

SARS-CoV-2 tests in occupational settings: what you look for is what you get

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SUMMARY

During the last month of 2019, a new Coronavirus from China started to spread all around the world causing a pandemic emergency still ongoing. The outbreak made imperative the need for diagnostic and screening tests that could identify the current and past infection state of an individual. Occupational medicine is facing a very demanding challenge, as the pandemic set off the need to re-evaluate many aspects of workplace safety. A fundamental role has been played by tests used to diagnose COVID-19 and to isolate infected asymptomatic subjects, with a view to the viral evolution and the emerging variants. However, the need for the urgent set-up of new methods for assessing both new and past infections has resulted in a large number of methods, not always comparable with each other, in terms of laboratory techniques, viral antigens used for detection, and class of antibodies detected. These factors make it difficult to understand the serological test results and their possible application. In this paper, we reviewed the types of assays currently available, to address some key aspects that characterize each technique, and might have an impact on results interpretation.

INTRODUCTION

Between the end of 2019 and the beginning of 2020, the emergence of several cases of pneumonia of unknown etiology spread from China to the rest of the world. Consequently, in March 2020, the World Health Organization (WHO) declared a pandemic (1). As of May 2021, the novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected more than 150 million cases in the world, with more than 3.23 million deaths attributed to COVID-19, and the pandemic is still ongoing.

In the last year, rigorous public health measures have been taken globally. In this context, occupa-

tional medicine is facing a very demanding challenge, as the pandemic set off the need to re-evaluate many aspects of workplace safety, such as the implementation on new procedures, the assessment of personal protective equipment (PPE), and the need to reorganize spaces and workflows (2-3). A fundamental role has been played by tests used to diagnose COVID-19 and to isolate infected asymptomatic subjects. However, the need for the urgent set-up of new methods for assessing both new and past infections has resulted in a large number of approaches, which are not always comparable with each other and measure different components of SARS-CoV-2 (4-6).

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STRUCTURE OF SARS-CoV-2

To give a better understanding of the different targets detected by each method, a brief description of the SARS-CoV-2 components is required (Figure 1). The SARS-CoV-2 is constituted by a single-strand positive-sense RNA (+ssRNA). The genome encodes four main structural proteins involved in the virus life cycle and replication: the nucleocapsid (N) protein, the membrane (M) protein, the spike (S) protein, and the envelope (E) protein (7-8). These proteins share high sequence similarity to the sequence of corresponding proteins of SARS-CoV, and middle east respiratory syndrome (MERS)-CoV (9). In addition, there are sixteen non-structural proteins (nsp1-16), and five to eight accessory proteins.

The nucleocapsid (N) protein is a very abundant viral protein whose expression is detectable in the host already during the early phase of SARS-CoV-2 infection. The M and E proteins are involved in the morphogenesis and assembly processes of new viral particles, and they confer a spherical shape to the virions. The S glycoprotein is a homotrimer that protrudes from the viral surface, giving to the virions the aspect of a solar corona. S-protein plays an important role in virus entry into the host cells. S-protein is composed of two functional subunits: S1 and S2. The S1 subunit can bind to the receptor on the host cell [the angiotensin-converting enzyme 2 (ACE2) receptor], through the interaction of its Receptor-Binding-Domain (RBD), which is also

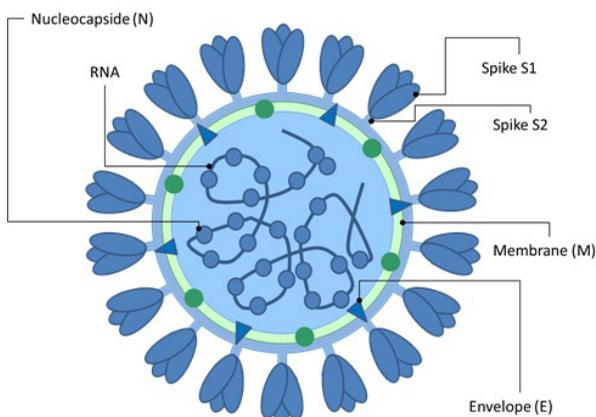


Figure 1. Structure of the SARS-CoV-2 virus.

the most specific protein of each coronavirus. The function of the S2 subunit is to fuse the membranes of viruses and host cells. Glycoproteins perform an important function for proper folding of the spike, and they contribute to stimulating the production of neutralizing antibodies (10-11).

