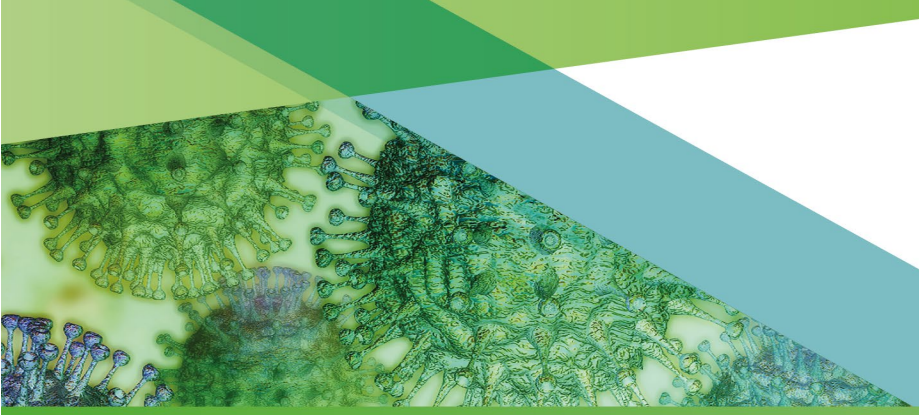

Considerations for the use of antibody tests for SARS-COV-2– first update

10 February 2022

Key messages

- At present, antibody tests are mostly used in research studies (mainly sero-epidemiological) at population level rather than for individual diagnosis of COVID-19 cases.
- A positive antibody test result can indicate a previous infection or vaccination but cannot be used to determine whether an individual is currently infectious or protected against infection.
- In the absence of a positive diagnostic test result, antibody tests cannot determine the time of infection.
- The antibody titres that correlate with protection from infection are currently unknown.
- There are a variety of antibody tests[1] available and it is extremely difficult to compare their results due to the diversity and lack of standardisation.
- Antibody tests that target the spike protein are unable to distinguish between those who have been previously infected and those who have received at least one dose of a SARS-CoV-2 vaccine.
- There is a risk that the antibodies detected by the commercial tests currently in use will not prevent infection with newly emerging SARS-CoV-2 variants.

Introduction

Antibodies are generated as part of an individual's immune response against SARS-CoV-2 and are produced by cells known as B cells. Antibody tests can detect these antibodies in the blood or saliva of individuals previously infected and/or vaccinated against SARS-CoV-2. Several different antibody tests have been developed using different parts of the virus as targets (epitopes - e.g. spike protein or nucleocapsid) and these are able to detect various antibody types, mainly the immunoglobulins IgM and IgG.

- IgM antibodies are one of the first isoforms of antibodies produced against viral infection; they are more short-lived and decline more quickly than IgG antibodies [1]. Interpreting their significance requires clinical and laboratory experience.
- IgG antibodies can be reliably detected 14 days after a SARS-CoV-2 infection or vaccination but are longer lasting than IgM. Depending on the epitope and factors such as disease severity, duration of infection, age, genetic factors, co-morbidities and the performance characteristics of the test used, IgG spike antibodies can be detected in most individuals for at least one year [2-4].

Antibody tests can be qualitative, indicating whether antibodies are present, or they can be quantitative, determining the level of antibody present in an individual.

[1] Qualitative or quantitative, detecting antibodies of different types (e.g. IgG, IgM), neutralising or binding, and against different proteins (e.g. the spike protein or the nucleocapsid).

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In most individuals, antibody levels peak four to five weeks after infection and will decrease in subsequent months after the infection has cleared [5]. So far, neutralising antibodies that block the virus entry to host cells have provided the best indication of protection from SARS-CoV-2 infection [6-10]. In addition to antibodies produced by B cells, memory T cell responses are also likely to contribute to protection, particularly from severe disease. Furthermore, as serum antibodies decline over time, long-lived memory B cells activate and expand quickly upon re-exposure to SARS-CoV-2, rapidly generating antibodies to compensate for waning immunity [11-14].

Serology tests are widely used in sero-epidemiological studies to assess the prevalence of SARS-CoV-2 antibodies in different population groups and areas. In a population where vaccines based on the spike protein are used, serological tests can identify the proportion of vaccinated (anti-spike antibodies only) and infected individuals (presence of anti-nucleocapsid and spike antibodies) [15]. Serological studies and surveys are performed in the European Union/European Economic Area (EU/EEA) countries and a sero-epidemiological study network in the World Health Organization (WHO) European Region is coordinated jointly by WHO's Regional Office for Europe and ECDC. An ongoing systematic review of serosurveys conducted worldwide can be found at the following link: https://serotracker.com/en/Explore.

