Rapid antibody testing

A critical tool in understanding COVID-19 prevalence

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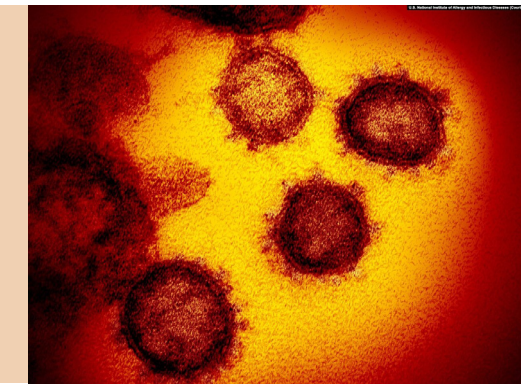

Detection of a serological response to SARS-CoV-2 is an integral part of the UK's national testing strategy.

Here, Carolyne Horner considers the applications of rapid SARS-CoV-2 antibody detection and discusses how the appropriate use of such testing could navigate the UK out of lockdown and beyond.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an unsegmented, single-stranded, positive-sense, enveloped RNA coronavirus causing the ongoing pandemic.[1] The 30kb genome encodes four structural proteins that form the viral particle: a spike (S) protein, a nucleocapsid (N) protein, a membrane (M) protein and an envelope (E) protein.[1]

SARS-CoV-2 is the cause of COVID-19 (coronavirus disease 2019), an acute respiratory illness with a disease spectrum ranging from asymptomatic or mild symptoms (in most cases), to severe illness and life-threatening acute respiratory distress syndrome (ARDS).[2]

Since the WHO announced pandemic status (11 March 2020),[1] SARS-CoV-2 has become a significant health burden. To date, there have been >132 million cases and >2.8 million deaths worldwide.[3]

Accurate diagnosis is paramount

Accurate diagnosis of COVID-19 is fundamental for appropriate, effective and timely patient care, and reduction of disease transmission in the community.[4] Diagnostic strategies serve different purposes and comprise detection of SARS-CoV-2 RNA or antigen, or detection of antibody raised to SARS-CoV-2.

SARS-CoV-2 antigen tests are used to determine if a person is carrying the virus, either symptomatically or asymptomatically, and as such could be a source of infection to others. Antigen tests commonly involve detection of virus from a respiratory sample, such as a nasopharyngeal/oropharyngeal/nasal swab.[5] The gold-standard method of SARS-CoV-2 detection remains the reverse transcription polymerase chain reaction (RT-PCR),[2] a laboratory-based technique that commonly detects more than one target sequence, such as RNA encoding ORF1b, the spike or nucleocapsid proteins. [4,6]

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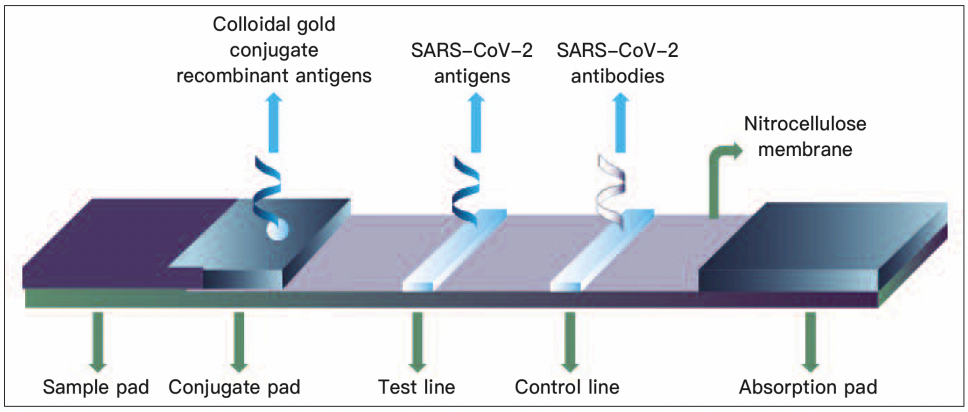
SARS-CoV-2 antibody tests, on the other hand, are used to determine if an individual has encountered the virus previously and usually require a whole blood, plasma or serum sample to be taken after the onset of symptoms (>10 days).[6] SARS-CoV-2 antibody tests commonly detect antibodies to the spike and/or nucleocapsid proteins.[6]

Unlike SARS-CoV-2 antigen detection, there is no gold standard method for SARS-CoV-2 antibody detection, meaning that it can be difficult to determine unbiased test sensitivity when evaluating new serology assays.[7] Laboratory-based chemiluminescence (CLIA) or enzyme-linked immunosorbent assays (ELISA) are commonly used to detect SARS-CoV-2 antibodies, and may be used in combination as a composite reference.[8] CLIA and ELISA methods have a high sample throughput, but usually require a venous blood sample, which has additional resource implications.



