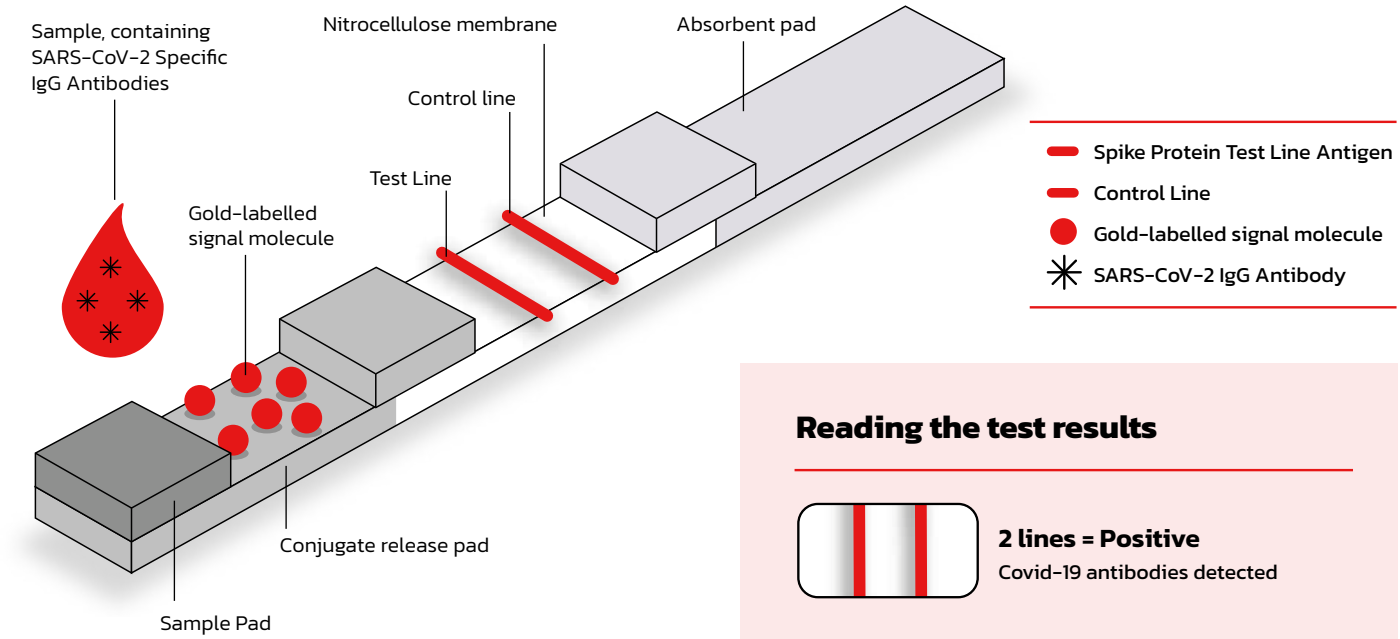

Figure 3: *schematic of AbC-19™ Rapid Test lateral flow immunoassay*

Reading the test results

2 lines = Positive
Covid-19 antibodies detected

1 C line = Negative
Test performed correctly but no antibodies detected

Validation results of AbC-19™ Rapid Test

Validation of three different production batches were tested using 653 patient samples. These evaluation studies 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, and 203 positive samples taken from patients who either had COVID-19 symptoms or a positive COVID-19 PCR result and had been verified as positive using a commercial IgG SARS-CoV-2 ELISA[20].

Part of this study was carried out at Ulster University. It has since been expanded to include 818 samples and is currently pending peer review[21].

					Sensitivity		Specificity	
Test name	**Manufacturer**	**IgG or Total**	**Target**	**Type**	No of samples	Overall Sens	No of negative samples	Overall Spec
Euroimmun Anti-SARS-CoV-2 ELISA (IgG) serology assay	Euroimmun	IgG	Spike protein S1	ELISA	68	94.4%	1344	99.60%
SARS-CoV-2 IgG kit (Abbott Architect i2000SR system)	Abbott Laboratories	IgG	Nucleocapsid protein	CLIA	22	86.46%	1070	99.60%
Elecsys Anti-SARS-CoV-2 serology assay	Roche	Total (IgM & IgG)	Nucleocapsid protein	ECLIA	59	88.1%	5272	99.81%
LIAISON SARS-CoV-2	DiaSorin	IgG	Spike protein S1/S2	CLIA	52	90.4%	1000	98.5%
Atellica-IM SARS-CoV-2 Total Assay	Siemens	Total (IgM & IgG)	Spike protein S1	CLIA	119	97.48%	1091	99.82%
AbC-19™ Rapid Test	UK-RTC (Abingdon Health)	IgG	Full spike protein	LFIA	653	98.03%	350	99.56%

Figure 4: *Comparison of leading SARS-CoV-2 immunoassays*[22]

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.

UK-RTC AbC-19™ Rapid Test shows sensitivity of 97.7%% and specificity of 100%.

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 no workflow is needed for storing, transporting, and analysing samples. The addition of an accompanying smartphone app to read and transfer the data securely and efficiently could afford the ability to register the level of IgG over a period of time and record it centrally.

