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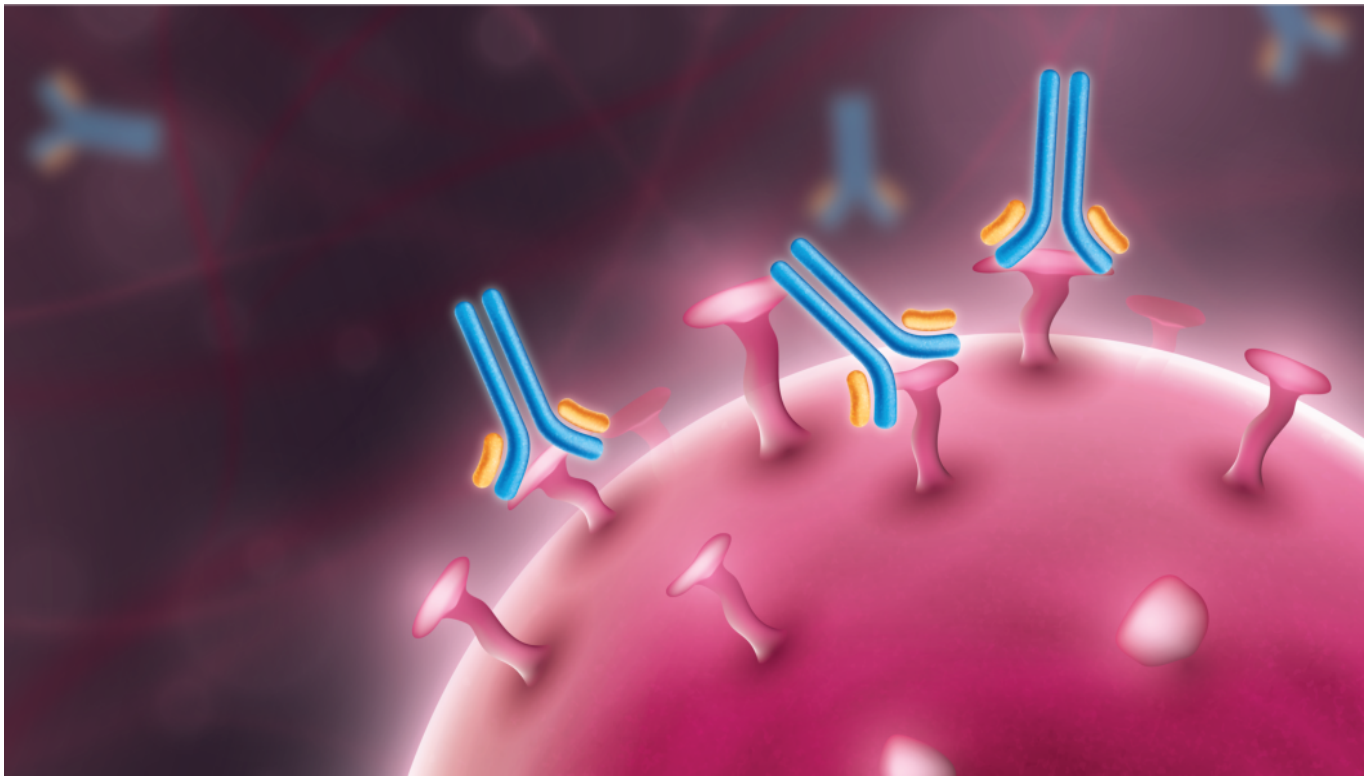
Issue: Oct-Dec 2021



UNDERSTANDING ANTIBODY TESTING IN SARS-CoV-2 INFECTIONS

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Since December 2019 researchers have tirelessly worked to develop diagnostic platforms for the timely and accurate diagnosis of SARS-CoV-2 infections.

Initially, molecular-based assays were the only means to confirm SARS-CoV-2 infections and its performance depended on many factors from sample types[1], the stage of infection in patients[2], the skill of sample collection, specimen transport and the quality of the assay used.

Along the course of the pandemic, serology tests were developed to detect antibodies against SARS-CoV-2 which became an important tool to augment diagnostics in SARS-CoV-2 infections.

To have some insights on how antibody test works, a brief overview of the virus and the humoral aspect of the adaptive immunity is discussed.

SARS-CoV-2 VIRAL ENTRY

The SARS-CoV-2 virus has 4 main structural proteins, the S (spike) protein, N (nucleocapsid) protein, M (membrane) protein and the E (envelope) protein (Fig.1).

