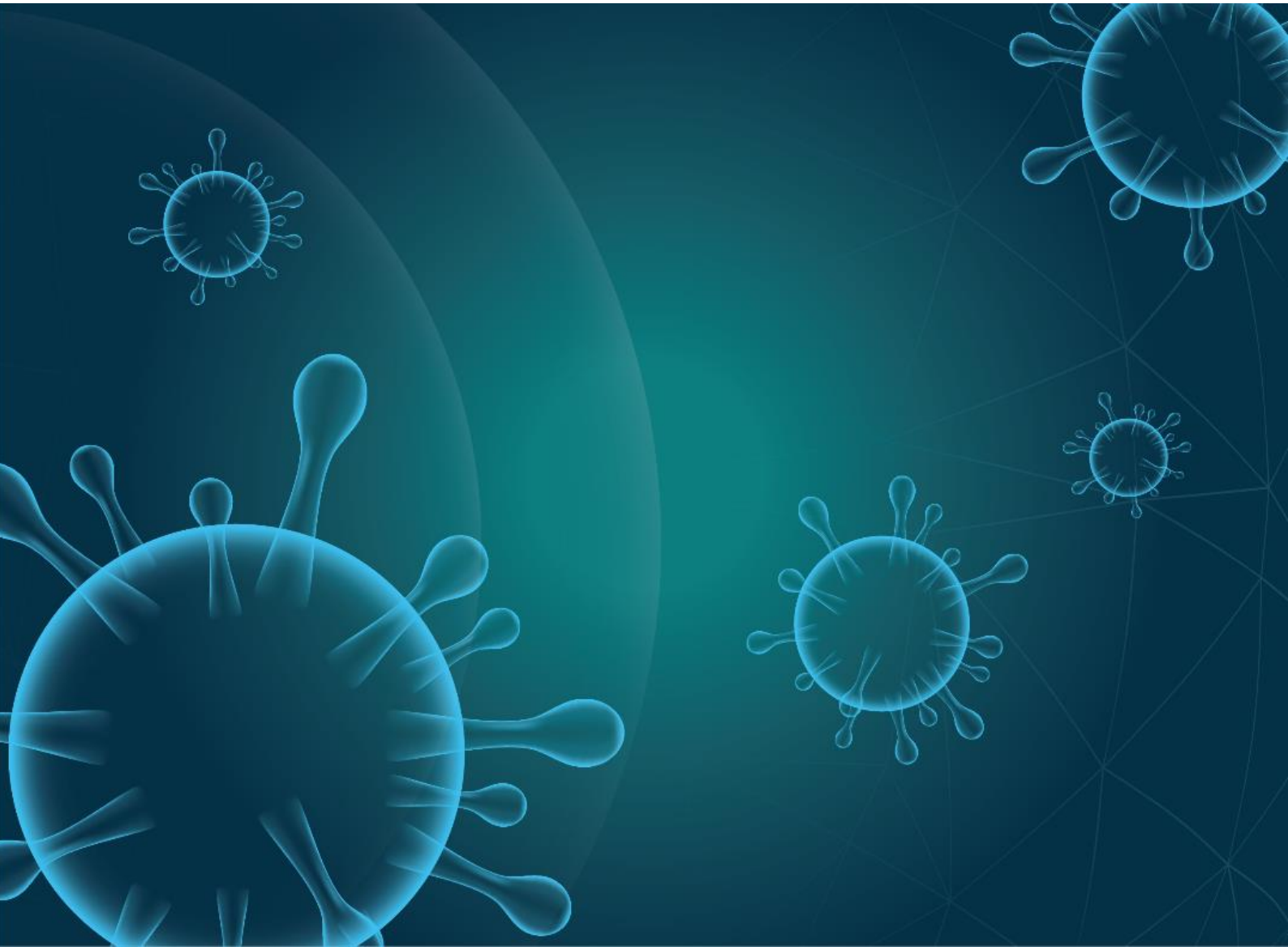
المختبر المرجعي الوطني
National Reference Laboratory
A Mubadala Health Partner

SARS-CoV-2 (COVID-19)

Guidance for Assay Test Selection



We are now well into the second year of the pandemic. Progress in treating, vaccinating and diagnosis have impacted who, how and when we test individuals. We can expect this to further evolve as we move through the stages of the pandemic towards the reestablishment of normalcy where SARS-CoV-2 no longer requires constant attention.

With the rapidly evolving testing needs for SARS-CoV-2 antibodies and antigens under different clinical scenarios, National Reference Laboratory (NRL) is providing you with this information guide to assist you in the selection of assays best suited for your patients under different clinical conditions.