OVERVIEW ON POSSIBLE TESTING FOR SARS-CoV-2

The choice of the right strategy of analysis is crucial to diagnose COVID-19 cases and to isolate infected asymptomatic carriers of the virus. In a very short time from the discovery of SARS-CoV-2, a huge number of tests has been developed (reviewed by the foundation for innovative new diagnostics - FIND, that is collating an overview of SARS-CoV-2 tests for the diagnosis of COVID-19, available at (12)). They can be grouped into two main categories: viral tests looking for SARS-CoV-2 (viral protein or RNA) and serological assays to detect immunological response against the virus.

Viral tests

Viral tests can detect a current or recent infection. Viral material can be researched in a specimen collected in the nasopharynx (nasal swab), in the oropharynx (oropharyngeal swab), or in the mouth (saliva). Although nasopharyngeal swab (NS) has been the most widespread biospecimen collected so far, recently, saliva diagnostic accuracy has been reported as comparable to the one of NS (13), especially in the ambulatory setting. These findings support larger-scale research on the use of saliva nucleic acid amplification test (NAAT) as an alternative to nasopharyngeal swabs.

A detailed description of procedures to collect those specimens can be found in the Guideline “Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19)” released from the Centers for Disease Control and Prevention (CDC) (14). Two main tests are available to date: 1) Tests based on nucleic acid amplification; 2) Tests detecting viral antigens.

Nucleic acid amplification allows the detection of viral ribonucleic acid (RNA), regardless of the infectivity status of the tested subject. They are very accurate but they require a laboratory analysis to

be performed, and therefore they cannot be used as self-diagnostic fast tests.

Assays developed to directly assess the presence of SARS-CoV-2 in biospecimens have been based on Real-time quantitative polymerase chain reaction (RT-qPCR) (15). RT-qPCR is very sensitive but the assays cannot distinguish between a positive result due to the presence of an actively replicating virus or rather an RNA residual of a past infection (i.e., no longer infectious) (16). Different laboratories are using different RT-qPCR panels (e.g., including ORF1a or ORF1b, N gene, S gene) to detect the viral RNA.

Many of them have implemented *in-house* testing based on the Berlin–Charité protocol (17), while others are using commercially available assays whose primers/probes sequences are not available due to proprietary information policies. As the degree of genetic similarity with other coronaviruses can vary (for example, S is more specific than N), the choice of PCR targets can affect the sensitivity of the method. Moreover, to achieve high sensitivity, many methods use a high threshold of positivity [often cycle threshold (Ct) >40], increasing the possibility of detecting false-positives. Although Ct is only a semi-quantitative value, low Ct generally indicates a high concentration of viral genetic material, typically associated with high risk of infectivity. A high Ct indicates a low concentration of viral genetic material, typically associated with a lower risk of infectivity. The clinical meaning of a positive result with high Ct (i.e. low viral load), often defined as “weakly positive”, is quite difficult to understand if the subject has always been asymptomatic. “Weakly positive” swab can be detected, in fact, in the early stages of infection or late in infection when the risk of transmission is low.

It has been described that some clinically-recovered COVID-19 patients, or even asymptomatic SARS-CoV-2 positive subjects, can show a persistent positivity of SARS-CoV-2 RNA after the resolution of symptoms (18-19). Whereas persistent fragments of the virus’ RNA can be present during the recovery period, the duration of infectivity (defined as the time during which SARS-CoV-2 can be transferred from an infected person to another) is harder to establish, but it is thought to decline

quite quickly (1-2 weeks after the onset of symptoms) (20-21).

