

Mass Antibody Detection: Can It be An Avenger for Infectivity

War against COVID-19

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Abstract

The ongoing pandemic of COVID-19 has not only commenced a global health emergency but agitated various aspects of humanity. During this period of crisis researchers over the world have ramped their efforts to constrain the disease in all possible ways whether it is vaccination, therapy, or diagnosis. Since the spread of the disease has not yet elapsed sharing the ongoing research findings could be the key to disease control and management. An early and efficient diagnosis could leverage the outcome until a successful vaccine is developed. Molecular tests both in-house and commercial kits are preferably being used worldwide in the COVID-19 diagnosis. However, the limitation of high prices and lengthy procedures impede their use for mass testing. Keeping the constant rise of infection in mind search for an alternative test that should be cost-effective, simple, and suitable for large scale testing and surveillance is a need of an hour. One such alternative could be the immunological tests. Therefore, in the last few months deluge of immunological rapid tests has been developed and validated across the globe. The objective of the present review is to share the diagnostic performance of various immunological assays reported so far in SARS-CoV-2 case detection. The article consolidated the studies (published and preprints) related to the serological tests such as chemiluminescence, enzyme-linked and lateral flow-based point-of-care tests in COVID-19 diagnosis and updated the current scenario. This review will hopefully be an add-on in COVID-19 research and will contribute to congregate the evidence for decision-making.

What we know about this real-world devil

The world today has been amalgamated like never before to fight a common enemy to mankind, the pandemic disease namely Coronavirus. Coronaviruses (CoV) belong to the family *Coronaviridae* responsible for the severe acute respiratory syndrome (SARS) that had first emerged as an epidemic in November 2003. Almost two decades later a novel coronavirus, SARS-CoV-2 originating in Wuhan city of China in December 2019 has led to an unprecedented global pandemic of COVID-19, a serious health challenge that our modern world was yet to see ¹. Despite the same family of viral origin as of SARS, transmissibility and disease severity of COVID-19 are much higher in terms of community spread. As of 31st May 2020, over 6.2 million confirmed COVID-19 cases reported so far with more than 370,000 deaths worldwide ² and the numbers are mushrooming by the day. After achieving a plateau in the COVID-19 infection graph in China, a shift in the epicenter of the disease is being seen with the astonishing numbers of new cases and deaths that are being reported in the USA, Iran, and many European countries.

Clinical manifestations of COVID-19 include fever, dry cough, fatigue, and pneumonia-like features. The coronavirus is reported to interact with host epithelial cell receptor, angiotensin converting enzyme 2 (ACE2), found in outer surfaces of most of the human body organs leads to endocytosis into the host cell ³. Severity of the disease occurs when COVID-19 virus (SARS-CoV-2) enters into the lungs through the oral or nasal passage, divides within the lung cells which lead to its death. Dead cells may generate an inflammatory response through IL-6, IL-1, and TNF- α etc., resulting in the consolidation of fluid in the alveolar and interstitial spaces, known as acute respiratory

distress syndrome (ARDS). The symptomatic clinical condition without ventilation may lead to death due to the insufficiency of oxygen in the vital organs thus multiple organ dysfunctions (kidney injury and cardiac arrest). Chances of this affecting the older age group are more due to their already present comorbidities.

Brief introduction of the current diagnosis

In the present scenario, while the vaccine is not available for COVID-19 and there is no short and sure treatment, rapid and extensive diagnosis could be the way to reach the grass-root level of the disease and break the widespread chain of this deadly COVID-19 infection ⁴. It will have a relevant role in the containment of the disease until a suitable therapy or vaccine strategy is attained. The conventional diagnosis is based on the clinical history and the radiographic findings of the chest but due to the lack of trained individuals in virus imaging and invasiveness of the process, it is routinely practiced only in the initial phase of the epidemic in China. In several areas temporary CT scans have been utilized for clinical diagnosis for false-negative results. These are non-invasive tests, however, was low on specificity, as lung images overlap with any common viral disease. A recent study reported a confirmed case of COVID-19 with no signs of pneumonia in chest CT image ³. Moreover, CT scans are expensive and constrained in the research labs and big hospitals requiring technical know-how.