Background

The COVID-19 pandemic has demonstrated the importance of diagnostics and frontline healthcare workers more than at any other time. NRL has been at the forefront of testing since the start of the pandemic, we were one of the first commercial laboratories to support the UAE government and swiftly implement COVID-19 testing at a national level early on during the pandemic in a time when the world was split between the importance of testing and social distancing measures. The pandemic was unprecedented territory and swift handling of testing was paramount in the early stages as the extent of the pandemic became known.

NRL was able to rapidly introduce a new molecular testing technique and significantly upscale the laboratory capacity in 3-days to meet an unknown level of demand. As a result, our BSL-3 facility had to be expanded whilst it was still being used for testing. The testing capacity increased from 500 samples daily to 7,500+ to meet the country's needs.

As the pandemic progressed we continued to innovate our processes and introduce new technologies and tests to ensure the optimal handling of the COVID pandemic in the UAE. Working with government and educational institutions, NRL has conducted several studies to develop a better understanding of the virus and impact on the UAE communities, including:

- Alatoom, A. et al. (2021). Evaluation of three commercial SARS-CoV-2 serology assays in a tertiary care hospital in the United Arab Emirates. *Journal of Infection and Public Health*, 14(7), 898-902. https://doi.org/10.1016/j.jiph.2021.04.003
- Alsuwaidi, A R. et al. (2021). Seroprevalence of COVID-19 infection in the Emirate of Abu Dhabi, United Arab Emirates: a population-based cross-sectional study, *International Journal of Epidemiology*, 1-14. https://doi.org/10.1093/ije/dyab077

What are the diagnostic and monitoring pathways for my patients?

Currently there are four different testing issues to consider:

1. How to detect active infection
2. How to determine immune status
3. Understanding the potential new and evolving assays to support clinical management post vaccination or convalescence
4. Identifying the assays developed by not recommended by the CDC.

The tables below provide an 'at a glance' overview of the current test selection.

Active infection test selection

Test selection	Utility	Benefit	Limitation	Availability
SARS-CoV-2 PCR	Detect infection in asymptomatic and symptomatic individuals.	Highly sensitive.	Window of detection is variable among individuals. May also detect past non-active infection. Typically performed on a nasopharyngeal swab.	Currently available with a turnaround time of approximately 8 hours.
SARS-CoV-2 Antigen	Detect active infection in asymptomatic and symptomatic individuals in a short period of time.	Will only detect active infection. Very useful for individuals with symptoms. Also beneficial for mass screening of high-risk groups such as airline passengers or mass screening in high prevalence populations.	Much less sensitive than PCR detection as a general population screen.	Under development.

Detection of immune status test selection

Test selection	Utility	Benefit	Limitation	Availability
SARS-CoV-2 Nucleocapsid Antibody	Can detect natural immune response due to natural infection.	Detection of a convalescent infectious individual whether immunized or not.	Not of use for post vaccination immunity.	Currently available daily.
SARS-CoV-2 Quantitative Total Antibody including IgG	Detects natural immune response and vaccination immune status.	Quantitative and the titer can be followed within an individual.	Cannot distinguish natural disease from vaccination. Not to be used interchangeably with other serologic assays to follow titer.	Currently available 24/7 on demand.

SARS-CoV-2 S1/S2 Spike Proteins Semi Quantitative Antibody IgG	Detects natural immune response and vaccination immune status.	Detects primarily IgG. Useful as one of the earliest released assays and commonly used in studies and vaccine testing follow up.	Cannot distinguish natural disease from vaccination. Not to be used interchangeably with other serologic assays to follow titer.	Currently available daily.
SARS-CoV-2 Neutralizing Antibodies	Surrogate assay for viral neutralizing antibodies.	Intended to estimate the neutralizing antibody capability in a given immune response. Largely correlates to the general immune response measured to spike proteins and closely correlates to pseudo or true viral neutralizing assays which are not widely available.	Minor limitation is that surrogate neutralization is not guaranteed to infer true immunity. No assay can prove future immunity.	Under development.
SARS-CoV-2 T Cell response assays utilizing gamma interferon measurement *in vitro*	The first assay to attempt to serve as a surrogate to acquired T Cell immunity in convalescent or vaccinated individuals versus non infected individuals.	Unknown.	There is no evidence that circulating T cells have any role in SARS-CoV-2 response. T cells located in central or distal regions such as the mucosal layer of the sinuses may not have the same response as circulating T Cells. There is no current means to measure central or distally located T cell immunity and no proof this is useful beyond clinical studies.	Under consideration.