An additional problem is the possibility that a subject develops a reinfection. According to the guidelines released by the Public Health England (22), healthcare workers or hospital patients who have tested positive for SARS-CoV-2 by PCR should be exempt from routine re-testing within a period of 90 days from their initial illness onset or test (if asymptomatic) unless they develop new COVID-19 symptoms. On the contrary, if a person is re-tested by PCR after 90 days from their initial illness onset or test and is found to be PCR positive, this should be considered as a possible new infection. If they have developed new COVID-19 symptoms, they would need to self-isolate again and their contacts should be traced.

Antigenic tests detect viral proteins (usually N or S proteins) and the analysis is fast to perform. However, the sensitivity of the method and its positive predictive value (PPV) are lower than the ones obtained for nucleic acid amplification and also lower in asymptomatic than in symptomatic subjects (23-25). Despite the reduced sensitivity compared with RT-qPCR, the use of antigen tests for serial testing might allow rapid identification of infectious persons. In fact, a good correlation between rapid antigen testing and infectivity has been reported and can be considered as an important indication of clinical utility in controlling virus transmission (26).

On the other hand, the benefit of having an immediate result, is balanced by the higher proportion of false positive and false negative results (27). In people with signs and symptoms of COVID-19, sensitivity is highest in the first week of illness when viral load is higher (28). Evidences in asymptomatic cohorts are still limited, and test ability to differentiate infectious subjects requiring isolation from subjects who pose no risk is still undefined, as there is no reference standard for infectiousness (29).

It is important to take into consideration the lower sensitivity and lower PPV when used for asymptomatic screening, by confirming the result of a positive test in an asymptomatic person, or a negative antigen test result in a person experiencing symptoms compatible with COVID-19, with a positive RT-qPCR.

Serological assays

Following the infection with SARS-CoV-2, antibody response can be multifaceted and include antibodies against several viral antigens. The most important targets are the nucleocapsid N protein and the S (S1 and S2) protein (Figure 2). The peak of production of these antibodies in symptomatic patients typically occurs between 14 and 21 days from symptoms onset (30). On the contrary, only a minority of positive asymptomatic subjects develop antibodies (31) and the underlying kinetics are still under debate.

The current literature agrees that anti-RBD antibodies are neutralizing (32). Anti-S can also include, but not be limited to, anti-RBD antibodies. In contrast, anti-N are produced earlier after infection but cannot prevent the binding of SARS-CoV-2 to

the ACE2 receptor. Actually, higher ratios of S1 or RBD to N IgG antibodies were seen in outpatients who had mild illness compared to severely ill patients (33).

The required performance of a serological assay depends on the specific aim of testing (34), which may span from population screening (either the general population or workers at high risk of infection), diagnostic support, or post-vaccination follow-up (35).

The commercially available serological tests are based on three main techniques:

- the qualitative rapid test lateral flow immunoassay (LFIA) can detect only the presence (or the absence) of antibodies in the sample, giving a yes/no result
- the semi-quantitative enzyme-linked immunosorbent assay (ELISA) assays
- the automatic quantitative chemiluminescent microparticle immunoassay (CMIA)/ chemiluminescent immunoassay (CLIA) assay, which can measure the titer of antibodies by quantifying chemoluminescence.

In all serological assays, a viral protein (e.g., N or S) or a protein-specific domain (e.g., RBD) is attached to a support, and then the patient's biological sample (usually blood, serum, plasma) is placed in contact with the support. As soon as a chemical reagent is applied, if specific antibodies that recognize the SARS-CoV-2 virus are present in the sample, they bind to the viral antigens on the plastic, causing a colorimetric reaction. When compared with a reference sample, the intensity of the signal indicates the number of specific antibodies in the sample and it is possible to obtain the antibody titer. Rapid tests or lateral flow assays, however, can only give a positive or negative result.

