ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 12, Issue, 12, pp.15214-15217, December, 2020

DOI: https://doi.org/10.24941/ijcr.40474.12.2020

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

PERFORMANCE EVALUATION OF VITROS® ANTI-SARS CoV-2 ANTIBODY ASSAYS (TOTAL & IgG): AN ADJUNCTIVE SCREENING TOOL FOR RTqPCR IN COVID–19 INFECTION DETECTION

[1]Dr. Manushri Sharma, [2,*]Dr. Amardeep Pathak and [3]Dr. Anurag Mehta

[1]Associate Consultant, Dept. of Biochemistry, Sir Gangaram Hospital, New Delhi – 110060
[2]Consultant & Head Dept. Transfusion Medicine, Rajiv Gandhi Cancer Institute & Research Centre, Delhi – 110085
[3]Director Laboratory & Transfusion Medicine, Rajiv Gandhi Cancer Institute & Research Centre, Delhi – 110085

ARTICLE INFO

Article History:
Received 01st August, 2020
Received in revised form
03rd October, 2020
Accepted 04th November, 2020
Published online 30th December, 2020

Key Words:
SARS-CoV-2 (Severe acute respiratory syndrome-Coronavirus-2), RTqPCR (Reverse transcriptase real time Polymerase Chain Reaction), ICMR (The Indian Council of Medical Research), COVID-19 (Coronavirus disease), CLIA (Chemiluminescence).

ABSTRACT

Background: Currently world is facing a pandemic caused by novel corona virus (SARS –CoV2) which causes a highly contagious infection affecting most commonly lungs and results in an array of clinical symptoms ranging from asymptomatic state to acute respiratory distress syndrome and may even lead to multi organ dysfunction.The diagnostic modalities include Reverse transcriptase real time PCR (RTqPCR) which is gold standard method for diagnosis of the infection using oro-pharyngeal or nasopharyngeal swabs from the patients. **Aim and Objectives:** Evaluation of the performance of VITROS® Anti SARS CoV-2 antibody Assays (Total &IgG) in RT-PCR positive symptomatic COVID -19 patients.A correlation of time gap between RTqPCR/CB-NAT tests and positive serology test will be done for Total antibody. **Materials and Method:** Blood samples of COVID 19 confirmed patients (by RTqPCR) were tested for Anti SARS CoV2 Total (IgM, IgG&IgA) (*ASCV2T*). All these ASCV2T positive samples were further tested for Anti SARS CoV2 IgG (*ASCV2G*) antibodies using VITROS® 3600 immunodiagnostics system as per the manufacturer protocol. **Results:** A total of 67 patient samples were collected in the period of 02 months from June 2020 & July 2020. Out of these 67 patients 34 were positive and 33 were negative for COVID-19 infection by RTqPCR method. 28 of the 34 RTqPCR positive samples were reactive for total antibody test. As per statistical analysis, ASCV2T assay showed sensitivity 82.35%, specificity 100%, PPV 100%& NPV 84.62%. **Conclusion:** Serological assay show fair sensitivity (82.35%) & specificity (100%). Both RTqPCR& serological assay as a combination can be used as an important screening tool for COVID 19 infection. This can be further validated by Randomized controlled trails or meta-analysis with large number of sample size for longer duration follow-up.

Citation: ***Dr. Manushri Sharma, Dr. Amardeep Pathak and Dr. Anurag Mehta.*** **2020.** "Performance evaluation of vitros® anti-sars cov-2 antibody assays (total & igg): an adjunctive screening tool for rtqpcr in covid–19 infection detection", *International Journal of Current Research*, 12, (12), 15214-15217.

INTRODUCTION

Currently world is facing a pandemic caused by novel corona virus (SARS–CoV2) which causes a highly contagious infection affecting most commonly lungs and results in an array of clinical symptoms ranging from asymptomatic state to acute respiratory distress syndrome and may even lead to multi organ dysfunction. The infection spread via respiratory droplets or from the surfaces where theses droplets may get deposited and fomites of the infected person. The incubation period of virus is around 02- 14 days (Sharma, 2020). The diagnostic modalities include Reverse transcriptase real time PCR (RTqPCR) which is gold standard method for diagnosis of the COVID 19 infection using oro-pharyngeal or nasopharyngeal swabs from the patients. In spite of being gold standard there have been multiple limitations associated with RTqPCR use. One of the important limiting factors is the sensitivity of the test. Across the globe it has been seen that the sensitivity of RTqPCR ranges anywhere from 60-90%.[(2,3)] The sensitivity of RTqPCR in patients can varry from 93% for broncho-alveolar lavage, 72% for sputum, 63% for nasal swabs, to only 32% for throat swabs (Wang, 2020) Serological testing for antigen and antibody detection is also available now. The Indian Council of Medical Research (ICMR) has validated and approved serological kits for the same, rapid diagnostic test (RDT) for antigen detection is widely used now as point of care testing (POCT) (Sharma, 2020).

