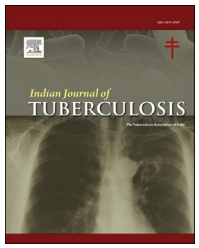
Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Review Article

Benefits and limitations of serological assays in COVID-19 infection

Zeeshan Sidiq, M. Hanif[*]*, Kaushal Kumar Dwivedi, K.K. Chopra*

New Delhi Tuberculosis Centre, JLN Marg, New Delhi, India

ARTICLE INFO

Article history:
Received 24 July 2020
Accepted 31 July 2020
Available online 6 August 2020

Keywords:
SARS CoV-2
COVID-19
Serology
IgM
IgG

ABSTRACT

Accurate and rapid diagnostic tests are critical for achieving control of coronavirus disease 2019 (covid-19), a pandemic illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Diagnostic tests for covid-19 fall into two main categories: molecular tests that detect viral RNA, and serological tests that detect anti-SARS-CoV-2 immunoglobulins. Reverse transcriptase polymerase chain reaction (RT-PCR), a molecular test, has become the gold standard for diagnosis of covid-19; however, this test has many limitations that include potential false negative results, changes in diagnostic accuracy over the disease course, and precarious availability of test materials. Serological tests have generated substantial interest as an alternative or complement to RT-PCR and other Nucleic acid tests in the diagnosis of acute infection, as some might be cheaper and easier to implement at the point of care. A clear advantage of these tests over RT-PCR is that they can identify individuals previously infected by SARS-CoV-2, even if they never underwent testing while acutely ill. Many serological tests for covid-19 have become available in a short period, including some marketed for use as rapid, point-of-care tests. The pace of development has, however, exceeded that of rigorous evaluation, and important uncertainty about test accuracy remains.

The COVID-19 pandemic caused by SARS-CoV-2 has affected living and working conditions of billions of people worldwide and has remarkably weakened the global economy as a result of lockdown in many cities. Reported for the first time in late December 2019, in Wuhan, China, this virus rapidly spread through China and then many other countries globally. As of July 19, 2020, the virus resulted in over 14.0 million laboratory-confirmed cases of corona virus disease 2019(COVID-19) and more than 597,583 deaths in 215 countries.[1] The World Health Organization (WHO) has declared COVID-19 a public health emergency of international concern and given a "very high" risk assessment on a global level.[2]

Coronavirus is positive-sense single-stranded RNA virus. It is a large pleomorphic spherical enveloped particle. The viral envelope consists of a lipid bilayer where the membrane (M), envelope (E), and spike (S) structural proteins are anchored.[3] The S glycoprotein is a large type 1 transmembrane protein consisting of two functional subunits S1 and S2. S1, comprises a receptor binding domain (RBD) which is responsible for binding to the host cell receptor. S2 contains elements needed

* *Corresponding author*. New Delhi Tuberculosis Centre, JLN Marg, Delhi Gate, New Delhi, 110002, India. Tel.: +11 23234270.
E-mail address: irldlndc@rntcp.org (M. Hanif).

https://doi.org/10.1016/j.ijtb.2020.07.034
0019-5707/

for the fusion of virus.[3–8] A subset of coronaviruses (in particular betacoronavirus) also have a shorter spike-like surface protein called hemagglutinin esterase (HE).[9] Inside the envelope there is the nucleocapsid, which is formed from multiple copies of the nucleocapsid (N) protein. This protein is bound to the single-stranded RNA genome.[10] The lipid bilayer envelope, membrane proteins, and nucleocapsid protect the virus when it is outside the host cell.[11]

COVID-19 exhibits a range of clinical manifestations, from mild flu-like symptoms to life-threatening conditions. The initial phase of the disease is clinically characterized by the appearance of cough, fever, generalized malaise, and myalgia. The laboratory tests show Neutrophilia, with normal or reduced lymphocyte count and elevated C-reactive protein (CRP).[12] Within approximately one week from infection, adaptive immunity is expected to rise. In some patients this transit of immunity is delayed due to Individual risk factors such as older age; and co-morbidities like diabetes, hypertension, cardiopathy, and obesity.[13] This factor could be the main cause of COVID-19 complications, occurring at about day 12. This is the time when circulating proinflammatory cytokines increase and inflammatory cells build up in target organs, particularly the lungs, causing tissue damage without providing any control over the infection.[14]

Currently, the real-time RT-PCR assay is the gold-standard method to diagnose SARS-CoV-2.[15,16] Unfortunately, the sensitivity of the RNA test in the real world is not satisfactory and, false-negative cases have also been reported due to problems with sample collection, sample transportation, RNA extraction, enzyme inhibitors, and the RT-PCR method. Infact, RT-PCR tests have many limitations by their nature of requiring high workload, needing skillful operators for testing and sample collection, and needing costly instruments and special operation places.