The sensitivity and specificity of serological assays for SARS-CoV-2 disease are still partially unknown, as a validated gold standard for diagnosis is missing (36). However, the sensitivity and specificity of the different assays may vary considerably between the different available methods, since the various implemented techniques (ELISA, CMIA/CLIA, LFIA) are diverse and have different intrinsic technical characteristics, which might also be affected from Covid-19 prevalence experienced in

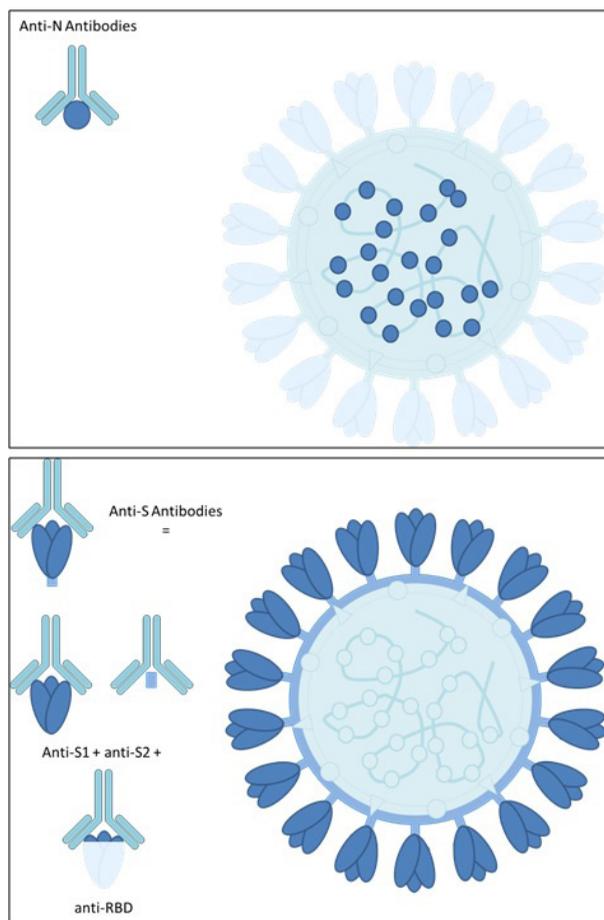


Figure 2. Immunological targets of SARS-CoV-2 commonly used in serological assays.

different places (37–38). In addition, the interpretation of results might be even more difficult, as memory T-cell against SARS-CoV-2 could be detected in subjects negative for antibodies even a relatively short time after infection (39).

Since each antigen used in the various tests has different immunogenic capacity, results must be interpreted in the context of the specific antigen used. For example, the Nucleocapsid protein is the most conserved between the different coronaviruses (40–41); as such, serological tests using N as the antigen can give rise to false positives as a result of incorrect detection of anti-N antibodies, which are present in the samples but are generated against the common coronaviruses (e.g. OC43, one of the coronaviruses responsible for common cold in humans). These antibodies are not specific to the SARS-CoV-2 nucleocapsid protein, but because they recognize the N protein of previous coronaviruses, which has an extremely similar structure and sequence to that of SARS-CoV-2, they can still bind to the N protein of the current virus. In contrast, the Spike protein is much more specific (RBD in particular): as such, methods focusing on S allow better discrimination between anti-Spike antibodies generated against SARS-CoV-2 or previous coronaviruses (42). In addition, as the current approaches are targeting vac-

ination toward RBD/S1, the measure of N-specific antibodies cannot be used to identify vaccine-related antibodies (anti RBD or anti S1) (33). The different available serological tests will be described in detail below.

LFIA

LFIA are rapid detection tests that can be used easily as point-of-care tests or in the laboratory, giving a result in approximately 15 minutes. With this method, antibodies contained in the biospecimen migrate through an adhesive matrix and interact with virus-specific antigens and a secondary antibody (for example, anti-IgM / IgG antibodies) bound to the matrix. When the antibody-antigen complex (formed in the Conjugation Pad) migrates through the membrane, IgM antibodies can interact with the secondary anti-IgM antibodies fixed on the M line of the support, while IgG antibodies interact with anti-IgG antibodies on the G line. A colored line means the presence of antibodies. LFIA can be targeted toward different viral proteins (Figure 3A).