Characteristics of lateral-flow immunoassays for antigen and antibody detection

Unlike RT-PCR and CLIA or ELISA methods, lateral flow immunoassays (LFIAs) offer a rapid point-of-care (POC) solution for COVID-19 diagnosis. They comprise a single use cassette that provide a result within 10-15 minutes without the need for a sample to be sent to the laboratory, thereby offering flexible, decentralised testing options for patient diagnosis and purposes of public health surveillance.[9]

Unreliable test worse than no test

According to a UK government policy paper, "an unreliable test is worse than no test".[10] As SARS-CoV-2 genome sequence information became available, manufacturers were quick to develop antibody LFIAs; by May 2020, there were over 200 antibody tests on the market or in development.[5] Concerns were raised about first-generation antibody LFIAs, which had notoriously poor sensitivity and specificity.[11,12]
While there were any number of published studies that proposed to evaluate performance, many were characterised by small sample size and associated with a high risk of bias.[11,12]

In the UK, the Medicines and Healthcare products Regulatory Authority (MHRA) regulates the quality and performance of *in vitro* diagnostic (IVD) medical devices. To do this MHRA publish Target Product Profiles (TPP), which are a series of desirable/acceptable characteristics for any particular IVD.[13]

Fortress COVID-19 Total Antibody Device

The Fortress COVID-19 Total Antibody Device is a single-use, closed system, cassette-based *in vitro* immunochromatographic device for the qualitative detection of total antibodies against SARS-CoV-2 in human serum, plasma or whole blood specimens.

The kit is intended for screening of patients suspected for infection with SARS-CoV-2, and as an aid in the diagnosis of COVID-19.

The device comprises a control line and two test lines, one for detection of IgM and the other for detection of IgG.

Details of rapid LFIA antigen/antibody tests and Fortress test information

Advantages of rapid LFIA antigen/antibody tests

- Point of care (not laboratory based)
- Rapid results (within 15 minutes)
- Inexpensive
- Easy to interpret (control line, test line or invalid)
- No technical requirements (equipment or knowledge)

	Fortress Coronavirus Antigen Nasal Swab Rapid Test	Fortress COVID-19 Total Antibody Device
Suitable population	Those suspected of being infected with the virus	Those suspected to have previous exposure to the virus
Molecule detected	Nucleocapsid protein antigen	Antibodies to spike protein
Sample required	Respiratory swab (nasopharyngeal or nasal)	Fingerprick blood (5–10 µL required)
Optimum time to diagnosis	Within 10 days of symptom onset	>21 days after symptom onset

Fortress Diagnostics: an introduction

Fortress Diagnostics

Fortress Diagnostics is a global provider of *in vitro* diagnostics.

With over 20 years of experience, the company designs, develops, manufactures and distributes anextensive portfolio of clinical diagnostic tests from its ISO1485:2016-accredited facility in the UK.

The company provides medical testing solutions to immunology, haematology and serological laboratories in hospitals, medical centres, clinics, blood banks and research institutions in over 100 international markets.
www.fortressdiagnostics.com

Applications of SARS-CoV-2 antibody tests

Diagnostically, SARS-CoV-2 antibody tests are useful for patients with suspected COVID-19 who have tested negative by RT-PCR; this may occur during the later stages of the disease when detectable virus may no longer be present.[14] SARS-CoV-2 antibody tests are also useful for the diagnosis of asymptomatic/mild cases who may not have been tested for SARS-CoV-2 at all.

In terms of disease prevalence, antibody tests may be used for community screening of immune response to SARS-CoV-2. Monitoring the seroprevalence in the wider population may serve to: [14,15]

- identify/quantify the extent to which a population has been exposed to the virus,
- make predictions for how the pandemic may continue in the UK,
- characterise the epidemiology of the disease, such as identification of groups most at risk of infection of severe disease, and
- inform public health policy during the pandemic and longer term.

Inexpensive point-of-care tests that require minimal training to complete and provide easy-to-interpret results, provide a source of inexpensive, widespread testing without direct laboratory support, enabling a much wider reach and impact of testing.

Rapid SARS-CoV-2 antibody tests: associated caveats

As with any diagnostic test, there are certain caveats associated with the application and widespread rollout of rapid antibody tests.

While rapid antibody tests offer a practical approach to identify individuals with previous exposure to SARS-CoV-2, further research is required to understand how antibody production maps to protection from infection. Currently there is no global standard for correlates of protection to SARS-CoV-2.[16]

Additionally, a negative result does not preclude the possibility of a previous encounter with SARS-CoV-2. There are multiple explanations for a negative result, not least the complexity of the human immune system. Various factors influence antibody production, such as when the blood sample was taken (LFIA are known to have variable sensitivity if used <21 days after symptom onset),[12] age (extremes of age may not produce antibodies at the same speed/level as other age groups), and general health/comorbidities, (conditions causing suppression of the immune system).