From a worldwide perspective, the most popular and gold standard technique which is being used for COVID-19 diagnosis globally is an initial symptomatic analysis followed by molecular-based nucleic acid detection from sputum, nasal and mouth swab and

serum. The COVID-19 bears a single-stranded RNA genome of approximately 30,000 nucleotides. The nucleic acid testing of COVID-19 is based on reverse transcription polymerase chain reaction (RT-PCR) technique ⁵. Several conserved COVID-19 regions have been targeted in the molecular tests such as RNA dependent RNA polymerase gene (RdRP gene), nucleocapsid protein gene (N gene), and envelop protein gene (E gene). Since this test can amplify even low levels of the virus and detect the disease at an early stage, this has been the most used and reliable detection method. However, the performance of RT-PCR is largely depends on the target viral RNA selected and the primers used. Therefore, the World Health Organization (WHO) time to time published the standard protocol for the parity of the assay. Secondly, variations in the viral load are noticed in different stages of disease manifestations and the biological samples used ⁶. Despite a large number of RT-PCR based tests is being conducted throughout the globe its accessibility to reach centers of testing from remote areas restricts its mass appeal. Moreover, they are expensive, equipment-based, time taking, and require developed molecular biology expertise. However, the results are preliminary and most of the in-house PCR tested worldwide have a high false-negative rate in COVID-19 diagnosis ⁷. Missed diagnosis of a large number of clinically suspected individuals may promote the spread of the virus that may ultimately lead to faster disease progression. Moreover, in many cases molecular testing often requires more than one test for disease confirmation ⁸. RT-PCR is valuable in the initial phase of infection when the virus is present in the body, however, it could not identify the past, recovered, and asymptomatic infections. Most of the countries are under lockdown for almost two months now, it is essential to have a vigorous door to door testing to counter the

pandemic. Molecular biology-based tests in such cases will become highly cumbersome and should be eased with easier and quicker tests.

Rapid diagnosis to test as much as we can at this point in time may be considered the only hope to fight the disease. As emphasized by the chief of WHO on 16th March just three days after declaring COVID-19 a pandemic to “test, test and test”. The Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland, has published the first result of independent evaluation and clinical performance of five molecular test kits on 16th April. 100% sensitivity was obtained by all the kits with 50 COVID-19 positive samples and 97-100% specificity achieved with 100 negative samples⁹. However, there may be asymptomatic or infected patients, who may have cleared the viral load without detection, thus an RT-PCR will fail to test such cases. Therefore, it is important to develop and introduce a rapid and point-of-care test to detect COVID-19 cases and carriers. The antibody-based tests can play an important role in the restraint of COVID-19, facilitate the rapid execution of a control strategy that limits the spread of the disease. The National Health Commission of China in the seventh edition of its novel coronavirus pneumonia diagnosis and treatment plan recently recommended the use of IgM/IgG antibodies testing for suspicious cases¹⁰. Additionally, the convalescent patients will have to be monitored closely for a long-term period, which will involve repeated testing for the presence of antibodies against the disease. For the presence of antibodies, accuracy will be needed, as they will serve as tests of cure. Since most of the countries have permitted the use of antigen or antibody-based serological tests the sensitivity and specificity of these tests against COVID-19 are being reported throughout the world. With the dynamics of the disease changing at the speed of its

spread, it seems highly essential to get all the information at a glance related to the performance of serological tests reported worldwide to pace our health care needs. This review aims to have a compilation of the performance of various serological assays across the globe for the detection of COVID-19 pandemic.

Key determinants of immunoassay

Although molecular tests are the gold standard and very specific in early COVID-19 detection its reach to large scale diagnosis is constrained in terms of its cost, feasibility, and rapidness. Immunological assays could be a good complement in this regard. However, concerning diagnosis, immunoassays are different from molecular assays because immunoassays stipulate some knowledge of the protein and the antibody response generated against the protein. Therefore, immunoassays can be engaged to detect either specific viral proteins (antigens) or the antibodies developed by the host B cells in response to that antigen.

The ~30kb SARS-CoV-2 genome codes for approximately 27 proteins that include 4 structural proteins, 8 accessory proteins, and 15 nonstructural proteins. The accessory proteins are involved in the replication of viral RNA and transcription. The functions of nonstructural proteins range from replicase, protease, and deubiquitinase, etc. The viral structural proteins comprise of spike glycoproteins (S), membrane glycoproteins (M), envelop proteins (E), and nucleocapsid phosphoproteins (N) ¹¹. The trimeric S protein (~180 kDa monomer) is postulated to be the first viral fraction to bind with host cell receptors through the receptor-binding domain (RBD) of S1 subunit and helps in the

fusion of virus and host membranes through S2 subunit. M protein plays its role in viral morphogenesis and budding through its glycosylated N terminal domain, three transmembrane domains, and a long C terminal domain. E protein is the smallest structural protein that constitutes the viral assembly, release, and pathogenesis. The N protein is the most abundant viral protein (~40 kDa) thus could be an ideal candidate for diagnosis¹². A high immune response has been demonstrated against this protein and detected in blood and urine samples both. Despite its abundance, S proteins have also been used in COVID-19 diagnosis because of its specificity¹³. Studies have been reported to map the SARS-CoV-2 proteins and its antibody interaction through proteome microarray. Krishnamurthy et al have demonstrated the antibody response against four SARS-CoV-2 proteins, S1, RBD, S2, and N proteins with infected serum samples and found 98.1% sensitivity¹⁴. Wang et al. have identified specific antibodies to M, N, and S proteins of the virus. More than 80% of COVID-19 patients showed antibodies against four immunodominant epitopes of N, S, and Orf3a (accessory protein) residue. Interestingly, no antibodies were detected to E protein¹⁵. In another proteome microarray, 29 convalescent sera of COVID-19 were used to demonstrate the IgM/IgG response against 18 SARS-CoV-2 protein constructs. The result showed 100% antibody response mainly for N and S1 protein where S1 proved to be the best in differentiating COVID-19 patients from controls¹⁶.