ELISA

ELISA tests need to be conducted in a laboratory. Usually, the analysis can require some hours, and this approach is considered semiquantitative. The

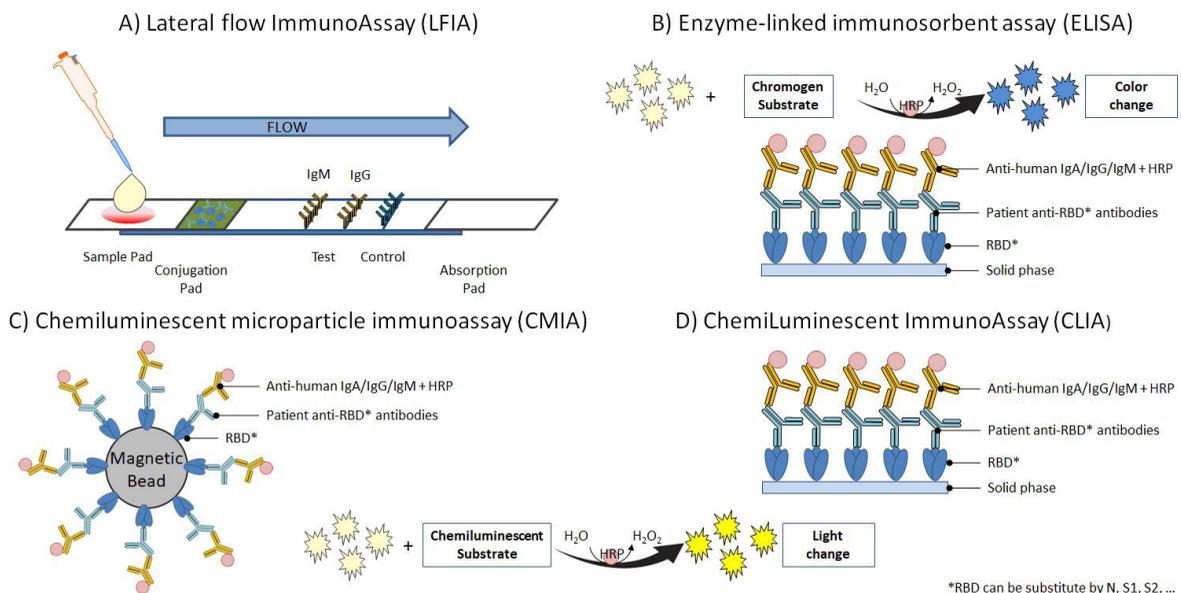


Figure 3. Principles for COVID-19 serological tests. HRP: Horse Radish Peroxidase (reagent).

method relies on the use of a plastic plate (usually a 96 or 384-well plate) coated with one or more viral antigens. Once the subject sample is added, if antibodies against the viral antigen are present, they bind to the plate. Then, the use of reporter molecules and chromogenic substrates allows to producing color changes to indicate the presence of anti-virus antibodies in the sample (Figure 3B). ELISA can detect different classes of antibodies, including IgG, IgM, and IgA.

CMIA/CLIA

Chemiluminescence tests are based on the detection of the high binding affinity between viral antigens and host antibodies, using chemical probes that produce light emission through a chemical reaction; the emission of light indicates a positive signal. CMIA (using chemiluminescent microparticles) and CLIA (using chemiluminescent surfaces) are both high throughput quantitative laboratory assays, which can be performed with dedicated platforms (Figure 3C and 3D). They can be used with whole blood, plasma, or serum samples. The patient's samples are mixed with a known purified viral protein, buffer reagents, and specific antibodies labeled with enzymes. The binding between the viral protein and the antibody mediates the production of a light-based luminescence. The amount of light emitted by the sample is proportional to the antibody (IgG, IgM, or IgA) titer.