During this current public health emergency of international concern, screening and diagnosing patients quickly to aid containment is a priority and these limitations make RT-PCR unsuitable for use in the field. Consequently, new tools, such as serological tests capable of tracking the virus through each phase of the disease, are in great demand. Conventional serological assays have a high-throughput advantage, and they avoid false-negative cases that may occur with the RT-PCRmethod.[17]

The current race to develop cost-effective point-of-contact test kits and efficient laboratory techniques for confirmation of SARS-CoV-2 infection has fueled a new frontier of diagnostic innovation. At present there is an increasing number of in vitro diagnostic companies that are either developing or have developed tests for antigens and antibodies (see https://www.finddx.org/covid-19/pipeline/). Five of the 27 antigen-detection rapid diagnostic tests and 26 of the 203 antibody detection tests reported on the website have been selected for the first round of evaluation. Additional tests will continue to be reviewed on a rolling basis. However, it is important to remember that these tests are often controversial and mainly involve small Chinese cohorts.

During the outbreak of SARS-CoV, different serological assays, including immunofluorescence assays (IFA), enzyme linked immunosorbent assays (ELISA), and Western blot (WB) analysis, were developed. Some studies suggested that virus-based IFA and ELISA were highly sensitive (85–100%) but lacked specificity. False positive results were due to antigens well-conserved among different CoV species[18,19] and to cross-reaction with autoantibodies in autoimmune diseases.[20] Consequently, serological assays based on recombinant antigens derived from both S and N proteins were widely used in laboratory diagnostics. The use of recombinant antigens offers the advantage of working without the need of stringent biosafety requirements and are also more appropriate for assay standardization. In particular, the N protein is easy to clone into prokaryotic or eukaryotic expression plasmids due to its small size and the absence of glycosylation sites. Researchers suggested that recombinant protein-based WB and ELISA are highly sensitive (73–100%) and have a low to moderate specificity.[21–26] Since S protein is difficult to express into prokaryotic in its full-length protein, only fragments can be used in immunoassays.

Several studies showed different reactivity of SARS patient sera with S protein, ranging from very low (13%) to 100%, depending on the method used. Moreover, a rate of false positive results ranging from 0 to 30%have also been shown using ELISA and WB assays.[27,28] The structural similarity and the sequence conservation of the immunogenic proteins of related CoV species that share common structural epitopes, can elicit cross-reactive antibodies in the host. Since cross-reactivity is more likely to occur with a high level of conservation of the proteins, this might explain why N protein-based serological assays were more often associated with cross-reactivity than S protein-based assays. Analysis of complete N sequences revealed that SARS-CoV N protein shared 25–29% identity with α-CoV and 33–47% with related β-CoV. The complete S protein sequence showed a lower degree of conservation, sharing 23–25% an identity with α-CoV and 29% with related β-CoV.

Various in-house and commercial ELISA methods have been used to validate and test different SARS-CoV-2 antigens. These include S, S1, RBD, and the N protein. It was shown that among these antigens tested, S1 was more specific in detecting SARS-CoV-2 antibodies while N protein was more sensitive than S1.[29] While describing an ELISA method involving the full-length S protein and the smaller RBD fragment, Anant et al suggested that antibodies mounted after Infection target both the full viral antigens as well as to the immunogenic fragments equally.[30] This suggestion is of great importance in that the assays involving the recombinant proteins are easier to standardize.