The immunoassays which are being used prominently for recent COVID-19 diagnosis are mainly chemiluminescence immunoassay (CLIA), Enzyme-linked immunosorbent assay (ELISA) and rapid diagnostic tests (RDT) such as lateral flow immunoassay (LFIA), these detect mainly the viral structural proteins or seroconverted IgM and IgG

antibodies in blood or serum. On 13th March 2020 FIND had called an expression of interest for evaluation of antigen and antibody-based immunoassays from the COVID-19 test manufacturers. Seven ELISA, 5 antigen detection, and 27 antibody detection RDTs have been selected for the first phase ¹⁷. Immunological assays listed in the site are either comply with research use only (RUO) or in-vitro diagnostics (IVD) guidelines. IVD assays are more stringent than RUO mainly in certification and validation with real clinical samples.

Antibody dynamicity through CLIA

Since the emergence of the COVID-19 pandemic many researchers have investigated the antibody response during the disease through chemiluminescence immunoassay. A study by Jin et al. demonstrated the dynamic variance of IgM and IgG antibodies in COVID-19 patients retrospectively. Positivity of IgM antibodies increased initially then decreased over time whereas IgG positivity increased to reach 100% and every time it was higher than IgM ¹⁸. Another retrospective study done by Hu et al. observed maximum concentration of antibodies on day 19-21 whereas the highest detection rate was found to be 73.6% on day 16-18 for IgM and 98.6% on day 19-21 for IgG. Additionally in critical COVID-19 patients the results suggested a significantly higher concentration of IgG than mild and moderate cases ¹⁹. A serological study in Italy observed 100% sensitivity for IgG than 88% for IgM at day 12 ²⁰. A study from China reported 97.7% and 95.6% sensitivity and 95.2% and 96.6% specificity of IgM and IgG antibodies respectively ²¹. 100% sensitivity and specificity were achieved in USA with

commercial IgG CLIA test at day 17 from the disease onset ²². In another study, seropositivity in confirmed COVID-19 patients was found within 7-12 days of disease onset and continues with disease progression. The overall specificity of IgM and IgG with non-COVID-19 suspected cases, other diseases, medical staff, and healthy controls were found to be 97% ⁸. Interestingly, Zeng et al. have shown comparatively higher IgG antibody in females during the early phase of SARS-CoV-2 infection and also tended to be elevated in severe cases than males ²³. A case-control retrospective study suggested maximum IgM level in the 4th week of disease onset and higher in deceased patients in comparison with mild to moderate COVID-19 patients ²⁴. In a similar kind of study Xiao et al have revealed 100% positivity of IgM and IgG in the 3rd week of disease onset. However, from the 5th week onward IgG remained positive but IgM continued to decline ²⁵. In contrast a study using peptide derived from S protein detected IgG earlier than IgM in CLIA and as early as two days from the onset of COVID-19 symptoms. The combination of IgM and IgG showed 81.52% positivity in comparison to 57.2% and 71.4% for IgM and IgG respectively with 100% specificity to other diseases and healthy controls ⁴. Four CLIA tests were evaluated and found to be different in performance with maximum sensitivity and specificity of 92% and 99.23% with total antibody ²⁶. In one of the studies comparing the two known antigens in CLIA suggested outperformance of RBD over N protein with early responses of IgA and IgM than IgG ²⁷. Additionally, the severity of the disease was positively correlated with the level of IgA antibody in many cases. Overall, sensitivities and specificities of IgA, IgM and IgG tests with laboratory-confirmed patients were 98.6%, 96.8%, and 96.8% and 98.1%, 92.3%, and 99.8%, respectively. However, after combining three antibodies the

sensitivity and specificity reached to 99.5% and 100% respectively. A multicentric study recently reported immunoassay with COVID-19 patients of 10 hospitals in Wuhan using recombinant fusion protein constructed with fragments of N and S proteins. Virus-specific IgG showed 96.62% and 86.54% sensitivity with RT-PCR confirmed and suspected cases respectively whereas IgM showed 85.88% and 73.08%. Moreover, IgM and IgG showed 97.33% and 97.43% specificity for hospitalized patients with other diseases while 99.49% and 99.15% with normal healthy individuals ²⁸. Liu et al. have demonstrated the superiority of antibody test than the nucleic acid test in moderate, severe, and critical COVID-19 cases of Wuhan retrospectively. For moderate cases positivity for IgM and IgG was 79.55% and 83.18% respectively than 65.91% for RT-PCR. Severe cases showed 82.69% and 100% positive ratio for IgM and IgG respectively than 71.15% of RT-PCR whereas in critical cases ratios found to be 72.97% and 97.30% for IgM and IgG respectively in comparison to RT-PCR with 67.57% ²⁹. The sensitivity and specificity obtained through CLIA are summarized in Table 1.