Table 1 reports a list of currently available tests to detect anti-SARS-CoV-2 antibodies. For each test, we reported the detected antibodies (IgM, IgG, or total antibodies) and the antigens they are directed against (N, S, or RBD).

These two pieces of information profoundly modify the interpretation of the obtained results, determining a method's suitability according to the different contexts. To make some examples, if the analysis aims to evaluate whether an individual produced an immune response after vaccination, it is mandatory to choose a technique able to detect anti-S or anti-RBD antibodies, since the vaccine only uses the RBD fragment (as it is the one inducing virus neutralization), and a vaccinated subject will not produce anti-N antibodies. On the contrary, if the purpose is to screen a large population in an early

phase of the disease (and positive serological assays will be confirmed with a viral RNA molecular test), anti-N antibodies would be more useful as they are produced earlier and in larger quantities than S antibodies. In any case, it is useful to point out that the use of diagnostics test for assessing the efficacy of vaccines, particularly among Healthcare Workers, might be often inappropriate.

According to the CDC guidelines, antibody testing is not recommended for vaccine decision makers (e.g. to prioritize vaccine administration in a context of lacking doses) or to assess immunity following vaccination (43) in order to define any timeline of further vaccination boosters. On the other hand, the application of these tests might be useful to assess the persistence of immune response, in the context of epidemiological studies, in order to better capture the evolution of the immune response.

Detection of viral variants

As of May 2021, one of the main concerns regards the development of new variants of the SARS-CoV-2 virus. The four main variants currently spreading include:

1. The "English" variant (B.1.1.7), which carries 23 mutations (the majority in the spike region) compared to the original reference sequence (NC045512-2-Wuhan-Hu-1) (44).
2. The "South African" variant (B.1.351), characterized by eight lineage-defining mutations in the spike protein, including three at important residues in the receptor-binding domain (K417N, E484K, and N501Y) that may have functional significance (45).
3. The "Brazilian" variant (p.1), carrying 17 mutations, including 3 in the S protein (46).
4. The "Indian" variant (B.1.617) is characterized by 13 mutations, three of which, located within the spike region, are of particular concern (L452R, E484Q, and P681R) (47-48).

Discrimination between variants cannot be made using traditional approaches, as the viral RNA needs to be sequenced (as a consequence, large-scale epidemiological investigations are still not available).

Several questions on this topic are still open, such as: how have these new variants spread in

Table 1: Overview of the currently available methods for SARS-CoV-2 IgM/IgG detection.