The immunodominant domain of the virus are the S and N protein. The viral ability to induce a clinically meaningful immune response renders these antigens the key target for antibody detection assays.

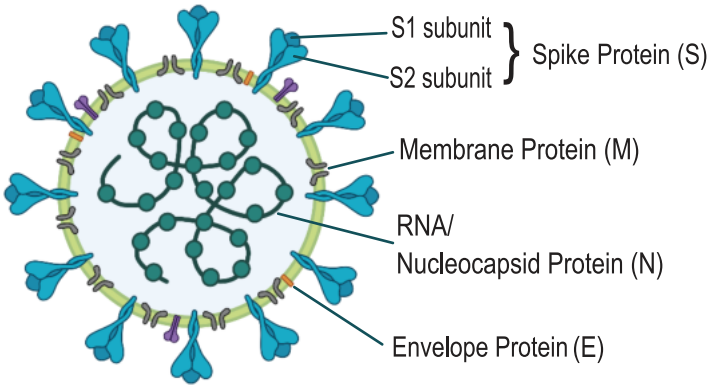


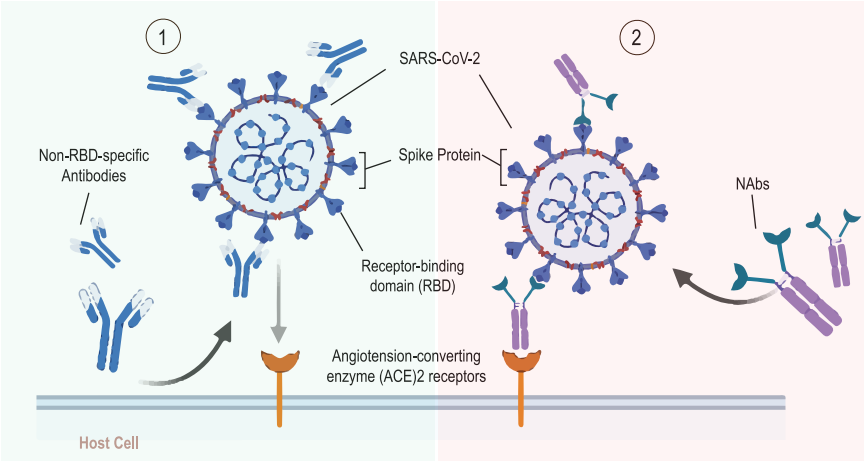
Figure 1: Structure of SARS-CoV-2 Virus

The spike protein comprises of 2 regions, the S1 and S2 subunits. The S1 subunit contains the receptor binding domain (S1-RBD) which binds to the ACE2 receptor on human cells thus initiating infection[3]. The S2 subunit mediates fusion of the host cell membrane and facilitate viral entry into host cells. The crucial properties of the S protein; namely host cell receptor recognition, viral attachment and entry into host cells, make the S protein an indispensable target for SARS-CoV-2 vaccines. The N protein, a structural viral protein that shields the viral RNA, is produced in abundance and is highly immunogenic. The N and the S antigens, given its immunogenic properties are potential targets in serology detection assays.

The virus, after entering into the host cells, uses the host cell machinery to replicate and produce more viral particles. The release of the viral particles from the host cells then triggers a cascading immune response. The B cells when activated (by the T cells) will replicate and differentiate into short lived and long lived plasma cells. The short lived plasma cells produces large amounts of antibodies targeted specifically and only against the stimulating viral antigen.

NEUTRALIZING ANTIBODIES

Antibodies formed to various antigens of the SARS-CoV-2 virus are binding with a subset having neutralizing properties[4]. Neutralizing antibodies are those which interfere with the binding of the virus to the host cell receptors thus preventing an infection (Fig. 2).



1

Antibodies that are non-RBD-specific ***could not fully*** block the RBD-ACE2 interaction; and thus the risk of viral infection is still present.

2

Neutralizing Antibodies (NAbs) specifically bind to the RBD of the viral S protein preventing the virus from interacting with the ACE2 receptor, hence 'neutralizing' the infection.

Figure 2: Non-RBD-specific Antibodies vs Neutralizing Antibodies (NAbs) in preventing viral infection

90% of the neutralizing antibodies targeted S1-RBD epitope of the spike protein[5]. Neutralizing antibodies are used as a surrogate marker for protective immunity, conferring immunity against potential future reinfection. To date, it is unclear what levels of neutralizing antibody correlates with protective immunity.