Antibody ELISA in COVID-19 diagnosis

Since conventional ELISA is a cumbersome and time-consuming process diagnostic companies provide precoated ELISA either with EUO or IVD labeling to detect virus-specific antibodies in human samples. However, the sensitivity and specificity of the ELISA kit largely relies on the type of viral protein used. Nevertheless, the seroconversion of antibodies in COVID-19 patients depends on the onset of symptoms, therefore, the day on which the test is being conducted should be an important

consideration. Liu et al. have used ELISA format to detect IgM/IgG antibodies in patient's serum against SARS-CoV-2 N protein. The test showed 81.5% sensitivity with IgM and/or IgG at day 10th of disease onset than 64.3% with viral RNA detection. The antibody positivity increased from 50% to 80% when tested before 5 days and after 10 days of symptom ⁷. Zhao et al. demonstrated that within 7 days the RNA test has 66.7% sensitivity than the antibody test of only 38.3% whereas after 12 days of onset antibody detection overtook the RNA test and found to be more than 90% sensitive ³⁰. Adams et al. have shown 85% sensitivity and 100% specificity of IgM or IgG ELISA using trimeric S protein and 100% sensitivity of IgG after 10 or more days of disease onset ³¹.

The dynamics of antibodies against SARS-CoV-2 showed that total antibodies can be detected first in the sample followed by IgM then IgG. Another similar kind of study revealed that antibodies increased from day 0 to day 5 and positivity rate observed from 50% to 81% for IgM and 81% to 100% for IgG ⁵. Serological test with combined IgM and IgG detection has increased the overall sensitivity as evident from a study where 44.4% and 82.5% sensitivities were observed for IgM and IgG independently whereas in combination sensitivity reached to 87.3% ³². Guo et al. demonstrated the sensitivities for IgM antibodies in PCR-positive confirmed and PCR-negative suspected cases which found to be 75.6% and 93.1%, respectively. Moreover, a combination of IgM ELISA with PCR significantly increased the positivity to 98.6% rather than the PCR test alone (51.9%) ³³. In contrast a cohort study in Hong Kong suggested the early seroconversion of IgG than IgM antibodies in confirmed cases. Moreover, antibodies after 14 days of disease onset showed 100% sensitivity for IgG and 94% for IgM against RBD antigen whose seropositivity was observed earlier than N protein ³⁴. Liu et al. confirmed that the

S protein has high sensitivity and early antibody response than N protein in COVID-19 diagnosis. For S protein-based ELISA, the sensitivity found to be below 50% at day 0-5 while 90.7% at day 11-15 for IgM and/or IgG detection with 100% specificity among healthy controls ³⁵. However, a study in France compared two in-house ELISA with N and S SARS-CoV-2 proteins and found early antibody response against N protein in patients than S protein ³⁶. Similar study in China with N and S protein-based ELISA showed better sensitivity of N protein than S protein for IgM and IgG detection ²¹. Okba et al. reported the higher specificity of S1 viral protein than S protein and showed the lower specificity of commercial serological ELISA against other coronavirus samples collected from different countries ³⁷. An ELISA in USA with S1 domain of SARS-CoV-2 showed 96.6% and 100% sensitivity for IgA and IgG detection respectively ³⁸.

Lassauniere et al. recently evaluated three commercially available serological ELISA kits in Denmark and observed 65-90% sensitivity with 93-100% specificity ³⁹. In Germany, Kohmer et al validated two commercial ELISA kits and found 58.8% and 70.6% sensitivity between day 5-9 and 93.8% and 100% between days 10-18 ⁴⁰. However, three commercial ELISAs in France demonstrated similar sensitivity of 86.7% with 80-100% specificity ⁴¹. Commercially available ELISA is cheaper than PCR diagnosis with high throughput competence and lenient sample requirement, however, unsuitable for field use is the limitation. The performance of ELISA for COVID-19 diagnosis in different countries is listed in Table 2.

Rapid point of care test in SARS-CoV-2 infection

In many infectious diseases, lateral flow immunoassay (LFIA) based immunochromatographic tests are popular for field adaptable, quick and user-friendly diagnosis. The test mainly uses colloidal gold as a tracer to detect either pathogens' antigen or its specific antibodies in the biological samples such as serum, urine, and oral fluid of patients. In the current pandemic apart from RT-PCR clinicians have searched for simple and rapid diagnosis for field settings. Therefore, many laboratories reported the LFIA based serological tests so far for COVID-19 diagnosis.

