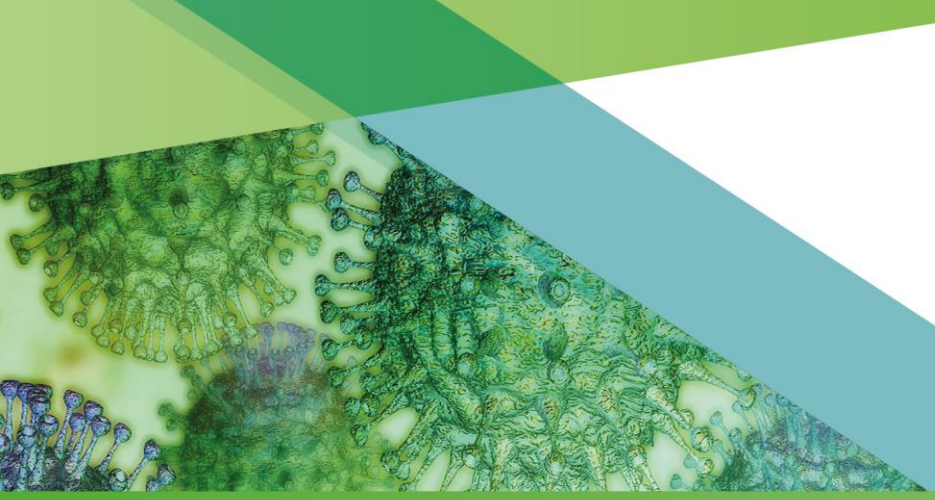

TECHNICAL REPORT

The use of antibody tests for SARS-COV-2 in the context of Digital Green Certificates

10 May 2021

Key messages

- This brief technical note is to inform the discussion on using Digital Green Certificates to facilitate the safe and free movement of citizens within the EU during the COVID-19 pandemic.
- At the moment, antibody tests are mostly used in research[i] studies of the population rather than for individual diagnosis of COVID-19 cases.
- A positive antibody test result can be proof of a past (including recent) infection, without providing any indication of the time of infection, and cannot exclude a current ongoing infection. Therefore, is not an absolute proof that a person is not infectious and/or protected against a new infection and cannot transmit the virus further.
- Even if antibody tests provide some evidence of an immune response[ii], it is not known if the antibody levels offer sufficient protection or how long such protection would last, i.e. how long this part of a Digital Green Certificate would be valid. It may well be that soon after a positive antibody test, the antibodies become undetectable.
- It is still unknown whether the antibodies detected by commercial tests currently in use would prevent infection with newly emerging SARS-CoV-2 variants.
- There are a variety of antibody tests[iii] and a comparison of their results is extremely difficult due to this variety[iv] and the lack of standardisation.
- The tests that target the spike protein will be unable to distinguish between people who have been previously infected and those who have received at least one dose of the vaccine.

[i] Sero-epidemiological studies.

[ii] The presence of neutralising antibodies against SARS-CoV-2 provides the best current indication for protection against reinfection for previously-infected individuals. Neutralising antibody tests (e.g. cPass) are now commercially available but are not widely used and most tests just detect so-called binding antibodies.

[iii] 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).

[iv] According to the 'COVID-19 Diagnostic Testing database' of the Joint Research Centre, at the time of writing this document there are 469 commercially available CE-marked antibody tests available in EU/EEA countries.

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Introduction

This brief technical note was developed at the request of the European Commission to inform the discussion on Digital Green Certificates to facilitate the safe and free movement of citizens within the EU during the COVID-19 pandemic[i].

Antibodies are generated as part of the individual's immune response against SARS-CoV-2 infection, or in response to vaccination, and indicate previous (including recent) infection or vaccination. Serological tests are developed to detect different types of antibodies:

- IgM antibodies are early antibodies and can provide the first indication of an immune response against SARS-CoV-2 infection. These antibodies are not as specific and generally not as long-lasting as IgG antibodies (see below), so interpreting their significance requires clinical experience.
- IgG antibodies are specific to the different antigens of the virus. So far, study results suggest that these antibodies can be reliably detected starting 14 days after SARS-CoV-2 infection or after vaccination.

