Original Research Paper

Volume - 12 | Issue - 08 | August - 2022 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Medical Microbiology

COMPARISON OF COMMERCIALLY AVAILABLE COVID-19 BINDING ANTIBODY ASSAY WITH SURROGATE VIRUS NEUTRALIZATION TEST (sVNT) IN VARIOUS PATIENTS FROM CENTRAL INDIA

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ABSTRACT Antibody response in COVID-19 was much talked about and quite debatable subject not only amongst the medical community but also in the general public. Govt. of India also carried out sero-surveys in various cities and towns of the country to evaluate antibody response among the general public. We performed and compared binding antibodies with neutralizing antibodies using Kawach Karwa test and surrogate virus neutralization test (sVNT) respectively. We also compared commercially available surrogate virus neutralization test kits.200 individuals were divided into four categories (50 each) as RT-PCR confirmed COVID 19 positive individuals, Non-vaccinated (C1), Close household contacts of C1 group (C2), Complete vaccinated individual, not reported positive for COVID 19 (C3). Non vaccinated, not reported positive for COVID 19, not come in contact with COVID 19 positive patient (C4). Serological test as ELISA Kawach Karwa and sVNT were perform using standard lab technique in BSL-2 environment.Our result showed maximum presence of binding antibodies in all the four groups and only 4% individuals came negative. By Genscript (sVNT) we found low presence of neutralizing antibodies with respect to binding antibodies due to its more specific and protective nature. We also found difference in levels of neutralizing antibodies titers by various commercially available kits.Neutralizing antibodies are more conclusive as protective antibodies with respect to binding antibodies but we should also consider other factors like cell mediated immunity personal hygiene pathogenicity of the virus etc. leading to chances of reinfection in the population though high level of neutralizing antibodies titers in the general public as overall represent a good herd immunity.

KEYWORDS : Neutralizing Antibodies (NAbs), IgG Binding Antibodies,Covid 19,Surrogate Virus Neutralization Test (sVNT),ELISA.

INTRODUCTION:

In December 2019 the coronavirus SARS-CoV-2 was reported in Wuhan city of China causing coronavirus disease 2019.1 The disease was manifested by mild flu like symptoms to severe pneumonia and fatal lung disease.2 This pandemic has resulted in more than 517 million cases and close to 6.0 million deaths as of May, 2022.3 During this pandemic scientific community, governments and policy makers went for diagnosis of the infected individuals in the community by large scale screening and contact tracing .There were several studies on the immune response of the COVID 19 infected individuals and the nature and longevity of the protective immunity. 4 Published literatures suggested that both T-cell and B cell immune response had played a key role in the protective immunity for SARS-CoV-2 infection.[5]

ELISAs (Enzyme-linked immunosorbent assays), is the test widely used to detect and estimate antibodies for various viral infections. IgG binding antibodies ELISA test is used to estimate the prevalence of SARS-CoV-2. In the development of a long term protective immune response Virus Neutralization has a paramount importance, hence the detection of virus neutralizing antibodies is of great importance. For neutralizing antibody detection, in vitro neutralizing virus assays are considered as the gold standard. These assays are performed in high containment Bio safety level -3 labs, but these are time consuming and not fit for large scale surveillance and vaccine trials. Due to high specificity and sensitivity of "surrogate virus neutralization tests" (sVNTs) and performing them takes less time. These newly developed assays could be supplemented for in vitro viral neutralization test and for estimation of neutralizing antibodies.6-11 In this study, we compare various commercially available binding antibody assay with the commercially available sVNTs assays.

MATERIALAND METHOD:

2.1 IgG Binding Antibody Enzyme linked immunosorbent assay

All Samples were tested for the IgG binding antibody by Kavach Karwa SARS CoV-2 IgG ELISA test kit. This SARS CoV-2 ELISA test kit has provided coated plates with SARS CoV-2 virus whole cell antigen, which binds with the IgG antibodies present in the human blood serum. The tests were done as per testing instructions and recommendations of manufacturer.

2.2 Surrogate Enzyme linked immunosorbent assay (sELISA)

In this study we used three commercially available surrogate virus neutralization test kits to detect SARS-CoV-2 specific NAbs. SARS-CoV-2 Surrogate Virus Neutralization test Kit (GenScript Biotech, USA), COVID-19 Neutralizing Antibody Microlisa (J.Mitra & Co. Pvt. Ltd., India) and Merilisa COVID-19 Neutralizing Antibody ELISA test (Meril Diagnostics Pvt. Ltd., India). Both Genscript and J.Mitra test kits can detect specific neutralizing antibodies against SARS-Cov-2 by competitive inhibition of the protein-protein interaction between the recombinant receptor binding domains of the viral spike glycoprotein (RBD) with the recombinant human ACE2 cell surface receptor coated on the titer plate. While the Merilisa is capture ELISA using purified receptor binding domain (RBD), Viral spike S-protein and the hACE2 protein. This test mimics the virus-host interaction same as in a conventional virus neutralization test by direct protein-protein interaction in ELISA plate. These tests were done as per the recommendations of the manufacturers and correlated accordingly. The result of the samples can be classified as either "Negative" (inhibition < 30%) or "Positive" (inhibition ≥ 30%) for Genscript and J.Mitra and for the Merilisa it is "Negative" (inhibition < 50%) or "Positive" (inhibition ≥ 50%).

2.3 Samples

A cross sectional study was done for the duration of 3 month in urban area of Gwalior city. By using purposive sampling method, we included a total of 200 participants above 18 years of age, 50 participants each from the four different group i.e.