The diagnostic indexes of LFIA based IgM/IgG test were demonstrated at different time points after onset of the symptom wherein at 0-7 days, 8-15 days and 16 days or more showed 18.8%, 100%, and 100% sensitivity and 77.8%, 50% and 64.3% specificity respectively ¹⁰. Similar kind of study by Pan et al with RT-PCR confirmed cases showed increase in sensitivity from 11.1% to 96.8% in LFIA within first week and after two weeks of disease onset respectively. However, in RT-PCR negative suspected cases the detection capacity by LFIA was 43.6% ⁴². Confirmed SARS-CoV-2 infection in China suggested 23% and 63.8% detectability of IgM and IgG LFIA test respectively in an early phase of illness which were poor than 92.3% with RNA test from sputum. Between 8-14 days the sensitivity of IgM and IgG increased to 50% and 87.5% respectively. However, at 15 days or more the sensitivity rose to 52.2% and 91.3% for IgM and IgG respectively than 60.8% for molecular test ⁶. Study in Germany with IgM/IgG LFIA showed 93.8% sensitivity from 10-18 days of PCR test than 62.5% from days 5-9 ⁴⁰. Another LFIA in Brazil showed 77.1% sensitivity and 98% specificity after at least 10 days when tested positive for RT-PCR ⁴³. A case report from Taiwan investigated IgG response against SARS-CoV-2 in LFIA and observed its appearance after 18-21 days

of exposure or the 11th day of illness ⁴⁴. A study from Spain suggested the use of antibody tests after at least 14 days of disease onset as they found 73.9% sensitivity at this time point as compared to 12.5% and 33.3% at 0-7 days and 7-13 days respectively ⁴⁵.

Li et al. developed and evaluated IgM and IgG based lateral flow tests using the recombinant RBD domain of S protein. Results interpreted with both IgM and IgG bands showed 88.66% sensitivity and 90.63% specificity which were better than single IgM and IgG tests ⁴⁶. One similar study showed 82.4% sensitivity including both serum IgM and IgG as compared to 57.1% and 81.3% for IgM and IgG alone with 100% specificity in each case ³². Similar study in the USA with defined cases estimated 91.8% sensitivity and 99.5% specificity ³². Moreover, Hu et al. have detected 63.4% of IgM/IgG positives in the blood of clinically suspected cases through LFIA than 46.3% from nasal or pharyngeal swab specimens through RT-PCR ⁴⁷.

Study by the National COVID Testing Scientific Advisory Panel, UK evaluated nine commercially available different LFIA tests for COVID-19 detection. The overall sensitivities of LFIA tests after 10 days of disease onset ranged from 55-70% as compared to RT-PCR with 95-100% specificities. Therefore, the report suggested inadequacy of the LFIA test to diagnose COVID-19 with the current performance ³¹. Low sensitivity of LFIA test was also observed in two separated studies in Iran and Germany with 47.9% and 36.4% sensitivities respectively ^{48,49}. Another study in Denmark validated four commercially available lateral flow tests with a similar number of confirmed COVID-19 cases and found sensitivities ranged from 83-93% with 100% specificity for all LFIA ³⁹. Ten LFIA tests were validated in USA with RT-PCR positive

samples and found 81.8-100% sensitivity and 84.3-100% specificity after 20 days of disease onset ⁵⁰. Rashid et al have reported a review on nine commercial rapid LFIA tests and observed 72.7-100% sensitivity and 98.7-100% specificity of antibody tests ⁵¹. A study in France with six POCs showed sensitivity ranges between 76.9% and 93.3% and specificity between 65% and 100% ⁴¹. The performance of a commercial IgM/IgG LFIA test was also reported with hospitalized and emergency department COVID-19 patients in Italy. At a median of 7 days only 63.3% of patients showed positivity with LFIA. Patients from the emergency department showed only 18.45% and 8.3% positivity with RT-PCR positive and negative cases respectively thus not recommended the use of LFIA for suspected COVID-19 cases ⁵². Study in Japan with a commercial one step test did not recommend the antibody test alone for initial diagnosis ⁵³. Research conducted throughout the world so far has mixed response for utility of LFIA test in COVID-19 diagnosis. The overall percentage of sensitivity and specificity of LFIA in different countries is compiled in Table 3. Figure 1 depicts the diagnostic performance of immune assays throughout the countries.