Currently, serology tests are used in sero-epidemiological studies to monitor the prevalence of SARS-CoV-2 in different population groups and areas. Such sero-epidemiological studies are performed in EU/EEA countries and the sero-epidemiological study network in the WHO European Region is coordinated jointly by the WHO Regional Office for Europe and ECDC. Studies are ongoing, but results are not yet available.

The situation and knowledge about immunity against SARS-CoV-2 is evolving rapidly. This technical note is based on current knowledge and will be re-assessed when new information becomes available.

Considerations for the use of antibody tests for issuing Digital Green Certificates

What do antibody tests tell us about a SARS-CoV-2 infection?

- Antibodies persist for at least six months, with the rate of decline varying according to factors such as age and severity of the previous COVID-19 infection. They also appear to be associated with some level of protection from reinfection. Studies with longer follow-up periods are ongoing. The relationship between antibodies in response to infection and immunity to infection with SARS-CoV-2 is under investigation, but not fully understood, e.g. some mild or asymptomatic COVID-19 cases do not develop detectable anti-SARS-CoV-2 antibodies following a laboratory PCR-confirmed infection.
- Serological tests target specific SARS-CoV-2-induced antibodies. However, results only provide a partial picture of the immune response against the virus since T-cell mediated responses are not considered. The induction of SARS-CoV-2-specific memory T-cells is also important for long-term protection and play a vital role in virus clearance. T-cells may be maintained even if there are not measurable levels of serum antibodies. This further complicates the assessment of the existence and duration of immunity based on antibodies only. Individuals may also have varying immune responses to infection.
- 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. Available antibody tests measure the presence or absence of IgM and IgG against SARS-CoV-2. The level of IgM antibodies begin to rise one week after the initial infection, while IgG antibodies appear later than IgM (usually within 14 days after infection); they only provide evidence of a past (including recent) infection. 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 the person, and will therefore not contribute to preventing virus transmission from an acute case.
- Antibody tests cannot determine the exact time of infection if it is unknown (i.e. no confirmation through a positive PCR or antigen test).

[i] https://ec.europa.eu/info/live-work-travel-eu/coronavirus-response/safe-covid-19-vaccines-europeans/covid-19-digital-green-certificates_en

- The detection of antibodies in a serological test may provide evidence of a past infection. However, there is uncertainty regarding how well, and at what level, the presence of IgM and IgG antibodies has a neutralising effect on the virus and how long this lasts. The relationship between antibody levels and clinical response is yet to be fully determined. Therefore, when the time of infection is unknown, a certain antibody level cannot be directly correlated to protection through neutralising capacity of these antibodies against SARS-CoV-2.
- It is unknown how well antibodies against previous SARS-CoV-2 lineages or variant viruses may protect against newly emerging variants. Reinfection after a first COVID-19 episode remains a rare event. However, there is a lack of data related to the emergence of new variants with different antigenic properties and there are uncertainties regarding the duration of protection from reinfection conferred by natural immunity. For example, it seems that people who have antibodies against older variants of the virus are not fully protected against the B.1.351 variant virus originally detected in South Africa.
- People who are vaccinated with one dose of a COVID-19 vaccine may already elicit a strong immune response and therefore test positive in the antibody test. Tests that identify antibodies to the spike protein of the SARS-CoV-2 virus will be unable to distinguish between people who have been infected previously and those who have received at least one dose of the vaccine.