Considerations for the use of antibody tests to issue or prolong digital COVID-19 certificates

What antibody tests do/do not tell us about a SARS-CoV-2 infection

- The detection and quantification of antibodies cannot be used as a direct indication of protective immunity at individual level.
  - Most, but not all individuals infected with SARS-CoV-2 will develop serum antibodies which can be detected with a SARS-CoV-2 antibody test. The level and rate of decline of antibodies vary greatly among individuals and antibody targets and depend on factors such as age, genetic and immune status, viral load and disease severity of the previous SARS-CoV-2 infection [2,4,16]. The presence of SARS-CoV-2-specific antibodies alone does not indicate that an individual is protected from re-infection. The relationship between antibodies and immunity to infection with SARS-CoV-2 is not fully understood and is still under investigation.
  - Higher antibody levels have been associated with some level of protection from re-infection [6-9]. So far, however, the threshold titres of antibodies conferring protection against re-infection are unknown.
  - The immune response against SARS-CoV-2 is multi-faceted and includes not only antibodies, but other cell-mediated responses, which can vary greatly between individuals. Therefore, when determining an individual's level of immunity/protection, results from an antibody test cannot be considered alone.
  - It is unknown how well antibodies against previous SARS-CoV-2 variants may protect against newly emerging variants. A potential increase in re-infection has been observed with the Omicron variant of concern, indicating that antibodies generated through vaccination or infections by other variants may not protect fully against infection with Omicron [17].
- Antibody tests cannot determine the time of infection or how long an individual might be protected from re-infection.
  - Antibody tests cannot determine the exact time of infection without further evidence (e.g. confirmation in the form of a positive nucleic acid amplification test (NAAT) or rapid antigen detection test (RADT) performed at the time of symptom onset).
  - As an antibody level protective against infection has not been determined and the rate of decrease of antibody levels over time is variable, antibody tests cannot determine a period in which an individual will be protected from re-infection.
- A serological test cannot differentiate between ongoing, recovered infections or vaccination.
  - While serological assays play an important role in research and sero-epidemiological investigations, they are not recommended for the diagnosis of an acute SARS-CoV-2 infection.
  - Around Day 7 after infection, the immune response can generate detectable levels of IgM and IgG antibodies against SARS-CoV-2. During the resolution of infection and when moving into the recovery phase, a patient may also continue to generate higher levels of these antibodies. Therefore, antibodies can be detectable in both the presence or absence of the virus and an antibody test alone cannot differentiate between active infection and a patient who has recovered.
  - Antibody tests do not detect the virus itself and cannot be used to identify people with an acute viral infection or assess the level of infectiousness of a person. Their use will therefore not help to prevent virus transmission from an acute case.
  - People who are vaccinated with one dose of a COVID-19 vaccine may already elicit a strong immune response and therefore test positive in an antibody test. Tests that identify antibodies to the spike protein of the SARS-CoV-2 virus will be unable to distinguish between those who have had an infection and those who have received at least one dose of the vaccine.

Interpretation of antibody test results for diagnosis of COVID-19

- Caution should be taken when interpreting serological test results to try and ascertain infection status. In individuals whose infection has resolved and who are now RADT- or NAAT-negative, an antibody test can indicate a past infection/recovery from SARS-CoV-2 infection. Where possible, an antibody test should be combined with an RADT or NAAT test. It should be noted that interpretating results from serological testing in elderly or immunocompromised patients is more complex. This is because their age or condition can have an impact on the individual immune response, including the production of antibodies, thereby affecting the interpretation of whether an individual is protected.
- Serum antibody tests are unable to identify if an individual is currently infectious and can transmit the virus. Antibody tests cannot replace NAATs or RADTs as the purpose and targets are different (antibody versus direct detection of virus genome or viral protein). As regards the potential timing when conducting such tests for digital COVID-19 certificates, it should be noted that recent antibody tests (i.e. one-to-two weeks before travel) will be unable to exclude the possibility of an active infection – with potential for transmission – at the time of travel.