A hunt for asymptomatic cases

The world has faced bigger pandemics in the past. The virus has infected humans before in the form of SARS and MERS. Then why the scare and why call the pandemic unprecedented? The severity of a disease depends on its transmission rate and death rate. Unlike SARS ($R_0=$), the transmission rate of COVID-19 is suspected to be very high especially through asymptomatic cases, with an average R_0 value of around 3.28 ⁵⁴. Thereby increasing the chances of spread due to these undetected asymptomatic cases

through leaps and bounds through contacts or expelling air droplets³. Li et al. demonstrated human to human SARS-CoV-2 transmission from asymptomatic when studied in two family clusters in China⁵⁵. A case report by Zhu *et al.* also highlighted a SARS-CoV-2 infection from asymptomatic contact⁵⁶. A study in Germany also suggested the transmissions of COVID-19 from asymptomatic during his incubation period⁵⁷. Since the inception of the COVID-19 infection, most of the studies are limited to the symptomatic cases thus not many details have been reported to access the asymptomatic cases. According to a report from the National Health Commission in China, 78% of the total identified SARS-CoV-2 cases within 24 hours on 1st April 2020 were asymptomatic⁵⁸. Moreover, a case study from the Diamond Princess Cruise ship estimated around 17.9% of asymptomatic cases occurred with this infection⁵⁹. Therefore, it is suspected that the undetected asymptomatic cases are underestimated and may result in an increase in the total number of reported COVID-19 infections⁶⁰. A community-based study in the USA County through rapid antibody test recognized population prevalence of 2.49%-4.16% for COVID-19. Thus an estimated 50 to 80 times higher number of cases than confirmed cases⁶¹. Another study in USA with 4856 individuals in population detected 87 (1.79%) SARS-CoV-2 positives with IgG test²². Similar kind of study in Iran found 22% prevalence of COVID-19 and estimated higher number of confirmed cases than expected⁶². A population-based survey in Brazil with 4188 subjects showed increase in positivity from 0.04% to 0.133% within two weeks of mid April⁶³. Recently a systemic review published with preliminary data of 83 asymptomatic cases in nine articles observed significant contribution of asymptomatic and pre-symptomatic patients in the spread of SARS-CoV-2 infection⁶⁴. Samples

collected from the oral swab, BAL fluid, and stool in one of the studies reported higher viral load in nasopharyngeal samples. However, this variation in viral load is negligible in symptomatic and asymptomatic cases. Confirmed tests were reported through RT-PCR in some individuals without having any COVID-19 symptoms⁶⁵. Interestingly, an asymptomatic case report from China showed positive nucleic acid test with urine samples before throat swab suggested the need for other means of diagnosis too for asymptomatic cases⁶⁶.

Since health care workers are the front runners in this pandemic many studies have focused on their screening for asymptomatic infection. Study with health care workers having mild respiratory symptoms from nine hospitals in Netherland identified 4.1% SARS-CoV-2 positive cases⁶⁷. Another test with residents of health care providers in Washington reported a 30.3% prevalence of infection in the facility⁶⁸. Seroprevalence of SARS-CoV-2 infection in healthcare workers from two community clinics in Japan observed 9.1% positivity rate in comparison to the overall rate of 5.9%⁶⁹. Samples collected randomly from health professionals in Spain depicted 11.2% of cumulative prevalence in antibody quantification⁷⁰. Study reported from Italy showed 5.25% seropositivity within the asymptomatic healthcare providers⁷¹. In contrast, another study from Italy screened health workers and observed discordant results between two immunological assays in reporting sensitivity⁷². It is evident from the knowledge gathered from the initial studies so far that asymptomatic cases have a significant role in COVID-19 spread. More studies are needed to understand the immune response and spread pattern in asymptomatic infection. To deal with the extra challenge of asymptomatic carriers a widespread testing is needed within the hotspot community

more systematically. The test should be ideally for onsite detection and rapid in which antigen or antibody-based immunoassays could be a critical success factor for mass level testing of COVID-19.

Conclusion

Like any previous pandemic, the answer to curing the disease will be an appropriate vaccine strategy. However, the solution to managing COVID-19 till then cannot be limited to any one thing, it has to be a holistic approach, like the WHO Director-General Tedros Adhanom mentioned, “Not testing alone. Not contact tracing alone. Not quarantine alone. Not social distancing alone. Do it all.”

The world cannot be kept in standstill and lockdown for an indefinite period. We may have to withdraw the lockdowns gradually expose our populations little by little to the virus so that a natural immunity is developed in our populations without causing a massacre. Until then, the most appropriate solution to combat this death-knell will be extensive diagnosis, identification of the asymptomatic carriers and symptomatic patients, marking and isolating them. While RT-PCRs are good options for detecting the virus, their cumbersome protocols, time and expense, does not make them suitable candidates for mass testing, urging for alternative tests. Rapid tests are quintessential for detecting a mass population because of its ease of use, less time consumption, and low cost, and thus may be used complementary to RT- PCRs. However, rapid tests too have their limitations of performance, and sensitivity. Since most of the serological tests have been reported at different time points it is difficult to access the correct time line for seroconversion against SARS-CoV-2 infection. The seropositivity of the antibody test

within the first week of disease onset is not satisfactory in many cases. However, sensitivity escalates to 80-100% in the second week combining both IgM and IgG in the interpretation. Nevertheless, serological tests can complement the nucleic acid tests largely for the false negative case detection. Additionally, serological tests mainly in the point-of-care format can be used for surveillance strategy within the community. However, un-regulation or misuse of these rapid tests can create an extra panic in society.

