

COVID-19 ANTIBODY TESTS: AN UPDATE

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Several developments have occurred since the authorisation and introduction of SARS-CoV-2 (COVID-19) antibody tests in South Africa in August 2020. The primary aim of the first COVID-19 serology assays that came to market was to determine whether a patient had previously been exposed to SARS-CoV-2. These antibody assays were largely directed to the COVID-19 Nucleocapsid (N) protein. COVID-19 vaccines have since been rolled out, and more SARS-CoV-2 serology assays, including quantitative Spike(S) IgG antibody tests, have become available. Here we provide an update on the latest COVID-19 antibody tests available at Ampath (Table 1) as of November 2021 in order to guide testing.

ANTIBODY RESPONSES TO COVID-19 AND COVID-19 VACCINES

Antibody responses have been shown to vary greatly between individuals. Trends may also differ depending on factors such as age and disease severity.

Not all individuals will mount an antibody response after vaccination or natural infection and antibody levels may wane over time.

Antibodies of the IgM and IgG classes may appear from one week after the onset of COVID-19 infection, but are only reliably detected from Day 14 onwards. As such, these antibody assays should not be used for the diagnosis of current COVID-19 infection. The IgM responses may become undetectable two to three months following infection. The IgG antibodies have been shown to remain detectable for at least six months after recovery from COVID-19, but the rate of decline in levels varies from one individual to the next.

Vaccine-induced antibodies may develop at a slower rate than following natural infection; as such, it is deemed best practice to delay testing until 21 to 28 days following the last dose of vaccination.

TABLE 1: COVID-19 ANTIBODY TESTS AVAILABLE AT AMPATH

TEST NAME	MNEMONIC	COMMENTS
SARS-CoV-2 Antibody Profile Includes: Nucleocapsid IgG Spike IgG (quantitative) Spike IgM	COVID19ABP	To determine both natural and vaccine-induced immune responses with a combined interpretation of the results. For further information about the individual components of the profile, refer to the relevant sections below.
SARS-CoV-2 Nucleocapsid IgG	COVID19AB	To determine previous exposure to COVID-19. Persons who have been vaccinated with COVID-19 vaccines based on the Spike protein (most vaccines with the exception of inactivated whole virus vaccines) will NOT mount a Nucleocapsid antibody response. The test is semi-quantitative, which means that the quantitative value and reference range reported is unique to the assay, and is merely intended for determination of the qualitative outcome of the test, namely positive or negative.
SARS-CoV-2 Spike IgG	COVID19SAB	This is a quantitative assay, which is intended to either determine post-vaccination antibody response or antibody response following natural infection with SARS-CoV-2. It can be used to determine vaccine-induced antibody response as most of the current COVID vaccines contain or target Spike proteins. Patients that had a natural COVID-19 infection may develop antibodies to both the Nucleocapsid and Spike proteins, whereas vaccinated individuals will usually test negative for Nucleocapsid antibodies, even if Spike antibodies are present.
SARS-CoV-2 Spike IgM	COVID19M	Spike IgM antibody, usually indicated for travel purposes to China together with SARS-CoV-2RT-PCR, but no longer restricted solely to travel.

INTERPRETATION OF SARS-COV-2 ANTIBODY RESULTS

Table 2 provides a basic outline of the interpretation of COVID-19 antibodies. It should be noted that this is a very condensed interpretation table and that further possible

scenarios such as waning of antibodies over time and the responses to inactivated whole virus vaccines have not been factored into it.

TABLE 2: INTERPRETATION OF SARS-COV-2 ANTIBODY RESULTS

NUCLEOCAPSID IgG	SPIKE IgG	Interpretion/Comments
-	-	Previous COVID-19 unlikely and no vaccination (or no detectable response)
-	+	Vaccine-induced immune response
+	+	Previous COVID-19 infection (with or without additional vaccination)
-	Not done	A negative result does not exclude past infection with SARS-CoV-2, due to the fact that a small percentage of people with COVID-19 never mount an antibody response, as well as the possibility that antibodies may wane over time
+	Not done	A positive result indicates likely past COVID-19 infection
Not done	-	A negative result does not exclude past infection or vaccination with SARS-CoV-2, due to the fact that a small percentage of people with natural COVID-19 infection never mount an antibody response, as well as the possibility that antibodies after natural infection or vaccination may wane over time
Not done	+	Patients that had a natural COVID-19 infection may develop antibodies to both the Nucleocapsid and Spike proteins, whereas vaccinated individuals will usually test negative for Nucleocapsid antibodies even if Spike antibodies are present

WHAT SPECIMEN SHOULD BE USED FOR SARS-COV-2 ANTIBODY TESTING?