Interpretation of antibody test results for diagnosis of COVID-19

- Serological tests are not appropriate for diagnosing an acute SARS-CoV-2 infection. Since antibodies (IgM/IgG) against the virus are detectable from day seven after the onset of symptoms, a negative serology result during the first seven days of illness cannot be used to rule out infection. Although the sensitivity of antibody detection increases after day seven, a negative serology result after day seven should be carefully interpreted before ruling out a case. On the other hand, a positive result between days 7-14 indicates a previous infection and cannot rule out the presence of the virus. For these reasons, serology alone should not be used to rule out an ongoing infection. Likewise, a patient with respiratory symptoms and a positive antibody tests, does not necessarily have an ongoing SARS-CoV-2 infection. It should also be noted that the sensitivity of serological testing in elderly or immuno-compromised people is unknown. This is because their age or condition can have an impact on the individual immune response. For these reasons, results from antibody tests should be interpreted with caution.
- For the purpose of Digital Green Certificates, a positive serum antibody test does not confirm if an individual is non-infectious and cannot transmit the virus. Antibody tests cannot replace RT-PCR or rapid antigen tests as the purpose and target are different (antibody vs. direct detection of virus genome or viral protein). Regarding the potential timing for conducting such tests for Digital Green Certificates, it should be noted that recent antibody tests (i.e. one to two weeks before travel) are unable to exclude the possibility of an active infection.

Considerations regarding available antibody tests

- Antibody tests currently used in Member States are not harmonised/standardised and results are not comparable. Laboratory methods used by Member States target different antibodies (IgM and/or IgG) and different epitopes of SARS-CoV-2, which makes comparison of results challenging. While a wide range of SARS-CoV-2 proteins are targeted in the different available antibody tests, only anti-spike and anti-receptor binding domain (RBD) IgG antibodies correlate well with neutralising antibodies, which is the best current proxy for protection against reinfection and therefore transmission.
- According to the 'COVID-19 Diagnostic Testing database' of the Joint Research Centre the list of all CE-marked antibody assays available in the EU/EEA, as of 10 May, includes 469 tests. Limited clinical validation data are available for these tests. The US Food and Drug Administration agency publishes a list of independent validations here. However, sensitivity and specificity estimates shown may not be indicative of the real-life performance of the tests. The sample type collected for each test (e.g. plasma, serum or whole blood, including finger stick blood) may affect the test results and their comparability to each other.
- WHO has developed an international standard to harmonise and standardise the different serological assays for detection of neutralising antibodies. The Joint Research Centre has also developed similar standards. These standards can serve as the basis for the calibration of tests that quantify antibodies. Calibration will need to be performed at individual laboratories that are using the different commercial antibody tests.

- Antibody tests are qualitative and are useful from a population, rather than an individual, perspective. Quantitative detection kits using ELISA are primarily used for research purposes, but the comparability between laboratories is hindered by the lack of available reference material, including the material and systems based on newly-emerging variants. 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). 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 (e.g. cPass) are now commercially available but not widely in use. As with other serological tests, even a positive neutralising antibody test does not guarantee protection against reinfection or the durability of neutralising antibody levels.
- There is evidence that in areas of low prevalence, the positive predictive value of antibody tests can be very low, meaning that there is a high risk that positive results would be false. However, the individual likelihood of a positive antibody test is dependent on the individual exposure and likelihood of infection over time, and it is challenging to estimate this for different populations across EU/EEA countries who have faced different epidemiological situations since the introduction of SARS-CoV-2[i]. In the context of potential co-circulation of other seasonal coronaviruses with SARS-CoV-2, the specificity of some antibody tests may decrease and thereby the risk of false positive tests may increase.

Antibody tests in the context of public health measures

- It is possible that individuals with certificates issued on the basis of a positive serology may be falsely reassured that they can relax attitudes towards behaviours that are essential to limiting risk of infection and onwards transmission, such as physical distancing, mask use and hand washing. As mentioned above, whilst positive serology result may be suggestive of prior infection, it may not guarantee protection from reinfection, or to newly-emerging variants with possible immune-escape potential.
- Any implementation of certificates on the basis of positive serology should be carefully considered and be accompanied by strong public messages and relevant communication about the importance of both vaccination and public health measures to reduce SARS-CoV-2 transmission.

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[i] https://www.nature.com/articles/s41598-021-84173-1

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