Technology	Test	Developer	Target
LFIA	CareStart COVID-19 IgM/IgG	Access Bio, Inc.	Spike and Nucleocapsid
LFIA	ACON SARS-CoV-2 IgG/IgM Rapid Test	ACON Laboratories, Inc.	Spike and Nucleocapsid
LFIA	Assure COVID-19 IgG/IgM Rapid Test Device	Assure Tech. (Hangzhou Co., Ltd)	Spike and Nucleocapsid
LFIA	WANTAI SARS-CoV-2 Ab Rapid Test	Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.	Spike
LFIA	Tell Me Fast Novel Coronavirus (COVID-19) IgG/IgM Antibody Test	Biocan Diagnostics Inc.	Spike and Nucleocapsid
LFIA	Biohit SARS-CoV-2 IgM/IgG Antibody Test Kit	Biohit Healthcare (Hefei)	Nucleocapsid
LFIA	qSARS-CoV-2 IgG/IgM Rapid Test	Cellex, Inc.	Spike and Nucleocapsid
LFIA	RightSign COVID-19 IgG/IgM Rapid Test Cassette	Hangzhou Biotest Biotech	Spike
LFIA	LYHER Novel Coronavirus (2019-nCoV) IgM/IgG Antibody Combo Test Kit	Hangzhou Laihe Biotech	Spike
LFIA	COVID-19 IgG/IgM Rapid Test Cassette	Healgen	Spike
LFIA	Innovita 2019-nCoV Ab Test (Colloidal Gold)	Innovita (Tangshan) Biological Technology Co., Ltd.	Spike and Nucleocapsid
LFIA	Orawell IgM/IgG Rapid Test	Jiangsu Well Biotech	Spike
LFIA	Rapid COVID-19 IgM/IgG Combo Test Kit	Megna Health, Inc.	Nucleocapsid
LFIA	Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit	Nirmidas Biotech, Inc.	Spike
LFIA	Sienna-Clarity COVIBLOCK COVID-19 IgG/IgM Rapid Test Cassette	Salofa Oy	Spike
LFIA	SGTi-flex COVID-19 IgG	Sugentech, Inc.	Spike and Nucleocapsid
LFIA	TBG SARS-CoV-2 IgG / IgM Rapid Test Kit	TBG Biotechnology Corp.	Spike and Nucleocapsid
LFIA	BIOTIME SARS-CoV-2 IgG/IgM Rapid Qualitative Test	Xiamen Biotime Biotechnology Co., Ltd.	Spike
ELISA	WANTAI SARS-CoV-2 Ab ELISA	Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.	Spike
ELISA	Platelia SARS-CoV-2 Total Ab	Bio-Rad Laboratories, Inc	Nucleocapsid
ELISA	SARS-CoV-2 RBD IgG test	Emory Medical Laboratories	RBD
ELISA	SARS-COV-2 ELISA (IgG)	EUROIMMUN	Spike
ELISA	SCoV-2 Detect IgG ELISA	InBios	Spike
ELISA	SCoV-2 Detect IgM ELISA	InBios International, Inc.	Spike
ELISA	COVID-SeroKlir, Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit	Kantaro Biosciences, LLC	Spike
ELISA	Mt. Sinai Laboratory COVID-19 ELISA Antibody Test	Mount Sinai Hospital Clinical Laboratory	Spike
ELISA	Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test	Quanterix Corporation	Spike
ELISA	Dimension EXL SARS-CoV-2 Total antibody assay (CV2T)	Siemens Healthcare Diagnostics	Spike
ELISA	Dimension Vista SARS-CoV-2 Total antibody assay (COV2T)	Siemens Healthcare Diagnostics	Spike
ELISA	OmniPATH COVID-19 Total Antibody ELISA Test	Thermo Fisher Scientific	Spike
ELISA	COVID-19 ELISA pan-Ig Antibody Test	University of Arizona Genetics Core for Clinical Services	Spike
ELISA	ZEUS ELISA SARS-CoV-2 IgG Test System	ZEUS Scientific, Inc.	Spike and Nucleocapsid
CMIA	Alinity i SARS-CoV-2 IgG	Abbott	Nucleocapsid

(Continued)

Table 1: Overview of the currently available methods for SARS-CoV-2 IgM/IgG detection.