***Corresponding author: Dr. Amardeep Pathak,**
Consultant & Head Dept. Transfusion Medicine, Rajiv Gandhi Cancer Institute & Research Centre, Delhi – 110085.

However with experience it has been noticed that this test also misses a significant number of positive cases as these tests possess sub-optimumsensitivity (50% - 60%) (European Commission, 2020; FIND evaluation update , 2020; COVID-19 In Vitro Diagnostic Devices and Test Methods Database, 2020). There can be two important reasons for high false negative results for the above mentioned both the diagnostic modalities - One is the sampling error and second is the low viral load. Antibody detection IgM/IgG by ELISA or CLIA (Chemiluminescence) is also available but they are strictly confined for epidemiological purpose and testing in the containment zone. All these described tests have very high specificity, but missing so many positive cases severely affects patient isolation and contact tracing method being used in India and across the globe currently. These false negative cases are spreading the infection in the community which is affecting the disease control. The need of the hour is to use two or more methods simultaneously to increase the sensitivity for disease diagnosis. We evaluate here the use of recently introduced VITROS®Anti SARS CoV-2 Antibody (Ab)Assays (Total &IgG) for screening of COVID-19 infection in symptomatic patients.

MATERIALS AND METHODS

The study was conducted at Rajiv Gandhi Cancer Institute and Research Centre, New Delhi.

Inclusion criteria

- Patients should be clinically suspected to have COVID-19 infection based on sign and symptoms or should have history of exposure to RTqPCR confirmed COVID-19 patient.
- The patients should have been tested for COVID-19 by RTqPCR/CB-NAT

Exclusion Criteria

- Patients diagnosed as COVID-19 based on other tests like antigen testing
- Those who are not willing to participate in the study

Methods

03 ml whole blood was collected in red topped plain vacutainer from all the patients. The vacutainers were kept at ambient temperature for 30 minutes to allow blood clotting. Then the vacutainers were centrifuged at 4000 RPM for 20 minutes. Serum was separated and kept in labeled 01 ml eppendorf tubes. The labeled serum samples were tested for Anti SARS CoV2 Total (IgM, IgG &IgA) antibody {ASCV2T}. All the ASCV2T positive samples were further tested for Anti SARS CoV2 IgGantibodies {ASCV2G}using VITROS® Anti-SARS CoV-2 Total & VITROS® Anti-SARS CoV-2 Ig Gassays, respectively. These assays were supplied by Ortho Clinical Diagnostics (OCD)& tests were run on VITROS®3600 immunodiagnostics system as per the manufacturer protocol.

Data & Statistical Analysis: Based on RTqPCR/CB-NAT results patient were labeled as COVID positive or negative. The sensitivity and specificity of Total antibody level were calculated using RTqPCR/CB-NAT results as gold standard.

RESULTS

A total of 67 patient samples were collected in the period of 02 months from June 2020 & July 2020. All these patients had been tested previously for COVID-19 infection by RTqPCR/CB-NAT. Out of these 67 patients 34 were positive and 33 were negative for COVID-19 infection by RTqPCR/CB-NAT. All these 67 samples were first tested for ASCV2T antibody test. 28 of the 34 RTqPCR positive samples were reactive for total antibody test. All these total antibody reactive samples were then tested for ASCV2G antibodies. 21 out of these 28 samples also showed reactivity for IgG antibodies. The sensitivity and specificity of the anti-SARS COV-2 total antibody tests is summarized in table 1. All the samples which had negative RTqPCR results were also negative for total SARS-COV2 antibody.

Correlation of time gap between RT-PCR and development of antibodies: 28 of the 34 samples positive for RTqPCR showed the presence of total antibodies towards ASCV2T and 06 samples were tested negative. These were not tested further.The 28 positive samples were further tested for ASCV2G antibodies. 21 tested positive with a range of S/CO values while 07 tested negative for ASCV2G.The 06 samples that tested negative for ASCV2T antibodies had a time gap between 01-03 days after the RTqPCR Test.Of the 28 samples that tested positive, for 04 samples a time gap correlation could not be done as their date of RTqPCR testing was not known. Out of 24 samples, 11 samples had a gap of between 10-24days and all these samples showed the presence of Anti SARS CoV-2 Total &IgG antibodies. 13 samples had a gap of between 01-04 days. Of these 06 tested positive while07 tested negative for Anti SARS COV-2 IgG antibodies.