Assays not recommended

Test selection	Utility	Benefit	Limitation	Availability
IgA and IgM antibodies to SARS-CoV-2	None. It is widely accepted that there is no reason to use conventional early serologic assays with this virus. Nor is it known to be useful to know the IgA values.	None as per current CDC guidelines. Circulating IgA or even localized IgA by biopsy is highly unlikely to have clinical utility. IgM is not a critical assay in terms of active versus convalescent infection in such a widespread disease at this time.	No known clinical utility.	This assay is without utility and will not be offered unless new clinical management is indicated.

Frequently asked questions

Q1. What is the assay timing for molecular testing?
PCR recommendations vary per Emirate. The timings should be aligned to the current DOH, MOH and DHA authorities' instructions.

Q2. Why are there so many different Immunoassays for serologic investigations? I just want to know a patient's immune status.
As this pandemic evolves so does the need for diagnostic versatility. Assay design impacts an assays clinical utility. SARS-CoV-2 has several protein structures or regions that trigger the generation of antibodies in humans. There is a membrane protein (M), envelope small protein (E), nucleocapsid protein (N), hemagglutinin-esterase (HE) and the spike protein. During the initial wave of the pandemic manufacturers developed antibody assays to detect infection of SARS-CoV-2. These assays were commercialized in an emergent manner to meet the clinical demand at the time. Further refined assays have been commercialized as we started to understand the natural course of the viral disease and our vaccination response. It is now well known that within the spike protein there are several antigenic regions. The subunit 1 (SB1) contains the receptor binding domain (RBD) which binds to the Angiotensin converting enzyme (ACE2) receptor on the cell surface. This permits subunit 2 (S2) to subsequently fuse to the host membrane which is required for the virus to enter the cell.

Q3. Do I need to follow titer for my patients in convalescence or post vaccination?
Measurement of antibodies post infection or vaccination to determine immune status has its limitations. While it is known that the vaccines do provide some protection there is little information on how antibody titer or even the presence of antibodies impacts immunity

Q4. Is it possible to follow antibody response in my patients?
Yes it is. However, please take care to order the same test code each time. Different assays give different and non-interchangeable values.

Q5. For other infectious disease it makes sense to follow IgM to determine acute responses or re-infection. Why is this not currently indicated with SARS-CoV-2?
It is unclear how antibody patterns will change with reinfection or over time. The use of an IgM in this situation may not be valuable as there is historical evidence and potential for IgG antibodies to dominate in subsequent reinfections. The classical example of this is CMV reinfection. IgG and IgM increase in titer in an unpredictable manner.

Q6. Why am I seeing different result values and interpretations for the assays I order on my patient?
All SARS-CoV-2 antibody assays lack a standardized method for comparison. There is a WHO first international standard for pseudo virus neutralization testing and some manufacturers of antibody tests will mention that their assay correlates well with the titer from this international standard.

This is a meaningless statement and should not be confused with an actual claim that is part of the actual validated intended use of an assay also known as the labeling.
To monitor patient response please order the same assay code consistently.

Q7. Why repeatably order the same code? How does this make sense? An antibody is an antibody. Every manufacturer assay is the same isn't it?

No. It is important to use the same assay if following an antibody titer. The SARS-CoV-2 assays are not interchangeable between manufacturers. With the use of quantitative SARS-CoV-2 antibody assays one manufacturers value is not comparable to another. For example, a numerical value of arbitrary units of 10 could be the same as another assay manufactures arbitrary units of 100 or 1000 for that matter.

Q8. How do I know which antibodies to test for?

There are IgM, IgG and IgA antibodies produced against SARS-CoV-2. IgA is protective at mucosal layer found in the nasal passages and other locations. The CDC[1] currently does not suggest that there is any clinical relevance in testing circulating IgA. Nor does the CDC recommend doing so. The CDC also does not indicate that it is important to measure IgM separately. The CDC recommends either a total or IgG assay for SARS-CoV-2.

Q9. What are the risks of thrombotic episodes with vaccines?

There has been extensive media coverage of rare cerebral vascular thrombotic (CVT) events occurring in recipients of the Janssen/Johnson & Johnson (J&J) as well as the AstraZeneca vaccines. To put the frequency of these events in perspective, the risk of CVT was over six times greater within two weeks of natural SARS-CoV-2 infection than the risk of CVT post vaccine. As is demonstrated by regulatory agencies and health authorities, globally, the benefits of vaccination continue to far outweigh the risks: https://www.gov.uk/government/news/mhra-issues-new-advice-concluding-a-possible-link-between-covid-19-vaccine-astrazeneca-and-extremely-rare-unlikely-to-occur-blood-clots

Q10. Is it possible to pre-determine the risk of having a thrombotic episode post vaccination?