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

The AbC-19™ Rapid Test LFIA is an effective diagnostic tool for SARS-CoV-2. The test is designed to specifically reveal antibodies to the full SARS-CoV-2 Spike protein. Furthermore, the test displays high levels of accuracy, in comparison not only with other LFIA assays, but also with those based on ELISA, CLIA and ECLIA platforms when all are compared in the same way to PCR positive and known negative samples.

With vaccines now being approved for use in the general population, large-scale immunisation programmes are under way, and with them comes the prospect of an eventual return to normal life.

However, many questions remain unanswered. We do not yet know how long the vaccines' protection will last, or the extent to which vaccination prevents the virus from spreading. Researchers are also still working to establish whether previous infection with SARS-CoV-2 can prevent people from becoming infected a second time.

These uncertainties serve to underline the importance of rapid, easy to use antibody tests suitable for use outside of the laboratory. 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 pandemic. Moreover, research studies have underlined the pivotal role of the SARS-CoV-2 Spike protein – and the body's response to it – in developing immunity.

As efforts intensify to build large-scale immunity to SARS-CoV-2, the AbC-19™ Rapid Test will be invaluable in helping governments, researchers and healthcare systems chart a course to a post-COVID-19 world.

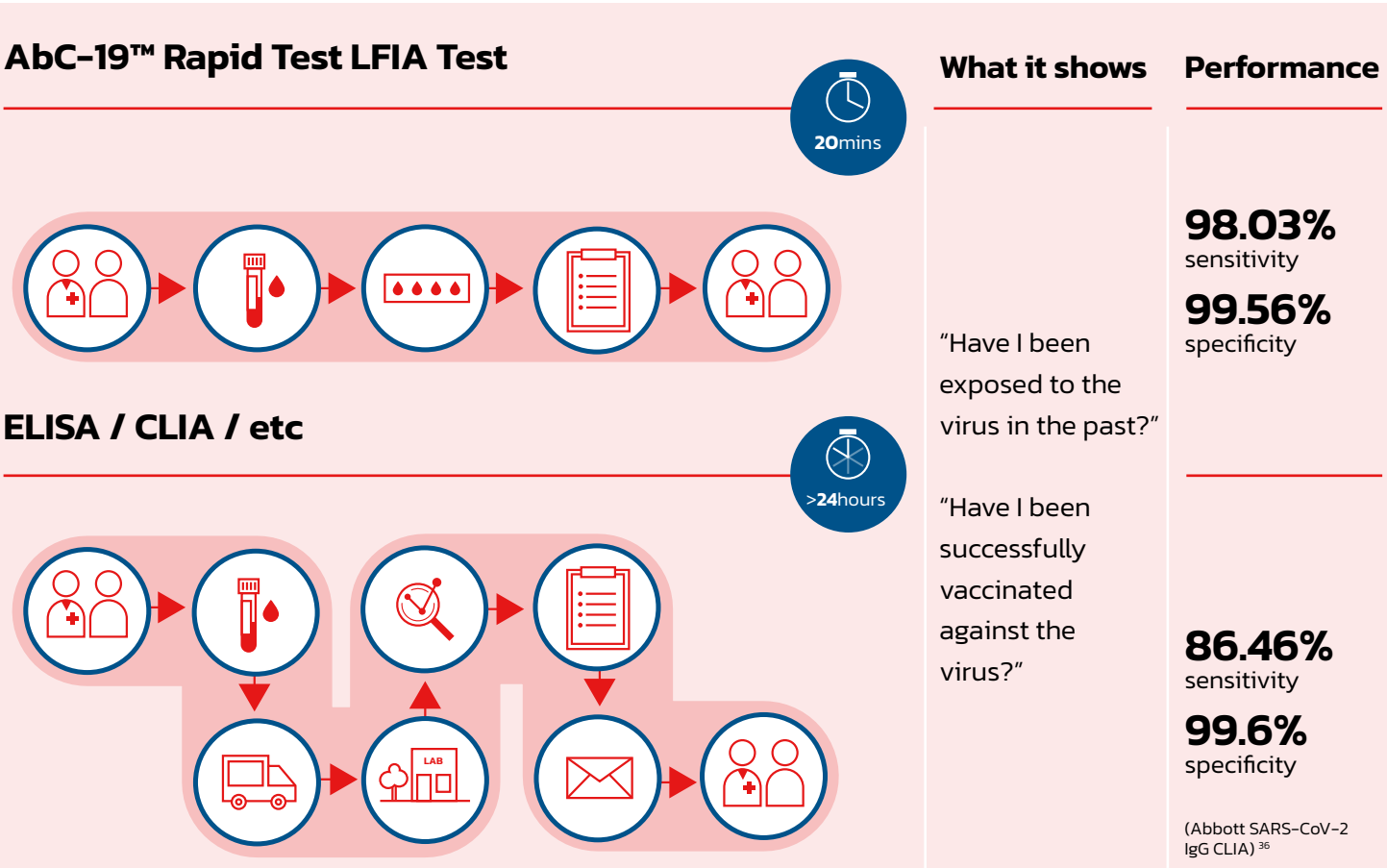

Figure 6: *AbC-19™ LFIA workflow is simpler, quicker and comparable to lab-based assays*

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