(1) Real Time Polymerase Chain Reaction (RTPCR) and/or Rapid Antigen Test (RAT) confirmed/ positive SARS-CoV2 individual found positive between 30-90 days before the date of sample collection. Also not vaccinated for COVID-19 (denoted as C1),

(2) Household uninfected and unvaccinated contacts of first strata i.e. C1 (denoted as C2),

(3) Individuals received COVID-19 vaccination (received all the recommended doses) who had neither declared positive by RTPCR and/or RAT nor any household contact of COVID positive patient (denoted as C3) and

(4) Individuals who are not vaccinated for any COVID vaccine and who had neither declared positive by RTPCR and/or RAT nor any household contact of COVID positive patient. (Denoted as C4)

Purpose of study was explained to the participants and a written informed consent was taken. All samples were collected and transported as per the standard sampling and sample transport procedure. The serum was separated and stored in the lab at 2-4°C to process it within 24 to 48 hours of collection. The study was reviewed, approved and conducted as per the guidelines.

All SARS-CoV2 positive individuals found positive less than 30 days before and more than 90 days after the day of sample collection, individuals partially vaccinated for COVID vaccine and not willing for the participation were excluded.

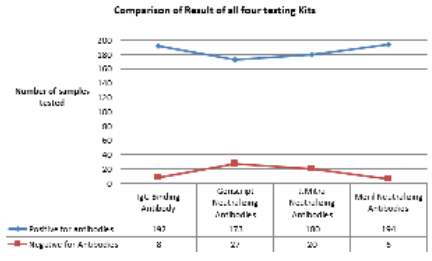
RESULT:

Out of total 200 samples, 96% individuals shows positivity for IgG binding antibodies, while only 4% individuals shows negative results in all the four groups as aggregate. In our study COVID 19 positive individuals (C1), and their contact unvaccinated individuals (C2), as well as non-vaccinated nor COVID 19 positive individuals (C4), showed 98%, 94%, and 92% of IgG binding antibody positivity respectively. This depicts that due to circulation of the virus in the community even non-infected nor exposed persons showed good binding antibody titer. The vaccinated persons showed 100% IgG titer which was represented as group C3. It also means that all the individuals who were vaccinated carry a good titer of binding antibodies.

While analyzing the data for neutralizing antibodies for which Genscript surrogate virus neutralization test (sVNT) kit was used which was compared with gold standard Plaque Reduction Neutralization Test (PRNT) 173 individuals (86.5%) showed positivity for neutralizing antibodies while 27 individuals (13.5%) were negative. This shows that neutralizing antibodies were less common as compared to binding antibodies in 19 (9.5%) individuals. From literature we know that NAbs are more protective as compare to binding antibodies though binding antibodies were produced more because of good immunogenicity. Further study on protectivity from the virus of these 19 individuals cannot be commented upon.

Groups(50 samples in each)	IgG Abs		Genscript NAbs test	
	Positive	Negative	Positive	Negative
C1	49	1	47	3
C2	47	3	44	6
C3	50	0	45	5
C4	46	4	37	13

C1= RT-PCR confirmed COVID 19 positive individuals, Non-vaccinated.



C2= Close household contacts of C1 group.
C3= Complete vaccinated individual, not reported positive for COVID 19.

C4= Non vaccinated, not reported positive for COVID 19, not come in contact with COVID 19 positive patient.

Genscript sVNT test which is comparable with Plaque Reduction Neutralization test (PRNT) in various other studies showed 94% positivity for neutralizing antibody in infected patients (C1), Showed 88% positivity in non-infected close contacts of the infected persons (C2). It also showed 90% positivity in fully vaccinated (C3) individuals and 74% positivity in non-vaccinated, non-infected persons. Thus it is evident that even in naïve population 74% positivity of neutralizing antibody was present giving an idea of good herd immunity. The binding antibodies vary from 100% in C3 to 92% in C4 group. Hence it is evident that in all the four groups the percentage of neutralizing antibodies was always less from binding antibodies up to 18% maximum.

Out of all the three neutralization antibody test kit only Genscript neutralizing test kit was compared with gold standard Plaque Reduction Neutralization Test (PRNT). Thus we will consider Genscript as gold standard surrogate virus neutralization test. It is evident from our study that Meril neutralizing antibody test kit gave maximum positivity for neutralizing antibody which was in line with binding antibody. Hence, it is difficult to comment that Meril neutralizing test kit was detecting neutralizing antibodies or binding antibodies or both or giving false positive results. Neutralizing antibody test kit by J.Mitra & co. showed results in between the two hence, it can be considered in more cost effective settings.

DISCUSSION:

In our study we found that binding antibodies was almost universally present in all the four categories of our study criteria giving an impression of protective antibodies titer but whether they are effective in real life situation or not cannot be commented upon. Though, it gives a false sense of security among the common masses. Neutralizing antibody test which is a proven test of protective level of antibodies was present in only 86.5% of all the categories whereas binding antibodies were present in 96% of the tested individuals. It is evident that 13.5% of people were deprived of neutralizing antibody were at risk of reinfection. In these people, reinfection not only depends on absence of protective antibodies but also on other in-vivo factors like cell mediated immunity, nature and pathogenicity of affecting variant, local antibody response in challenged individual or personal hygiene measures like use of face masks, hand washing etc.

Performing a Plaque Reduction Neutralization Test (PRNT) not only requires BSL-3 facility but also the presence of live virus thus to avoid complexities associated with PRNTs, surrogate virus neutralization test (sVNT) came into picture but not all assays which are available commercially were compared and evaluated against the gold standard. In our study also we found remarkable difference between three commercially available kits in terms of cut off value of results, clarity of results and the cost varies quit remarkably. Hence it is important to choose a product which should give comparable reproducible and quality results so that there is no false impression among general public of the level of protection and they should be informed that general hygiene measures will always remain paramount.

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