While these rare post vaccination thrombotic events have occurred in the presence of PF4/polyanion antibodies that are observed in some patients after heparin therapy, there is currently no evidence to suggest that this biomarker is causative, or if so, the extent of its impact. Performing tests to determine antibodies to PF4/polyanion complexes is not currently recommended, nor advised by any health authority at this time regarding SARS-CoV-2 vaccination. The test serves as a screen for patients on heparin prophylaxis who have a high pretest probability for Heparin Induced Thrombocytopenia (HIT) and is not currently indicated for any other use regarding vaccinations and may potentially misguide the decision to vaccinate.

[1] https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html. Date accessed 4 March 2021

Clinical scenarios matched to tests

Test No.	Test Description	Notes
9918190	SARS-CoV-2, PCR (NAA), Saliva	• Useful for symptomatic or asymptomatic person that was in prolonged close contact with a diagnosed infected person or is suspected of early infection. PCR is the test of choice. • Useful for mass high sensitivity screening. PCR is the test of choice. Refer to the most recent DOH Circular for the most up to date testing requirements.
9917140	SARS-CoV-2, PCR (NAA)	
9917150	SARS-CoV-2 Nasopharyngeal swab PCR	
TBD - Under development	SARS-CoV-2 Antigen	SARS-CoV-2 antigen testing from a nasal swab or nasopharyngeal swab will soon be possible. The importance of this assay is that it will demonstrate active infection by the direct detection of the virus particles. The assay is much less sensitive than PCR and is not a replacement. At the current time this assay is valuable in highly symptomatic individuals as a complement to PCR or to rapidly identify an individual that may be shedding virus. Like all SARS-CoV-2 assays we can expect them to serve in areas that fulfill need in our evolving combat of SARS-CoV-2. It is of interest to note that the UK has approved this assay for mass asymptomatic testing. High prevalence of the disease and socio-economic factors drive regional and country decisions.
14560	SARS-CoV2 Nucleocapsid (N) Antibody, IgG Serum	This is an assay that detects antibodies to the nucleocapsid antigens. It is useful for post-COVID infection detection of antibodies or to observe immune status in asymptomatic individuals. It is **not** useful for the detection of antibodies after vaccination.
14570	SARS-CoV-2 Spike (S) Protein Antibodies Quantitative	This is an assay that detects total antibodies including IgG to the Spike protein on the surface of the virus in a qualitative manner. The assay is useful for detecting the presence of antibodies either post infection or post vaccination. Generally, antibodies can be tested 2-4 weeks after the second vaccine dose.
14580	SARS-CoV-2 S1/S2 IgG Semi Quantitative	This assay is like test number 14570 however it is less sensitive to the detection of IgM antibodies. In most situations this is not relevant. Please keep in mind that the values from each test are not interchangeable. This is an assay that detects IgG antibodies to the spike protein domains S1 and S2. The assay is useful for post infection and post vaccination with an estimated titer. Less than 11.9 Arbitrary Units (AU/mL) is considered non-reactive. Primarily this test is useful for ongoing studies that have been using this assay. Assay 14570 can be used for general use due to its continuous availability 24/7.
TBD - Under development	SARS-CoV-2 Blocking Antibody Surrogate Assay	This assay is under investigation and may be offered shortly. True neutralization assays for SARS-CoV-2 blocking antibodies requires strict biological safety precautions and few laboratories in the world can perform this test. The tests are not considered practical for any use beyond research investigations. A surrogate virus neutralizing assay has been developed however it is impractical for use. A more efficient approach is to use an antibody identification test that mimics blocking activity. Antibody competition with ACE2 protein at a receptor binding site serves as a surrogate to blocking assays. This is the best commercially available and practical approach as the assay is correlated to PRNT and is typically aligned to other SARS-CoV-2 antibody assays. At some point we may be able to have spike protein antibody claims for existing assays that can replace this assay however much more information is required to make a claim.

Test No.	Test Description	Notes
N/A	**T Cell response assay**	The assay is under consideration and may or may not be of clinical use at this time. NRL is committed to state-of-the-art testing that has merit.

TBD – Test number to be determined once validated. Not currently available.
N/A – Not available.

Please note that this information does not supersede the information provided in the most recent government circulars. Please follow the guidance and requirements of the most current circulars as applicable.