Llywodraeth Cymru
Welsh Government

Technical Advisory Group

Consensus statement on guidance for the use of SARS-CoV-2 antibody testing in a diagnostic setting using laboratory-based platforms

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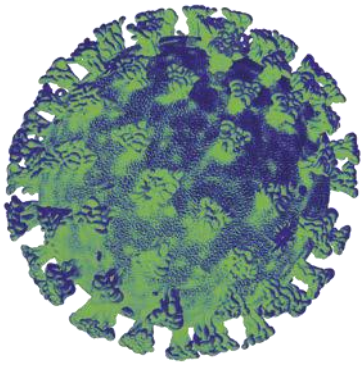

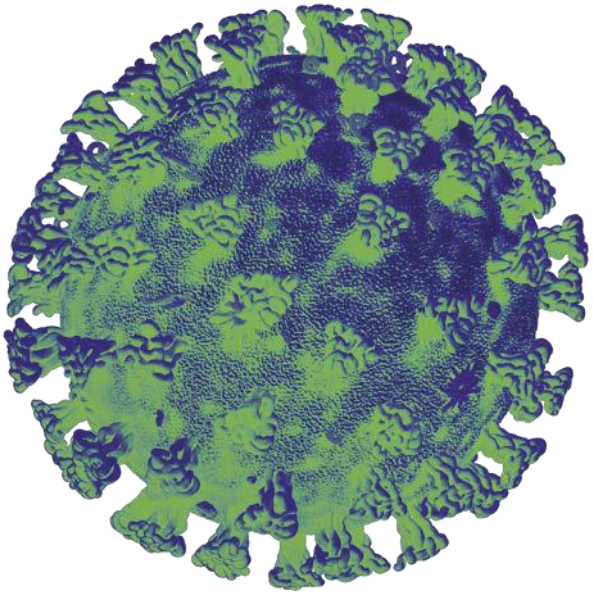

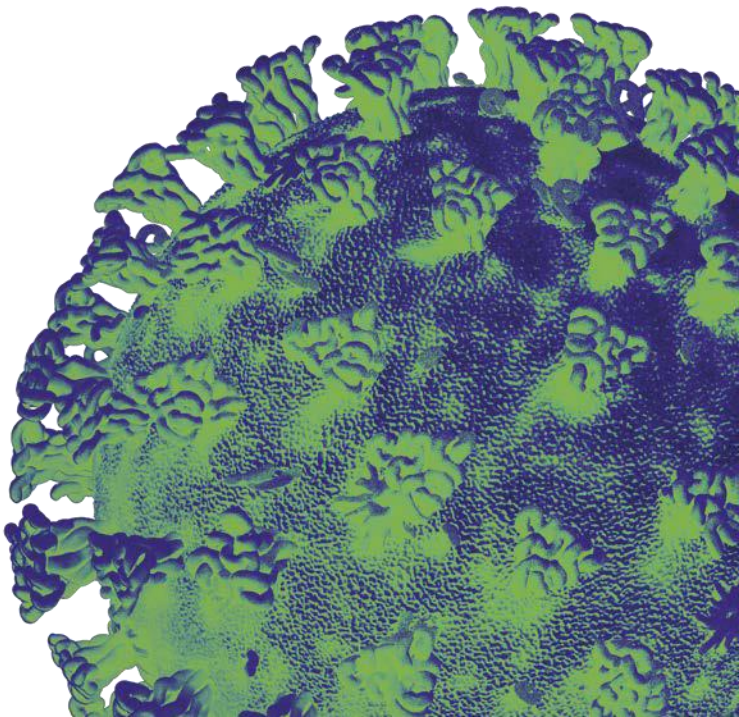

TAG Consensus statement on guidance for the use of SARS-CoV-2 antibody testing in a diagnostic setting using laboratory-based platforms

Objective

In the absence of national UK guidelines for the use of SARS-CoV-2 antibody tests as a diagnostic test, the purpose of this document is to provide some advice around their use as a diagnostic tool and to highlight the limitation of the assay and the requirement for careful interpretation of the results.