There are studies which suggest that combined use of nucleic acid tests (NAT) and serological tests can significantly improve diagnostic sensitivity as well as the positive detection rate. While studies have reported sensitivity ranging from 78.7% to 100%, positive detection rate of 98.6% have been reported using combined IgM assay with NAT compared to only 51.9% using a single RT-PCR test.[31–34] It has also been suggested that highest overall sensitivity can be achieved from an IgM-IgG combined assay compared to NAT and IgM, IgG singly. Jia et al reported a positive detection rate of 72.73% using combined IgM and IgG for patients with SARS-CoV-2 negative nucleic acid test.[35] It should however be noted that most of the studies available today have a great bias considering the various time lapses between the initial exposure to the virus

and the serological assays confirming the detection. As a consequence, data are still of little use today, both when considering the different isotypes and comparing single or double positivity of different isotype antibodies.

We actually still do not know if serological assays perform better as a screening test, as a diagnostic tool alongside molecular diagnostics to achieve the greatest accuracy, or with the epidemiologic aim of getting a real picture of the pandemic at its end. Some of the potential applications of serology can be: Screening and management of clinical patients and close contacts especially the ones with a negative RNA test; Screening of key populations such as travelers and people returning to school or work from high incidence areas; Conduct population-based serological survey, to understand the actual prevalence and pathogenicity of SARS-CoV-2 infection in different regions and populations; Analyze the antibody level and spectrum of antibody epitopes in convalescent COVID-19 patients, to provide scientific guidance for the design and evaluation of vaccines and therapeutic antibodies in the future. Nevertheless, what is important to know is that no laboratory test could ever substitute clinical observation and practical experience. If there is a clinical suspicion, a negative test result cannot exclude the possible presence of the disease.

Conflicts of interest

The authors have none to declare.

REFERENCES

1. World Health Organization. Coronavirus disease (COVID-19) outbreak.https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200624-covid-19-sitrep-156.pdf?sfvrsn=af42e480_2.
2. World Health Organization. Coronavirus disease 2019 (COVID-19) Situation Report -181. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200719-covid-19-sitrep-181.pdf?sfvrsn=82352496_2.
3. Belouzard S, Chu V, Whittaker GR. Activation of the SARS coronavirus spike protein via sequential proteolytic cleavage at two distinct sites. *Proc Natl Acad Sci U S A*. 2009;106:5871–5876.
4. Bosch BJ, van der Zee R, de Haan CA, et al. The coronavirus spike protein is a class I virus fusion protein: structural and functional characterization of the fusion core complex. *J Virol*. 2003;77:8801–8811.
5. Burkard C, Verheije MH, Wicht, et al. Coronavirus cell entry occurs through the endo-/lysosomal pathway in a proteolysis-dependent manner. *PLoS Pathog*. 2014;6, e1004502.
6. Kirchdoerfer RN, Cottrell CA, Wang N, et al. Pre-fusion structure of a human coronavirus spike protein. *Nature*. 2016;3(531):118–121.
7. Millet JK, Whittaker GR. Host cell proteases: critical determinants of coronavirus tropism and pathogenesis. *Virus Res*. 2015;16(202):120–134.
8. Tortorici MA, Veesler D. Structural insights into coronavirus entry. *Adv Virus Res*. 2019;105:93–116.
9. de Groot RJ. Structure, function and evolution of the hemagglutinin-esterase proteins of corona- and toroviruses. *Glycoconj*. 2006;23(1–2):59–72.
10. Chang CK, Hou MH, Chang CF, Hsiao CD, Huang TH. The SARS coronavirus nucleocapsid protein-forms and functions. *Antivir Res*. 2014;103:39–50.
11. Neuman BW, Kiss G, Kunding AH, et al. A structural analysis of M protein incoronavirus assembly and morphology. *Struct Biol*. 2011;174:11–22.
12. Huang C, Wang Y, Xingwang L, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395:497–506.
13. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. 2020;395:1054–1062.
14. Infantino M, Damiani A, Gobbi FL, et al. Serological assays for SARS-CoV-2 infectious disease: benefits, limitations and perspectives. *Isr Med Assoc J*. 2020;22(4):203–210.
15. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019, China novel coronavirus investigating and research team. *N Engl J Med*. 2020;382:727–733.
16. Coronaviridae study group of the international committee on taxonomy of viruses, the species severe acute respiratory syndrome-related coronavirus:classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol*. 2020;5:536–544.
17. Xiao SY, Wu Y, Liu H. Evolving status of the 2019 novel coronavirus infection: proposal of conventional serologic assays for disease diagnosis and infection monitoring. *J Med Virol*. 2020;92:464–467.
18. Che XY, Qiu LW, Liao ZY, et al. Antigenic cross-reactivity between severe acute respiratory syndrome-associated coronavirus and human coronaviruses 229Eand OC43. *J Infect Dis*. 2005;191:2033–2037.
19. Dijkman R, Jebbink MF, Gaunt E, et al. The dominance of human coronavirus OC43 and NL63 infections in infants. *J Clin Virol Off Publ Pan Am Soc Clin Virol*. 2012;53:135–139.
20. Wang Y, Sun S, Shen H, et al. Cross-reaction of SARS-CoV antigen with autoantibodies in autoimmune diseases. *Cell Mol Immunol*. 2004;1:304–307.
21. Leung DT, Tam FC, Ma CH, et al. Antibody response of patients with severe acute respiratory syndrome (SARS) targets the viral nucleocapsid. *J Infect Dis*. 2004;190:379–386.
22. Carattoli A, Di Bonito P, Grasso F, et al. Recombinant protein-based ELISA and immuno-cytochemical assay forthe diagnosis of SARS. *J Med Virol*. 2005;76:137–142.
23. Guan M, Chen HY, Foo SY, Tan YJ, Goh PY, Wee SH. Recombinant protein-based enzyme-linked immunosorbent assay and immunochromatographic tests for detection of immunoglobulin G antibodies to severe acute respiratory syndrome (SARS) coronavirus in SARS patients. *Clin Diagn Lab Immunol*. 2004;11:287–291.
24. Liu X, Shi Y, Li P, et al. Profile of antibodies to the nucleocapsid protein of the severe acute respiratory syndrome (SARS)-associated coronavirus in probable SARS patients. *Clin Diagn Lab Immunol*. 2004;11:227–228.
25. Shi Y, Yi Y, Li P, et al. Diagnosis of severe acute respiratory syndrome (SARS) by detection of SARS coronavirus nucleocapsid antibodies in an antigen-capturing enzyme-linked immunosorbent assay. *J Clin Microbiol*. 2003;41:5781–5782.
26. Woo PC, Lau SK, Tsoi HW, et al. Relative rates of non-pneumonic SARS coronavirus infection and SARS coronavirus pneumonia. *Lancet*. 2004;363:841–845.
27. Maache M, Komurian-Pradel F, Rajoharison A, et al. False-positive results in a recombinant severe acute respiratory syndrome-associated coronavirus (SARS CoV) nucleocapsid-based western blot assay were rectified by the use of two subunits (Si and S2) of spike for detection of antibody to SARS-CoV. *Clin Vaccine Immunol*. 2006;13, 409-314.

28. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure,function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell*. 2020;S0092:8674, 30262.
29. Nisreen MAO, Müller MA, Wentao Li, et al. *SARS-CoV-2 specific antibody responses in COVID-19 patients*. medRxiv; 2020. preprint on March 20.
30. Amanat F, Nguyen THO, Chromikova V, et al. *A serological assay to detect SARS-CoV-2 seroconversion in humans*. medRxiv; 2020. preprint on March 18.
31. Zhao J, Yuan Q, Wang H, et al. *Antibody Responses to SARS-CoV-2 in Patients of Novel Coronavirus Disease 2019. Pre-print in medRxiv Preprint on March 03*. 2020.
32. Woo PC, Lau SK, Wong BH, et al. Longitudinal profile of immunoglobulin G (IgG), IgM, and IgA antibodies against the severe acute respiratory syndrome (SARS) coronavirus nucleocapsid protein in patients with pneumonia due to the SARS coronavirus. *Clin Diagn Lab Immunol*. 2004;11:665–668.
33. Guo L, Ren L, Yang S, et al. Profiling early humoral response to diagnose novel coronavirus disease (COVID-19) [published online ahead of print, 2020 mar 21] *Clin Infect Dis*. 2020. ciaa310.
34. Li Z, Yi Y, Luo X, et al. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. *J Med Virol*. 2020:1–7. https://doi.org/10.1002/jmv.25727.
35. Jia X, Zhang P, Tian Y, et al. *Clinical significance of IgM and IgG test for diagnosis of highly suspected COVID-19 infection*. medRxiv; 2020. on March 12.