A single blood specimen is collected in a clotted tube (red or yellow top). There are no special specimen handling requirements and specimens can be collected at any routine Ampath laboratory or depot.

IMPACT OF VARIANTS ON ANTIBODY TESTS

While there is a theoretical risk that mutations in the Spike region of SARS-CoV-2 may affect the binding between antibodies directed against the variant Spike antigen and the wild type Spike antigen used in commercial antibody assays, this has not been seen in practice to date.

QUANTITATIVE SPIKE ANTIBODY TESTS AND STANDARDISATION

Not all antibodies that are formed in response to a pathogen will necessarily have the ability to neutralise that particular pathogen. Although neutralisation assays remain the best available method to determine whether the antibodies detected have the ability to neutralise a virus such as SARS-CoV-2, these assays require facilities that can safely handle the live SARS-CoV-2 virus. Alternative methods such as ELISA tests are often used as surrogate assays to circumvent the technical difficulties

that prevent the widespread use of neutralisation assays. As the majority of neutralising antibodies against SARS-CoV-2 target the receptor binding domain (RBD) of the Spike protein, it has been demonstrated that anti-Spike antibody tests (and in particular those that target the RBD) correlate well with neutralisation assay results.

Manufacturers have developed newer SARS-CoV-2 Spike antibody assays that incorporate measures to better quantify the amount of anti-Spike antibodies present. A number of laboratory assays (including those that measure anti-nucleocapsid antibodies) are semi-quantitative, where the quantitative value generated is intended to determine whether the assay is positive or negative. Quantitative assays, on the other hand, offer an extended dynamic range and high precision, and are one step closer to standardisation between COVID-19 antibody assays. Although the World Health Organization (WHO) has launched a WHO Standard for SARS-CoV-2 antibodies, work is still underway to establish an international unit of measurement. At this stage, the reference ranges and units of measurement are still unique to each manufacturer's assay, and should not be used interchangeably.



CORRELATES OF IMMUNE PROTECTION

Quantitative Spike antibody assays are a good candidate for determination of an antibody level that may confer protection against SARS-CoV-2. However, the exact correlates of protection are yet to be established. Although it is not yet known whether a particular level of antibody can indicate immunity to COVID-19, researchers have correlated the quantitative values of commercial Spike antibody assays with neutralising antibody results,

which may assist in interpreting these results until more formal guidance is available. Table 3 depicts the findings from a peer-reviewed scientific publication, where results generated by the same Spike IgG assay that is currently in use within Ampath were correlated with neutralisation values. It should be noted that the emergence of future variants with the ability to evade pre-existing immunity will likely affect the correlates of immune protection.

TABLE 3: CORRELATION OF SPIKE IGG QUANTITATIVE ANTIBODY VALUES WITH NEUTRALISING ANTIBODY VALUES

ABBOTT IgG II assay	NEUTRALISATION VALUES
50 AU/ml (positive cut-off)	18% of viruses neutralised
107 AU/ml	30% of viruses neutralised
369 AU/ml	50% of viruses neutralised
2 340 AU/mL	80% of viruses neutralised

FROM: Bradley BT, et al. Anti-SARS-CoV-2 antibody levels are concordant across multiple platforms but are not fully predictive of sterilizing immunity. J Clin Microbiol doi:10.1128/JCM.00989-21

Amphath does not recommend that COVID-19 antibody assays be used as tools for recommendations on vaccination, as correlates of immune protection have not yet been defined and could be influenced by new variants, e.g. Omicron.

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REFERENCES AVAILABLE ON REQUEST

It should also be kept in mind that serology assays only provide insight into the humoral immune response, and do not take into account T-cell mediated immune response to COVID-19, which likely assists in viral clearance, and is generally considered to be maintained for a longer period of time than specific antibodies. Amphath offers T-cell testing to COVID-19 against both N- and S-protein antigens, or in the case of persons who received a COVID-19 vaccine, against S-protein antigen only.