Antibody Testing Associated with Vaccination

Testing for antibody to SARS-CoV-2 undertaken as a component of the investigation of the immune response to vaccination and antibody testing as a component of the investigation of vaccine failure or escape is outside the scope of this paper. While such applications of antibody testing may have both value and utility, it is essential that that they are considered in the wider context of the investigation and evaluation of vaccination as a whole rather than separately in the context of clinical diagnostic testing.

Background

SARS-CoV-2 antibody tests are widely available and have been used to gain information regarding sero-prevalence in a variety of groups, such as healthcare workers and teachers, and in community settings.

Even though these assays are now widely available, the SARS-CoV-2 antibody test is still a relatively new test and as such there are no national recommendations, from organisations such as NICE, on their use diagnostically. There remains uncertainty regarding the interpretation of the test result and the length of time the antibody will remain detectable in a laboratory assay. This variability is known to be assay-dependent. Such inter-assay variation remains an unquantifiable limitation at the present time and is likely to affect ‘measurements of uncertainty’, when used diagnostically.

Since the advent of molecular diagnostics for respiratory viral infections, antibody tests have had little clinical utility in this field; as such, there is limited experience of the use of serological tests for diagnostic purposes for other respiratory viral infections. The presence of SARS-CoV-2 antibody markers provide limited information regarding the immune response and there remains uncertainty regarding what a positive antibody test means, in the context of immunity to SARS-CoV-2.

Laboratory assays for the detection of SAR-Cov-2 antibody

There are several types of immunoassay available, using different viral antigens for antibody detection. The target markers are epitopes on the spike protein (S), membrane protein (M), envelope protein (E), and nucleocapsid protein (N) proteins. The most common antigens used for indirect assays are the recombinant spike protein, which contains the domain for attachment to the host cells, and the nucleocapsid protein, involved in viral replication, transcription and assembly.

In addition, assays may also look for different classes of antibody such as IgA, IgG and IgM. Each antibody may require a different interpretation depending on the clinical scenario.

Prior to the implementation of a national sero-prevalance service for Wales, national evaluation of the testing platforms was undertaken. Panels of sera from known PCR positive individuals, known negative samples and samples from individuals with seasonal coronavirus, were tested on six platforms and the results compared. The four platforms selected demonstrated high concordance with serum panels, providing results consistent with the sensitivity and specificity quoted by the manufacturers. The sero-prevalance work did not inform individual patient management, but gave a broad overview of the sero-prevalance within specific groups. This information was used by Welsh Government to inform future management plans in response to the pandemic.

The diagnostic uses of the SARS-CoV-2 antibody test have not been evaluated. The number of different assays and markers available have increased over the last few months. The evaluation and verification work carried out for the sero-prevalence work previously, has reduced relevance now as these tests have continued to evolve and develop.

Key areas for interpretation of results

Optimal time for taking a sample

Sensitivity and specificity of the IgG, IgM and total antibody tests are optimal if samples are taken between 14-30 days after the onset of symptoms. There will be some variation within this range, depending on the assay used and the antibody class. Individuals may also have a delayed response in developing detectable antibodies.

Waning of antibody titres

The detection of antibody markers will wane over time. This is also assay specific and will also be affected by the class of antibody being detected. In some assays the positive signal is lost around 3 to 4 months after infection. It is not yet clear how this will be affected by vaccination and whether the signal will be maintained for a longer period and what the variation within the available assays will be.

Qualitative and Quantitative results

Assays will be qualitative or quantitative.

A qualitative results is one where the results fall into two or three broad categories, Positive, Negative +/- ‘greyzone’. In a qualitative assay the results are not correlated to a standardised value and therefore a relationship between amount of antibody and a numerical value cannot be assumed.

Quantitative assay are able to give a value against a standards that allows for some interpretation as to the amount of antibody present and how this value relates to neutralising antibody.

Standardisation and comparability of assays

The variability of assay performance has been an area of concern. As assays have been developed at pace, the only way to look at the quality of the products has been direct comparison studies in conjunction with the manufacturers' estimates of sensitivity and specificity. WHO have recently made available an International standard for anti- SARS-CoV-2 immunoglobulin. This is welcomed and will aid the development of quality assurance systems and allow for more informed interpretation of results.