Technology	Test	Developer	Target
CMIA	Architect SARS-CoV-2 IgG	Abbott	Nucleocapsid
CMIA	AdviseDx SARS-CoV-2 IgM (Alinity i)	Abbott Laboratories Inc.	Spike
CMIA	AdviseDx SARS-CoV-2 IgM (Architect)	Abbott Laboratories Inc.	Spike
CLIA	Babson Diagnostics aC19G1	Babson Diagnostics, Inc	Spike
CLIA	Access SARS-CoV-2 IgG	Beckman Coulter, Inc.	Spike
CLIA	Access SARS-CoV-2 IgM	Beckman Coulter, Inc.	Spike
CLIA	BioCheck SARS-CoV-2 IgG and IgM Combo Test	BioCheck, Inc.	Spike
CLIA	BioCheck SARS-CoV-2 IgG Antibody Test Kit	BioCheck, Inc.	Spike
CLIA	BioCheck SARS-CoV-2 IgM Antibody Test Kit	BioCheck, Inc.	Spike
CMIA	LIAISON SARS-CoV-2 S1/S2 IgG	DiaSorin	Spike (S1/S2)
CLIA	DiaSorin LIAISON SARS-CoV-2 IgM Assay	DiaSorin, Inc.	Spike
CLIA	Diazyme DZ-Lite SARS-CoV-2 IgG CLIA Kit	Diazyme Laboratories, Inc.	Spike and Nucleocapsid
CLIA	Diazyme DZ-Lite SARS-CoV-2 IgM CLIA Kit	Diazyme Laboratories, Inc.	Spike and Nucleocapsid
CLIA	VITROS Anti-SARS-CoV-2 IgG test	Ortho-Clinical Diagnostics, Inc.	Spike
CLIA	Q-Plex SARS-CoV-2 Human IgG	Quansys Biosciences, Inc.	Spike
ECLIA	Elecsys Anti-SARS-CoV-2	Roche	Nucleocapsid
ECLIA	Elecsys Anti-SARS-CoV-2 S	Roche Diagnostics, Inc.	Spike
CLIA	MAGLUMI 2019-nCoV IgM/IgG	Shenzhen New Industries Biomedical Engineering Co., Ltd.	Spike and Nucleocapsid
CLIA	ADVIA Centaur SARS-CoV-2 IgG (COV2G)	Siemens Healthcare Diagnostics	Spike
CLIA	Atellica IM SARS-CoV-2 IgG (COV2G)	Siemens Healthcare Diagnostics	Spike
CMIA	ADVIA Centaur SARS-CoV-2 Total (COV2T)	Siemens Healthcare Diagnostics	Spike
CMIA	Atellica IM SARS-CoV-2 Total (COV2T)	Siemens Healthcare Diagnostics	Spike
CLIA	Vibrant COVID-19 Ab Assay	Vibrant America Clinical Labs	Spike and Nucleocapsid

different countries? What kind of disease do they cause? And how may these variants affect existing therapies and vaccines? In particular, one of the most important open questions regards how mutations (the ones reported above or other future variants) might affect the performance of currently available viral tests for SARS-CoV-2 detection. This would be a particularly pronounced problem for methods targeting a single position of the viral genome if this is mutated. Recently, preliminary investigations assessed if the B.1.1.7, B.1.351, and p.1 lineages can be detected by traditional methods of RT-qPCR, showing that these lineages do not affect the large majority of the publicly available RT-qPCR assays, such as Berlin-Charité protocol. However, they can challenge the available commercial kits directed to the S gene (49-51). Up to date, no information has

been published about the ability of conventional diagnostic kits to detect the Indian variant.

In addition, since most antigen-based tests target the N protein, minimal impact has been recently reported for the detection of the “English” B.1.1.7 lineage (50, 52), such as for the “South African” (53) variant. This observation, however, emphasizes the need to follow a multi-target approach interrogating different regions of the viral genome to increase test sensitivity (50).

Diagnostics in Occupational Settings

During SARS-CoV-2 pandemics, workplaces have often led to the formation of clusters of infections. This occurred both in particularly high-risk work environments (e.g. slaughterhouses, hospitals) (54) and in more “standard” environments, such as

offices. Occupational physicians were often asked to plan and perform surveillance campaigns, highly relevant for both the individual worker and public health (55). Several strategies have been proposed, including point-of care tests such as antigenic rapid assays. It is important to point-out that the success of each strategy is profoundly impacted by the pre-test probability of the infection. It is essential that the evaluation of the infection also takes into account the symptoms, to complement the result of diagnostic tests: negative results cannot exclude infection if the patient is experiencing Covid-like symptoms (56).

CONCLUSIONS

When hypothesizing to perform SARS-CoV-2 tests, it is fundamental to properly consider the questions to be addressed. The biology of the virus and each tests' peculiar characteristics guide the path toward the appropriate answer.

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