DISCUSSION

Diagnostic tests always play a critical role in any epidemic or pandemic situations. They are the important tools to confirm the diagnosis of COVID 19, contact tracing with confirmed cases, to identify population at risk and to assess the effectiveness of the control strategies. Unfortunately, in this COVID 19 pandemic condition all the diagnostic tests i.e. RTqPCR, Rapid antigen kits and serological antibody assayshave higher rate of false negative results because of their low sensitivity, thus being the weakest link. In this global COVID 19 outbreak, RTqPCR for SARS CoV 2 serve as a diagnostic gold standard. Rapid antigen testing can also be a viable, simple & point of care testing alternative to RTqPCR.

Studies showing suboptimal sensitivity of these rapid antigen tests limits its usage at large scale (FIND evaluation update, 2020). The current study was done with an aim to evaluate the performance of the total SARS-CoV2 antibodies (serological testing). It has been observed that all the anti COVID-19 positive samples were also positive by RTqPCR testing. This shows high positive predictive value (PPV) of the test. However, there were 06 RTqPCR positive cases that were missed on antibody testing. Thus it can be concluded here that antibody testing alone cannot be an ideal screening method. It has been seen here that the sensitivity of the test with RTqPCR taken as gold standard is high. We must consider here that this is not the true sensitivity as RTqPCR test itself misses 10-40% (Watson, 2020; FIND, 2020) of the total cases.

Table 1. The Sensitivity, Specificity, PPV and NPV of total anti-SARS CoV-2 antibody assay

		RTq PCR/CB-NAT		Sum
		Positive	Negative	
Total Antibody (Anti SARS COV-2 Total)	Positive	28	00	28
	Negative	06	33	39
Sum		34	33	67
Sensitivity	82.35%			
Specificity	100%			
PPV	100%			
NPV	84.62%			

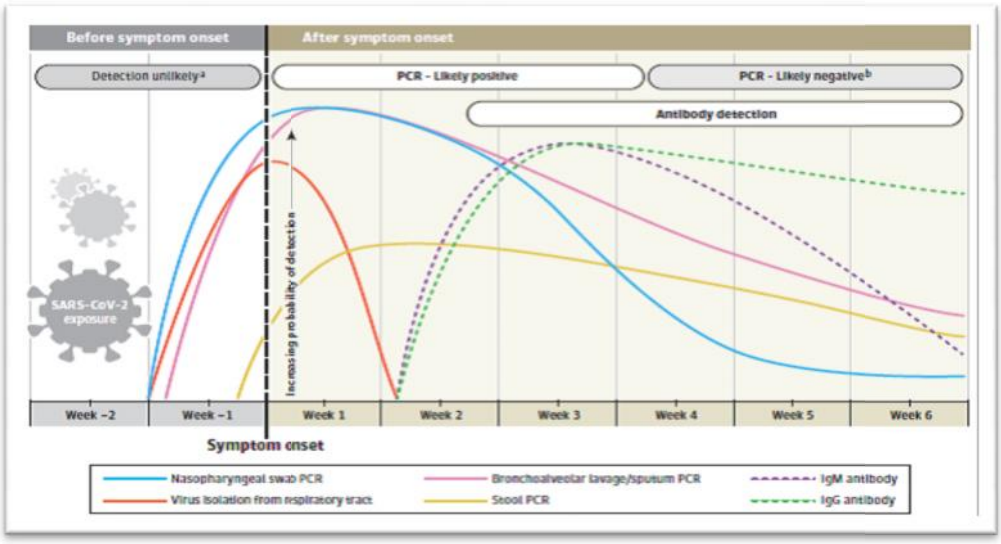

Figure 1. Estimated Variation over Time in Diagnostic Tests for Detection of SARS-CoV-2 Infection

Although we were not able to find cases that were antibody positive and RTqPCR negative, it is possible that some of false negative cases by RTqPCR may get detected on antibody testing. This can only be identified if paired samples are tested by both the methods simultaneously. In a study of 140 patients done by Guo L *et al*, combined sensitivity of RTqPCR and IgM ELISA directed at nucleocapsid (NC) antigen was 98.6% Vs 51.9% with a single PCR test (Guo, 2020). The cause for negativity for such a case in our study may be due to small sample size.
The sensitivity (82.35%) & specificity (100%) of anti SARS CoV2 assay in our study is in concordance with other studies (Lin, 2019; Ma, 2020; Jia, 2020).