The current phase can very well be called the testing times, where various diagnostic tests, vaccines, drugs are in trials and tremendous effort is being put by many across the globe to put an end to this pandemic. The human race has faced various virus attacks, and has forever fought to strive through the battle. The fight too will be a long one, and the key to success will be continuous efforts. Herein our review aims to enumerate all the important diagnostic findings together to help strategize ways to deal with this global pandemic.

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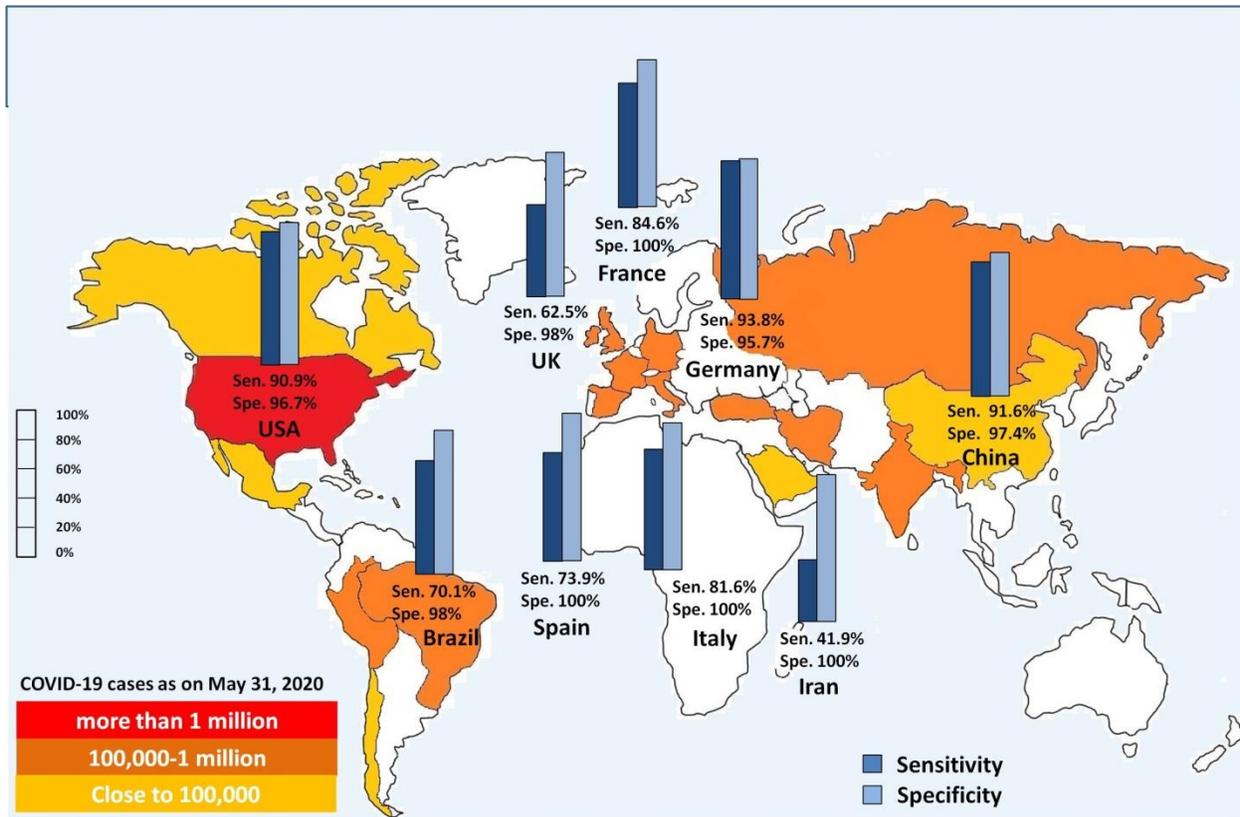


Figure 1: The figure represents the combined sensitivity and specificity of immunoassays including chemiluminescence (CLIA), enzyme-linked (ELISA) and lateral flow tests (LFIA) reported across the countries. The sensitivity and specificity were aggregated from 19 studies in China, 4 studies from USA, 2 studies each from Italy, Germany, and France, and single study each from UK, Spain, Iran and Brazil.

Table 1: Performance of CLIA serological test in different countries.