Considerations relating to the antibody tests currently available

- The antibody tests currently used in Member States are not harmonised/standardised and therefore the results are not comparable. Laboratory methods used by the Member States target different antibodies (IgM, IgA and/or IgG) and different epitopes of SARS-CoV-2, which makes comparison of results challenging. Although a wide range of SARS-CoV-2 proteins are targeted in the different antibody tests available, only anti-spike and anti-receptor binding domain (RBD) IgG antibodies correlate well with neutralising antibodies. High neutralising antibody levels are currently the best proxy for protection against re-infection.
- Clinical validation data are limited for the antibody tests available. A list of all CE-marked antibody assays available in the EU/EEA can be found in the Joint Research Centre's 'COVID-19 Diagnostic Testing database'. The US Food and Drug Administration (FDA) has published a list of independent validations[2]. The Global Alliance for Diagnostics (FIND) has conducted a multicentre diagnostic evaluation study of 16 manual enzyme-linked immunosorbent assays (ELISAs). However, the sensitivity and specificity estimates shown may not be indicative of the actual performance of the tests. The specimen type collected for each test (e.g. plasma, serum or whole blood, including finger stick blood) may affect the test results and their comparability.
- WHO has developed an international standard (IS) to harmonise and standardise the different serological assays. The Joint Research Centre and the National Institute for Biological Standards and Control (NIBSC) have also developed similar secondary standards which can be used if the WHO IS is unavailable. These standards can serve as a basis for calibrating tests that quantify antibodies. Calibration will need to be performed at individual laboratories that are using the different commercial antibody tests.
- Qualitative antibody tests are useful from a population-wide, rather than individual perspective. Most commercially-available detection reagents used for rapid detection of antibodies (lateral flow based rapid antibody assays) or large-scale automatic immunoassays only provide qualitative results (i.e. presence or absence of antibodies). Quantitative detection kits using ELISA are primarily used for research purposes but comparability between laboratories is hindered by the lack of reference material, and this also includes the material and systems based on newly emerging variants. Neutralising antibody tests that allow for the rapid detection of total neutralising antibodies in a sample by mimicking the interaction between the virus and the host cell are commercially available, but not widely used. As with other serological tests, even a positive neutralising antibody test does not guarantee protection against re-infection, or the durability of neutralising antibody levels.
- There is evidence that in areas of low prevalence, the positive predictive value of some antibody tests can be low, meaning that there is a high risk of positive results being false. However, the individual possibility of a positive antibody test is dependent on the individual exposure and likelihood of infection over time. It is challenging to estimate this for different populations across the EU/EEA, given that countries have all faced different epidemiological situations since the introduction of SARS-CoV-2 [18,19].

Antibody tests in the context of public health measures

- Tests should preferably be ordered from the local public health authority (or a central source) to ensure the use of approved test kits and to track, if possible, the number of tests distributed/used. Use of self-tests (lateral-flow based rapid antibody assays) to detect antibodies is not recommended. It is also challenging for lay individuals to interpret results since a positive result may not be proof of protective immunity.
- It is possible that individuals with certificates issued on the basis of positive serology may take a more relaxed attitude and not observe the behaviour required to limit the risk of infection and onward transmission (e.g. physical distancing, use of masks and hand washing). As mentioned above, while a positive serology result may be suggestive of infection, it does not guarantee protection from re-infection, particularly as regards newly emerging variants with immune escape potential.

[2] https://open.fda.gov/apis/device/covid19serology/

- A positive serology result may also discourage an individual from taking the correct decision in relation to the need for a booster dose. National recommendations should always be followed in relation to this issue.

Use of certificates issued on the basis on positive serology may affect public attitudes towards the importance of vaccination. Several studies have shown that vaccination reduces the risk of re-infection and enhances the immune responses in previously recovered individuals [17,20-22]. It is therefore important to consider public health messaging carefully in order to mitigate vaccine hesitancy. Recovered individuals need to understand that both vaccination and compliance with public health measures are still important in order to reduce SARS-CoV-2 transmission.

Critical limitations preventing the use of antibody tests for the digital COVID-19 certificate

At present, antibody tests cannot be used to issue or prolong the digital COVID-19 certificate for the following reasons:

- Positivity of an antibody test alone does not indicate protection from re-infection.
- The range of antibody titres that can confer protection against infection or severe disease have not yet been determined.
- The rate of decline in antibody titres between individuals is variable, therefore a standard predictable rate cannot be calculated.
- Detected antibodies may not protect against emerging variants.
- Antibody tests lack standardisation, both globally and within the Member States.

What can antibody tests be used for?

- Seroprevalence studies can provide an estimate of humoral immunity within a population, thereby giving an indication of the under-ascertainment of reported infections. Using different assay targets (nucleocapsid and spike), these studies can also help to estimate the proportion of individuals who have evidence of natural or vaccine-induced immunity. These results are of great importance for developing accurate modelling forecasts.
- Serological tests can be used to identify whether a vaccination series was successful in inducing antibody responses in individuals with a compromised immune system. When correlates of protection are better established, this information can be used to determine whether an individual requires an additional vaccine dose.
- Serological tests can be used for research purposes, such as establishing correlates of protection and cross-protection from variants and understanding different immune responses and the duration of antibody responses.
- In a similar manner to vaccination, individuals who become re-infected/exposed to SARS-CoV-2 can experience an increase in their prior antibody level state. Quantitative antibody tests could be therefore used as an indicator of whether current immunity has been naturally 'boosted' compared to earlier antibody results.

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