Tests for antibodies against SARS CoV2. July 2020. Wendy Barclay and Peter Openshaw.

The purpose of this paper is to address three questions:

1. Are antibody tests able to provide information on a person's immunity?
2. Is someone with antibodies likely to be able to still transmit the virus without becoming infected themselves?
3. How reliable are the antibody tests that are commercially available?

Background on antibodies to the antigens of SARS CoV2.

SARS CoV2 is a newly emerged coronavirus that is the causative agent of COVID-19, a respiratory disease.

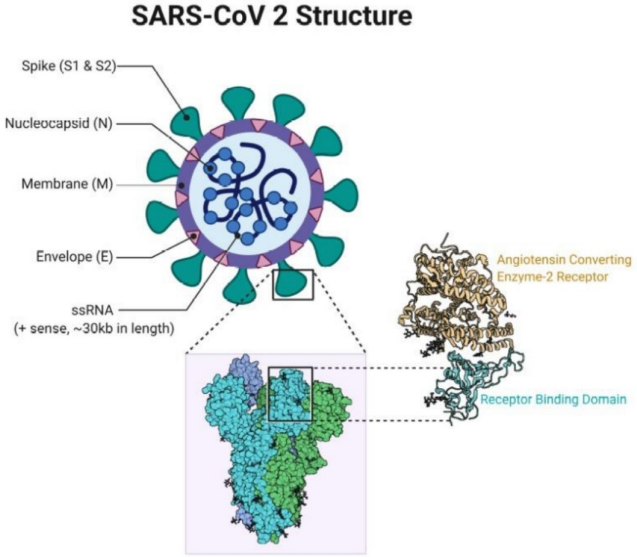
Viral antigens N and S

The virus has a large positive sense RNA genome, that is associated in the virus particle with many copies of a virally encoded nucleocapsid protein, N. The virus particle has an external envelope with lipids derived from the host cell on which many copies of a trimeric spike protein, S, are displayed.

S is the virus attachment protein through which virus binds to target cells, by interacting with the primary receptor, ACE2. The domain of S that interacts with ACE2 is known as the receptor binding domain RBD. Since the viral antigens are foreign proteins, people infected by SARS CoV2 generate an immune response and make antibodies that bind N and S, and other viral proteins.

The presence of antibodies specific for SARS CoV2 viral proteins therefore indicate that a person has been infected by the virus. Tests that measure antibodies to N or S can be used in seroprevalence studies. Antibodies that bind the RBD of S and block the ability of virus to attach to target cells are said to neutralize the virus.

SARS- CoV 2 Structure. Contributed by Rohan Bir Singh, MD; Made with Biorender.com



Neutralizing antibodies mediate protection in animal studies

In animal models, the presence of neutralizing antibodies correlates with protection. Animals that had been previously infected and mount a polyclonal antibody response (i.e. diverse antibodies against different sites/proteins) that includes neutralizing antibody did not become infected when re-challenged after 28 days (Imai et al PNAS.2020).

Animals to which single (monoclonal) neutralizing antibody had been passively transferred are also protected from infection at high doses of transferred antibody, and from disease at lower doses. **This indicates that neutralizing antibodies are necessary and sufficient for protection**, at least in those animal models. This concept underpins the use of therapeutic antibodies or convalescent sera for treatment of patients with COVID.

What is not currently known is what level of antibody is required to confer protection in humans against a natural dose of SARS CoV2, such as would be faced during a transmission event.

In answer to the question:
Are antibody tests able to provide information on a person's immunity?
Tests that measure antibodies to RBD might be a correlate of immunity, but

a) we don't yet know what levels of such antibodies are required for protection,
b) we don't know for certain that protection can be conferred by neutralizing antibody in humans, as opposed to animal models, because there has not been a human challenge either in an experiment or during a known natural re-exposure.

Do antibodies protect against transmission?

In studies carried out at the Common Cold Unit in the 1980s, volunteers were infected deliberately with a seasonal coronavirus and then challenged one year later. Antibodies that had been induced during the immune response in the first infection had waned to low levels after one year and some of the volunteers became re-infected. However, those volunteers did not become ill, even though they did shed virus.

Thus there is a chance that people with naturally induced or vaccine induced antibodies to SARS CoV2 might become re-infected upon exposure to virus at a later time point but not show symptoms.

The question is:

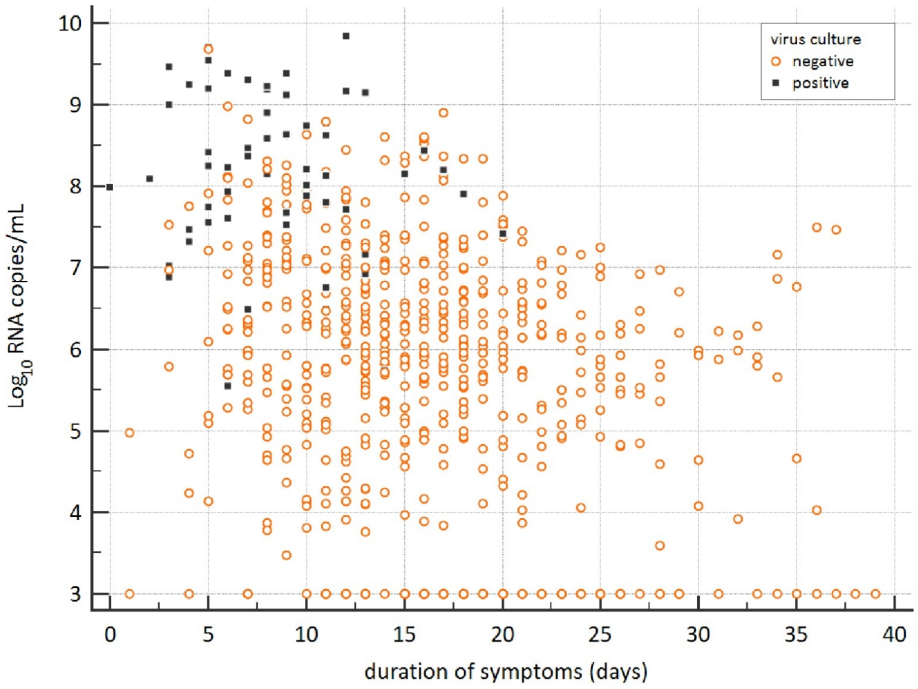
Is someone with antibodies likely to be able to still transmit the virus without becoming infected themselves?