Hence, detecting neutralizing antibodies rather than all antibody types is fast gaining recognition. Conventional gold standard techniques to assess neutralizing antibodies in vitro is by plaque reduction neutralization tests (PRNT). However, PRNT requires working with live virus demanding containment laboratory facility and is not amenable to high throughput formats[6]. Hence, alternate serological assays benchmarked against PRNT are commercially available to detect neutralizing antibody against SARS-CoV-2 following an infection or post vaccination.

ANTIBODY TESTING: PROS AND CONS

Validated serological assays are used in conjunction with molecular test to complement and expand the detection window of SARS-CoV-2 infection. Serology assays has its strengths to overcome the limitations of molecular testing, first and foremost the cost may be prohibitive in resource limited settings and secondly the varied sampling methods for molecular test that can impact its sensitivity.

SARS-CoV-2 serology assays are used together with molecular assay for diagnosis of SARS-CoV-2 infections in individuals. It can be used to establish prevalence data of SARS-CoV-2 disease in the community besides monitoring immune responses post infection or post vaccination aiming to define vaccine efficacy. It has a role to identify donors for convalescent plasma therapy[7].

There are concerns regarding the commercially available serological assay:

Availability of different testing methodologies:

1) Enzyme Linked Immunosorbent Assay (ELISA)

2) Chemiluminescent Immunoassays (CLIA)

3) Lateral Flow Immunoassay (LFAs)

With varying classes of antibody detected (IgM, IgG, IgA or Total IgM/IgG) with different target antigen of the SARS-CoV-2 virus utilized in the assay (recombinant NP, full length Spike protein, or the S1- RBD of the S protein).

These varied testing formats require stringent laboratory verification, understanding fully its clinal utility to ensure accurate reproducible results.

The potential for cross reactivity between the SARS-CoV-2 antibodies with that of the other members of the family of coronavirus:

Potentially yielding false positive results.

However, recent preliminary studies by many groups are defying the possibility of cross reactivity between these antibodies[8].

Due to the lack of concreate data the FDA currently warrants inclusion of a comment in laboratory reports stating the possibility of cross reactivity between viruses in the family of coronaviruses.

Given the complexity of serology assays, the importance of providing a brief, relevant clinical history by the clinicians so that appropriate serology platform is used for the right clinical indication to eventually provide meaningful interpretable results by the laboratory.

In conclusion, serology assays to detect antibodies to SARS-CoV-2 provides an additional tool for the diagnosis of SARS-CoV-2 infections. It is, however, not advocated to be used as a stand alone diagnostic assay for acute infection. It has provided vital clinical information during the course of the SARS-CoV-2 pandemic and its routine application in the diagnosis and management of SARS-CoV-2 infections is established.

ANTIBODY TESTS AVAILABLE:

TEST TYPES	COI RTK Covid-19 IgG/IgM	CO9 Covid-19 Ab Total IgG/IgM/IgA	COQ Total (Quantitative) Antibody Test	CON Neutralizing Antibody Assay
Applications	Detects **anti -N** antibodies (IgM/IgG)	Detects total **anti -N** antibodies (IgM/IgG/IgA)	Detects total **anti-S1 (with RBD)** antibodies (IgM/IgG/IgA)	Detects **neutralizing** antibodies
Methodology	Rapid Test Kit	Electrochemiluminescence Immunoassay (ECLIA)	Electrochemiluminescence Immunoassay (ECLIA)	Electrochemiluminescence Immunoassay (ECLIA)
Test Performance	IgG: 87.90% IgM: 97.20%	Specificity: 99.80% Sensitivity: 99.50%	Specificity: 99.80% Sensitivity: 98.80% **(100% concordance with NAbs Assay)**	Specificity: 99.69% Sensitivity: >99.99%
Results	Qualitative	Qualitative	Quantitative, in U/mL	Qualitative
Interpretation Limitations	Does not detect anti-S antibodies	Does not detect anti-S antibodies	Does not detect anti-N antibodies	Does not detect anti-N antibodies
TAT*	2 hours	Within 24 hours	Within 24 hours	Within 24 hours

*Upon sample receipt at core laboratory.

Note:
A negative test result does not exclude SARS-CoV-2 infection or failure of seroconversion.

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