A time gap correlation could not be clearly established from the samples tested in this study. It was clear that those with a time gap of 10 days and more, showed the presence of Anti SARS CoV-2 Total as well as IgG antibodies. However, few samples (06) with a time gap between 01-04 days also showed a combination of status like only Total antibodies being present or both Total &IgG antibodies being present. This could be probably because these patients get their RTqPCR tested very late (after 7-10 days of symptoms) or during sero-conversion phase. As mentioned earlier, in these cases patients need to be followed up for a longer period to confirm their sero-conversion. Serological diagnosis is especially important for patients with mild to moderate illness who may present late, beyond the first 2 weeks of illness onset (Sethuraman, 2020). The most sensitive and earliest serological marker is total antibodies, levels of which begin to increase from the second week of symptom onset.[(12)]

Although IgM and IgG ELISA have been found to be positive even as early as the fourth day after symptom onset, higher levels occur in the second and third week of illness (Figure 1) (Sethuraman, 2020). Most of the studiesshows that in confirmed COVID 19 cases, IgM antibodies can be detected around 05-10 days after onset of symptoms and IgG antibody concentration appears on detectable range after 10-14 days of symptoms. [(14-18)]

Conclusion

As the diagnostics strategies are in the evolving phase in this pandemic situation and a lot more are changing day by day. The limited knowledge on dynamics of the immune response to COVID 19 infection has led to reluctancy on recommending the use of serological tests. In such scenarios with current knowledge & understanding of viral infectivity Vs immune response, we should focus on detecting maximum number of positive cases. So, it is imperative to use a combined approach with both serological testing for SARS-CoV2 antibodies and RTqPCR/CB-NAT.

REFERENCES

Covid-19 In Vitro Diagnostic Devices and Test Methods Database: European Comission; 2020. Available from: https://covid-19-diagnostics.jrc.ec.europa.eu/

European Commission (EC). Current performance of COVID-19 test methods and devices and proposed performance criteria (16 April 2020). Brussels: EC; (21 April, 2020). Available from: https://ec.europa.eu/docsroom/documents/40805.

FIND evaluation update: SARS-CoV-2 immunoassays 2020. https://www.finddx.org/covid-19/sarscov2-eval-immuno/ (accessed June 29, 2020).

Find. Sars-COV-2 Molecular Assay Evaluation: RESULTS 2020 (22 April, 2020). Available from: https://www.finddx.org/covid-19/sarscov2-eval-molecular/ molecular -eval-results/.

Guo L, Ren L, Yang S, *et al*. Profiling early humoral response to diagnose novel coronavirus disease (COVID-19). *Clin Infect Dis*. 2020;ciaa310. Published online March 21, 2020. doi:10.1093/cid/ciaa310

Jia X, Zhang P, Tian Y, *et al*. Clinical significance of IgM and IgG test for diagnosis of highly suspected COVID-19 infection. *MedRxiv*(Preprint). doi:10.1101/ 2020. 02.28.20029025

Lin D, Liu L, Zhang M, *et al*. Evaluations of serological test in the diagnosis of 2019 novel coronavirus (SARS-CoV-2) infections during the COVID-19 outbreak. *Med Rxiv* (Preprint).doi:10.1101/2020.03.27.20045153

Long Q-X, Liu B-Z, Deng H-J, *et al*. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med* 2020; 26: 845–48

Lou B, Li T, Zheng S, *et al* Serology characteristics of SARS-CoV-2 infection since the exposure and post symptoms onset. *Med Rxiv*. Preprint posted March 27, 2020. doi:10.1101/2020.03.23.20041707

Lou B, Li T, Zheng S, *et al*. Serology characteristics of SARS-CoV-2 infection since exposure and post symptoms onset. *Eur Respir J* 2020; published online May 19. DOI:10.1183/13993003.00763-2020.

Ma H, Zeng W, He H, *et al*. COVID-19 diagnosis and study of serum SARS-CoV-2 specific IgA, IgM and IgG by a quantitative and sensitive immunoassay. *Med Rxiv* (Preprint). doi:10.1101/2020.04.17.20064907

Sethuraman N, Stanleyraj S, Ryo A. Interpreting Diagnostic Tests for SARS-CoV-2 (view point/Opinion); JAMA Published online May 6, 2020 doi:10.1001/jama.2020.8259

Sharma A, Sharma RP, Agarwal S, Sharma M, Pathak A, Mishra G. The covid-19 pandemic- a comprehensive insight on the recent updates. International Journal of Medical Science and Diagnosis Research (IJMSDR);Volume 4, Issue 8; August: 2020; Page No. 34-38. DOI: https://doi.org/10.32553/ijmsdr.v4i8.641

Tan W, Lu Y, Zhang J, *et al*. Viral kinetics and antibody responses in patients with COVID-19. *medRxiv*2020; published online March 26. DOI:10.1101/2 020.03.24. 20042382 (preprint). 22

To KK, Tsang OT, Leung WS, *et al*. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis* 2020; 20: 565–74

Wang W, Xu Y, Gao R, etal . Detection of SARS-CoV-2 in different types of clinical specimens(JAMA.). JAMA 2020. 10.1001/jama.2020.3786. 32159775

Watson J, Whiting PF, Brush JE. Interpreting a covid-19 test result. BMJ 2020;369:m1808 doi: 10.1136/bmj.m1808

Zhao J, Yuan Q, Wang H, *et al*. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. *Clin Infect Dis* 2020; published online March 28. DOI:10.1093/cid/ciaa344.

****