(we *assume this means without becoming* ***symptomatic****- if a person is not infected they can't transmit*)

It is reasonable to assume that people with antibodies are less likely to be infected, but we at present do not know how much protection is conferred by an immune response, and for how long. It is possible that those with antibody can be infected and that symptoms might be supressed despite localised infection (i.e. in the nose).

Virus transmission can take place from presymptomatic and asymptomatic individuals. Therefore, people without symptoms can still infect others if they have sufficient replicating virus in the nose, lungs or throat.

However, some people who are recovering from infection may have viral RNA (detected by the swab PCR test on a nasal swab) but do not shed live virus that can be grown in a lab or infect others. The loss of viral infectivity happens at about day 8-14 when the viral load (detected by PCR) is in decline and antibody is starting to appear (MEDRXIV-2020-125310v1-vanKampen).



It is unlikely that people recovering from SARS-CoV-2 infection and have developed antibody in the nasal secretions, blood or serum are still infectious. However, this has not been formally proven.

Modelling shows that peak transmission coincides with or even slightly precedes the onset of symptoms (https://doi.org/10.1038/s41591-020-0869-5). This time-point is when highest viral loads are present in the upper respiratory tract, as indicated by the levels of viral RNA or of infectious virus recovered from nose and throat swabs.

The presence of antibodies is associated with declining viral replication. In the animal studies mentioned above, peak virus titres were lower in animals that received passive transfer of antibody. In vaccinated non-human primates that made

an serum antibody response, viral titres were lower in lungs but not significantly reduced in the upper respiratory tract (https://doi.org/10.1101/2020.05.13.093195).

If recapitulated in humans, this leaves open the possibility of nasal infection in people in whom lung infection is prevented by antibody, a situation that is seen in some other viral diseases (e.g. RSV prevention by palivizumab). This means that nasal infection might still occur and the virus be transmitted to others if the antibody is systemic (circulating) and not local (mucosal).

Commercial tests for antibodies to SARS CoV2

There are many different antibody tests now described for the new virus. A recent systematic review and meta-analysis covered reports of 40 studies in both peer reviewed and posted literature (Bastos et al. BMJ.2020).

Three different types of commercial antibody tests are available:

Enzyme Linked Immunosorbent Assays, (ELISAs), and Chemiluminescent Immunoassays (CLIAs) must be run in a laboratory using specialist equipment whereas Lateral Flow Immunoassays (LFIAs) could be used as point of care PoC tests, even in the home, by people using a fingerprick bleed. In general the CLIAs and ELISAs have higher sensitivity than LFIAs, and all tests have similar specificity.

CLIA and ELISA tests could be run on blood samples collected by individuals in their own home using a fingerprick that could be collected into capillary tubes or as dry blood spots and then sent in to testing centres but the tests have not been optimized for this purpose.

In the BMJ meta-analysis, the pooled CLIA sensitivity (ability to detect antibodies in people who were known to have been infected) was 98%, ELISA 84% and LFIA 66%, but there is wide variation across the same test type depending on the manufacturer.

Most of the studies available so far used sera from hospitalized patients, sometimes collected less than 21 days after symptoms, even though higher antibody titres are found after this time point. Fewer studies have assessed antibody following asymptomatic infection but a recent paper found lower antibody titres that waned more rapidly in this group (https://doi.org/10.1038/s41591-020-0965-6).Studies rarely use fingerprick blood to evaluate the tests, but rather serua in a lab, even for validating PoC tests.

The DHSC funded REACT2 study aims to use PoC tests in the home to measure seroprevalence in the UK population. A substudy assessed LFIAs from different manufacturers for sensitivity and specificity using blood taken from NHS staff who had a known PCR positive infection with SARS CoV2 more than 21 days previously. NHS staff were asked to perform the LFIA test themselves and also to give a venous blood sample for sera to be tested in the laboratory using either the same LFIA or two different laboratory ELISAs. So far 14 different LFIAs have been assessed.

In brief, the best of the LFIA tests show sensitivity of 96.4% to detect using fingerprick blood and specificity of 99.8% against 500 sera collected pre 2019, which is in line with ELISA or even CLIA.

In conclusion, although early reports suggested poor performance that would preclude utility, some of the commercial tests that have come to market more recently are performing well.

Current UK seroprevalence levels are 7.8% nationwide, 13.6% in London (PHE latest report). Using even the best of the tests available there will be a substantial number of people whose test results will be called wrongly: if seroprevalence is 10%, a test with 97.8% sensitivity and the same specificity calls incorrectly 2 people in every 1000 as not having been infected when they had been, and 20 people in every 1000 as being potentially 'immune' when they have not been infected.

If immunity passports based on the commercial antibody tests change behaviour, or practise, the low specificitycould have serious implications.
At present there is insufficient evidence that knowledge of an individual's immune status can be relied upon to enable a change in behaviour. This is because the tests themselves have lower than ideal specificity, and we do not yet know that a positive results in such a test guarantess protective immunity.