Immunity (natural infection)

The Spike target provides a better correlation with neutralising antibody than the nucleoprotein target, although the presence of detectable antibody from any marker cannot yet be taken as evidence of immunity.

Vaccination and antibody detection

The rollout of the SARS-CoV-2 vaccine also adds a layer of complexity to the interpretation of serological results.

It is important to know which vaccine has been given as each vaccine will cause the production of a specific antibody response. The table below summarises antibody responses following natural infection and vaccination with a spike antibody inducing response, as an example.

	'S' antibody IgG / total antibody	'N' antibody IgG / total antibody
Natural infection	Positive	Positive
Vaccination	Positive	Negative
Vaccination and previous infection	Positive	Positive / Negative
No recent previous infection or vaccination history	Negative	Negative

Antibody results will need to be interpreted with a knowledge the vaccine given, and date of doses given.

Antibody class

The detection of a specific class of antibody is relevant to interpretation. IgG antibody and total antibody are likely to be positive for longer periods than IgM antibodies. IgM antibody assay are subject to cross reactivity and therefore cross reactivity profile of SARS-CoV-2 IGM assays are relevant in the interpretation of the results.

The timing of antibody detection is also relevant to interpretation. Biologically IgM is produced in response to an acute primary infection or following re-infection/ re-exposure. It may be short lived and therefore detectable for a brief period of time, or more persistently. IgG may be detected at the same time as IgM or may become detectable later in the course of the infection. The relationship between antibody classes can be both assay specific and infection specific.

Diagnosis of SARS-CoV-2 acute infection

There is no evidence that antibody tests alone can be used for the diagnosis of the acute infection; RT- PCR or antigen detection remain the tests of choice in the acute phase.

Interpretation of the results

A positive nucleocapsid antibody test can be interpreted as evidence that SARS-CoV-2 infection has occurred at some time in the past 1-3 months, but cannot determine exactly when the infection happened. The result does not presume immunity.

A positive spike antibody test result can be interpreted as either evidence of previous SARS-CoV-2 vaccination or that SARS-CoV-2 infection has occurred at some time in the past 1-3 months, but cannot determine exactly when the infection happened. The result does not presume immunity.

A negative test result does not exclude previous infection with SARS-CoV-2, as we know that some patients who have had SARS-CoV-2 infection may not have a detectable antibody. Immunosuppression and treatments, such as immunoglobulin therapy, may affect antibody results.

As with all diagnostic tests positive and negative predictive values will be affected by the sensitivity and specificity of the assay in conjunction with the prevalence of the infection and whether the test was being used in a symptomatic or asymptomatic population.

Diagnostic use of anti SARS-CoV-2 assays

When used for a diagnostic purpose all results must be interpreted with the full clinical picture, and take into consideration any RT-PCR results and relevant vaccination history.

As yet, there are no nationally agreed guidelines. Clinicians should consider how the result will impact on an individual patient’s management and be clear regarding the limitations of assays being used. Results cannot be interpreted in isolation. The decision to use a SARS-CoV-2 antibody test in a diagnostic setting should be made by a clinician with a sound understanding of the limitations of such a test as an investigative tool.

<u>Recommendation</u>

Currently SARS-CoV-2 diagnostic antibody tests should be limited to specific testing schemes that overseen by a senior accountable clinician, with the responsibility for interpretation of the result, in the context of other relevant investigations and history,

lying with the requesting clinician. Guidance regarding the scope and limitations of the assay must be available from the diagnostic laboratory performing the test, to support clinical decision-making, and this diagnostic service should not be offered by any NHS laboratory service that cannot provide this. Laboratories offering this as a diagnostic test should, of course, ensure they are working to the required accreditation standards.

References

Covid-19 serology and post-vaccine surveillance in England; Presented by Kevin Brown at the PHE Virology cell presented 11th Feb 2021

NERVTAG immune certification update 0.2 Ref: NERVTAG FC-44-07

https://www.nibsc.org/documents/ifu/20-136.pdf