Identifying the most suitable rapid antibody test for REACT 2 national survey

In the UK, the REACT (REaltime Assessment of Community Transmission) programme[17] aims "to improve understanding of how the COVID pandemic is progressing across England".

Commissioned by the Department of Health and Social Care, REACT is a collaboration comprising Imperial College London, Ipsos Mori (a market research company), and Imperial College Healthcare NHS Trust.

The programme comprises two parts:

- REACT 1: monthly home-based antigen testing to determine current SARS-CoV-2 infection levels
- REACT 2: six weekly home-based antibody testing to determine how many people have been exposed to SARS-CoV-2 and have developed antibodies

The REACT 2 arm of the programme requires a reliable, rapid antibody POC test for use throughout the longevity of the study. To identify the highest performing LFIA available on the market, the REACT team have evaluated the suitability of a number of rapid antibody kits in two rounds of testing. [8,18] LFIA were selected for inclusion based on sensitivity and specificity data reported by the manufacturer and the availability of kits for large scale testing.

Table 1. Summary of REACT 2 SARS-CoV-2 antibody lateral-flow immunoassay evaluations: performance data of fingerprick self-test compared with S-ELISA and/or hybrid double antigen binding assay.[8,18]

REACT Evaluation Period	Lateral-flow Immunoassay	Antibody detected	Target for antibody (proteins)	Sensitivity		Specificity		Invalid (n)
				(95% CI)	n/N	(95% CI)	n/N	
Round 1 (May 2020)	Menarini	Separate IgM & IgG	S1, S2, N	96% (84.9–99.5)	43/45	97.8% (96.1–98.9)	489/500	3
Round 1 (May 2020)	Fortress	Separate IgM & IgG	S	84% (70.5–93.5)	38/45	98.6% (97.1–99.4)	493/500	3
Round 1 (May 2020)	Biopanda I	Separate IgM & IgG	S, N	67% (55.5–76.6)	56/84	99.8% (98.9–100)	499/500	0
Round 1 (May 2020)	Biosure/ Mologic I	IgG only	N	61% (46.2–74.8)	30/49	97.2% (95.3–98.5)	486/500	8
Round 1 (May 2020)	Wondfo	IgM/IgG combined	S	22% (13.1–33.1)	16/73	99.4% (98.3–99.9)	497/500	0
Round 2a (June-July 2020)	Surescreen I	Separate IgM & IgG	S	86% (72.7–94.8)	38/44	99.8% (98.9–100)	499/500	0
Round 2a (June-July 2020)	Panbio (Abbott)	Separate IgM & IgG	N	77% (61.4–88.2)	33/43	99.8% (98.9–100)	499/500	0
Round 2a (Sept 2020)	AbC-19 (Abingdon)	IgG only	S	69% (53.8–81.3)	33/48	99.8% (98.9–100)	499/500	4

S: spike; N: nucleocapsid

In Phase I of Round 1, five LFIAs were assessed by finger prick test in the research clinic and with sera tested in the laboratory (Table 1). In Phase II of Round 1, another six LFIAs were identified as eligible for inclusion and were tested with sera in the laboratory.[8]

To assess concordance, the evaluation comprised the results of a finger prick LFIA as read by the participant, the results of a finger prick LFIA as read by a member of the study team, and comparison of the results of testing sera using two in-house ELISA, one targeting the spike protein and the other a double antigen binding assay (targeting the spike protein, plus the receptor binding protein). A composite of the two ELISA results was considered the gold standard. Of the 11 kits tested in Round 1, only four had sensitivity >85% against sera tested in the laboratory.[8]

Based on performance results (Table 1), plus concordance between participant and observer finger prick result interpretation, and the availability of the kits on a large scale, the Fortress rapid antibody test was identified as the LFIA of choice for the REACT 2 study. [8]

Rapid home-based SARS-CoV-2 antibody testing

Given that the basis of REACT 2 is a home-based antibody test every six weeks, it was also necessary to determine how rapid antibody LFIAs performed in a non-laboratory, non-clinic, unsupervised use setting. Participant useability of the Fortress rapid antibody LFIA plus one other LFIA was assessed.[19] Compared with the other LFIA considered in the study, the Fortress antibody test had "almost perfect agreement" between participant and clinician interpreted results (98.9% [154/164] agreement for positive results, 97% [228/235] agreement for negative results and 98.4% [120/122] agreement for invalid results). Based on these concordance results, the high level of usability and acceptability, low invalid result rate (4.8%, 137/2848) and results of the laboratory evaluation, the Fortress rapid antibody test was confirmed as the LFIA of choice for the REACT 2 study.[19]