Country	COVID-19 cases/ nonCOVID-19 cases	Antibodies	Days from disease onset	Sensitivity %	Specificity %	Reference
China	43/33	IgM/IgG	Median 16d	48.1/88.9	100/90.9	[18]
China	276/367	IgM/IgG	--	57.2/71.4	100	[4]
		IgM+IgG		81.52		
China	34	IgM/IgG	3 rd week 5 th week	100/100 94.1/100	--	[25]
China	3/736	IgM/IgG	7-12d,	100	97.3-100	[8]
China	133	IgM/IgG	--	72.9-82.6/ 93.1-100	65.9-71.1	[29]
China	87/483	IgA/IgM/IgG	--	98.6/96.8/96.8	98.1/92.3/99.8	[27]
China	555/1558	IgM/IgG	3-35d	73.0-85.8/ 86.5-96.6	97.3-99.4/ 97.4-99.1	[28]
China	221	IgM/IgG	16-21d	73.6/98.6	--	[19]
US	125/1020	IgG	17d	100	99.9	[22]
China	50/130	IgM/IgG 4 CLIA	13d	92	99.23	[26]
Italy	87	IgM/IgG	12d	88/100	--	[20]
China	47/300	IgM/IgG	--	97.7/95.6	95.2/96.6	[21]

Table 2: Performance of antibody ELISA test in different countries.

Country	COVID-19 cases/ nonCOVID-19 cases	Antibodies	Days from disease onset	Sensitivity %	Specificity %	Reference
China	238/120	Ab/IgM/IgG	5d	81.5	94.2-100	[7]
China	173	Ab/IgG/IgM	<15d	100, 94.3, 79.8	--	[30]
China	178	IgM/IgG	0d 5d	50/81 81/100	--	[5]
China	63/35	IgM/IgG	1-28d	87.3	100	[32]
China	214/100	IgM/IgG	0-5d 11-15d,	45.5 90.7	100	[35]
China	208/285	IgA/IgM/IgG	5-14d median	92.7/85.4/77.9	100	[33]
China	23	IgM/IgG	14d<	88-94/94-100	--	[34]
Denmark	30/82	IgG/IgM, IgA 3 ELISA	--	65-90	93-100	[39]
UK	40/142	IgM/IgG	<28d	85	100	[31]
France	51/200	IgM/IgG	5-14d	65-69	100	[36]
Germany	17/13-26	IgA, IgG	5-9d	58.8-70.6	95.2-95.7	[40]

France		2 ELISA	10-18d	93.8-100		
	15/20	IgA, IgG	15d<	86.7	80-100	[41]
China		3 ELISA				
	47/300	IgM/IgG	--	89.1-7.9/ 95.7-97.9	97-99.7/ 85.7-99.7	[21]
USA	30 /57	IgA/IgG	3-4d<	96.66/100	92.98/98.24	[38]

Table 3: Performance of LFIA serological test in different countries.

Country	COVID-19 cases/ nonCOVID-19 cases	Antibodies	Days from disease onset	Sensitivity %	Specificity %	Reference
China	397/128	IgG/IgM	-	88.66	90.6	[46]
China	90/89	IgG/IgM	0-7d	18.8	77.8	[10]
			8-15d	100	50	
			16d<	100	64.3	
China	38	IgM/IgG	0-7d	23/53	--	[6]
			8-14d	50/87.5		
			15d <	52.2/91.3		
China	91/35	IgM/IgG	--	57.1/81.3	100	[32]
		IgG+IgM		82.4		
China	41	IgM/IgG	2 weeks	29.3/46.3	--	[47]
		IgG+IgM		63.4		
China	86	IgM/IgG	1-7d	11.1	--	[42]
			8-14d	92.2		
			15d<	96.8		

Italy	80/30	IgM/IgG	median7d	63.3	100	[52]
Denmark	30/82	IgM/IgG 6 POC	7-23d<	80-100	80-100	[39]
Spain	55 /45	IgM/IgG	<7d 7-13d 14d<	12.5 33.3 73.9	100	[45]
USA	37/30	IgM/IgG	--	91.8	99.5	[61]
UK	40/142	IgM/IgG 9 LFIA	0-28d	55-70	95-100	[31]
Japan	112 /48	IgM/IgG	<1 week 1-2 weeks 2 weeks<	27.8 48 95.8	98	[53]
USA	11/108	IgM/IgG 10 LFIA	20d<	81.8-100	84.3-100	[50]
Iran	114/198	IgM/IgG	5-53d	47.9/47	99/100	[48]
Germany	10 /13	IgM/IgG	5-9d 10-18d	62.5 93.8	100	[40]
France	15/20	IgM/IgG 6 POC	15d<	76.9-93.3	65-100	[41]
Germany	27/22	IgM/IgG	median 18.5d	36.4	88.9	[49]
Brazil	83/100	IgM/IgG	10d	77.1	98	[43]