Fortress antibody test most suitable for REACT 2

As the pandemic has progressed, new rapid antibody LFIAs have become available and existing antibody LFIAs have been improved. REACT 2 continually evaluate and search for the highest performing antibody LFIA on the market and the Fortress COVID-19 antibody test has been included in a second round of evaluation based on the same testing principles as Round 1.[18]

In Round 2, nine LFIAs (seven new to the evaluation and two identified from Round 1 Phase II) were assessed with sera tested in the laboratory, as before. In order to proceed to specificity testing, any newly evaluated LFIA had to reach >84% sensitivity (that of the in use LFIA); three of the nine LFIA reached this requirement and achieved comparable results in a laboratory setting using serum samples (Table 1).[18]

Only one other LFIA had comparable sensitivity (86%) to the Fortress LFIA (84%) on finger prick testing, however, this result was not considered "significantly superior" to justify a change in test and the Fortress rapid antibody test remains the kit of choice for REACT 2 surveillance.[18]

The REACT 2 evaluation emphasizes the need for validation of LFIA to be completed in their intended use situation (i.e., finger prick tests using peripheral blood, the intended sample for home-based testing). There were significant differences in sensitivity for two LFIA when tested in the laboratory with sera versus finger prick peripheral blood.[8,18] While whole peripheral blood is a crude sample type when compared with serum, a finger prick blood sample is an easy sample to take without the need for venepuncture.

Pioneering results of seroprevalence studies in the UK

As yet, none of the LFIAs evaluated by REACT have achieved the stringent sensitivity and specificity requirements set by the MHRA for a LFIA to be used as a clinical decision-making tool at an individual level;[13] this limitation is not seen as a barrier to population-based seroprevalence studies when home-based testing is required.[18]

By taking into account the sensitivity and specificity of the LFIA used, it is possible to estimate the seroprevalence within the community.[18] Participants must be made aware that the results of the LFIA are for research purposes only and that government guidelines must be continued to be followed, whatever the result of the antibody self-test.[19]

Early results from the REACT 2 programme, up to mid-July 2020, indicated that 6% (5,544/109,076) participants had antibodies to SARS-CoV-2, with one third (32%), antibody positive individuals reportedly being asymptomatic. The study estimated, in England up to the end June 2020, that 3.36 million people had been infected with SARS-CoV-2, and identified certain ethnic groups, and health and care home workers were most at risk of SARS-CoV-2 infection.[20] Later results from the programme indicate a declining prevalence of antibody positivity in adults across all age groups in England following the first peak of infections (6% [20 Jun - 13 July], 5% [31 Jul – 13 Aug], 4% [15 - 28 Sept]).[21]

The most recent REACT 2 report[22] describes SARS-CoV-2 antibody prevalence in England during early 2021 (26 Jan – 8 Feb). In this period of widespread vaccine rollout and following the second wave of infection, 13.9% participants had antibodies to SARS-CoV-2; 37.9% among vaccinated participants and 9.8% among unvaccinated participants. Seroprevalence was high among participants who had received two doses of Pfizer-BioNTech vaccine (91.1%) and among those who had received a single dose of vaccine following natural infection (88.8%).[22]

In addition to the REACT programme, UK Biobank,[23] a unique, large scale biomedical database and research resource, has completed a SARS-CoV-2 Serology Study, which also used home-based rapid antibody tests to detect antibodies to SARS-CoV-2.[23]

Recruiting 88,000 participants throughout the UK the study identified an increase in seroprevalence between May 2020 (7%) and December 2020 (9%); 99% participants had SARS-CoV-2 antibodies at three months after infection compared with six months after infection (88%), and significant differences in seroprevalence by age, ethnicity, geographic region and socioeconomic status across the UK.[23]

A second UK Biobank seroprevalence study, the Coronavirus Self-Test Antibody Study, is ongoing. This study aims to investigate the long-term health effects of COVID-19. Phase I recruited between February – March 2021 and included the Fortress rapid antibody kit; Phase II is currently recruiting.[24]

Rapid antibody testing supporting the roadmap out of lockdown

Rapid SARS-CoV-2 antibody tests are an important complement to laboratory-based testing and quantitative research. Within a year of the pandemic, the use of rapid SARS-CoV-2 antibody tests have become a fundamental tool in our understanding of the prevalence of COVID-19.

The availability and systematic validation of rapid antibody LFIAs available in the UK has enabled widespread population-based, surveillance of the seroprevalence of SARS-CoV-2. Results from longitudinal studies, such as REACT and UK Biobank, will further inform our understanding of the virus, the disease spectrum it causes, and the future course of the pandemic.

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