A Luminex based serological assay for detecting IgM and IgG antibody response in SARS-CoV2 patients

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Abstract

Nucleic acid real-time PCR test has become the main diagnostic tool for SARS-CoV-2 infection, and there are also drawbacks to these real-time PCR test kits. Additionally, it has reported high false-negative rates. A reliable and rapid test method is urgently required to briskly detect antibody response in a large number of infected patients and ensure prompt care for patients. Therefore, we report here a Luminex bead based multiplexed immunoassay to serologically diagnose the SARS-CoV2 infection and detect IgM and IgG response in COVID-19 patients. The technology can provide sensitive and specific detection using a low amount of serum samples in high-throughput screening platforms.

Introduction

The recent global outbreak of COVID-19 caused by SARS-CoV2, a member of coronavirus family originated from China (Wuhan, Hubei province) imposed catastrophic impacts on every society, infecting at least 8.79 million people worldwide resulting in 0.46 million deaths as of today [1- 4]. There are several reasons for this pervasive problem; however, there is scarce information on the assessment and diagnosis of patients infected with COVID-19, and the potential risk of respiratory illness which may be mild or severe. The current approach to diagnose the viral infection is based on molecular detection using RT-PCR assay which detects the viral load in clinical samples. However, as a consequence of low virus titer, there have been instances when the test has failed to detect the viral RNA from clinical samples [5].

SARS-CoV-2 antibodies in patient blood is a reasonable choice for immediate, quick, highly responsive diagnosis of COVID-19. It is commonly agreed that immunoglobulin M (IgM) offers the first line of protection during viral infections prior to the generation of adaptive, high-affinity IgG responses that are essential for long-term immune and immunological memory [6]. We believe that the mechanism of antibody production against SARS-CoV-2 would be an indicator of infection. Hence, to ameliorate the current diagnosis, in this article we report a bead-based multiplex immunoassay to detect IgG and IgM responses from the serum samples of COVID-19 patients. This would eventually allow for a deeper analysis, as antibodies persist in serum for a longer period of time and henceforth increase the chances for detection, which would benefit from adopting Luminex based serology assay as a routine diagnostic technique for SARS-CoV2 infection.

We sampled 20 clinical specimens that were symptomatic and RT-PCR tested, from the total of cases reported to the THSTI-Biorepository collected under the DBT India consortium for the COVID-19 research program and IRB approvals from all partnering clinical sites to conduct this study. The 20 clinical specimens which were either positive by RT-PCR or suspected from the hospital (data not shown) were tested using the Luminex platform against the Receptor-Binding Domain (RBD) and Spike antigens of SARS-CoV2

and 10 clinical specimens collected pre-pandemic from enrolled participants were used as negative controls. To develop the aforementioned Luminex based serology assay capable of detecting COVID-19 infection, we expressed RBD and Spike proteins and purified using a mammalian expression system and metal affinity chromatography. Both the purified proteins were coupled separately on Luminex beads as described previously [7] and confirmed by Western blot using the anti-His-HRP antibody. Aiming to unveil specificity of the assay, strict cut-off (5X SD + Mean) was used for RBD and spike proteins to rule out any non-specific readings.

Our results showed a higher antibody reaction to spike protein relative to RBD (Table I), therefore decided to continue using only spike protein for future tests. The developed Luminex platform against the RBD and spike protein was able to detect both IgG and IgM responses in patient serum samples (Figure 1 & Table I). Precisely, we were able to detect IgM and IgG response for 11 out of 12 patient serum samples (Table I) that tested positive by RT-PCR, with an exception of patient # 16 for the IgM response to the Luminex assay. Contrarily the patient # 18 which was earlier tested RT-PCR positive and showed high IgM response by Luminex did not show the IgG response. Moreover, patient # 1 and # 6 which were IgM negative showed IgG antibody responses. Apart from these few exceptions, IgM and IgG were detected in most of the analyzed samples by Luminex assay (Figure 1 & Table I), suggesting that the assay could be a strategy for the adoption of manageable and scalable research practices for community screening during COVID-19.

In addition to the diagnostic benefit, our results showed a clear positive association between clinical seriousness and Ab titer in the COVID-19 patients. Whereas the causal association between hormonal reaction and seriousness of the disease was still uncertain, the findings pose a theoretical use of the strong Ab titer as a proxy predictor for bad clinical prognosis. As most of the recovering patients encountered with viral infections show the presence of neutralizing antibodies (NAbs), which are strong predictors of defensive immunity. We further patrolled the results of the serology platform to determine the presence of NAbs in the patient serum samples. All the samples were heat-inactivated at 56°C for 1 h before testing with the SARS-CoV2 virus [8]. The virus was obtained from the BEI resources (Isolate USA-WA1/2020) [9], passaged once in Vero cells, titrated and 1 x 10^2 $TCID_{50}$ virus (diluted in 50 µl of the serum-free media) was incubated with 2.0 fold serial dilutions for 90 mins followed by 1 h adsorption on Vero cells. The cells were subsequently washed and DMEM media supplemented with 2% FBS was added to cells. The presence of cytopathic effect (CPE) was observed in cells using a microscope after incubation for 4-5 days at 37°C with 5% CO_2. We found that, following infection, antibodies were elicited against the spike protein and when tested in a serum neutralization assay these sera were able to neutralize SARS-CoV-2. Results from serum neutralization experiments revealed, 7/11 IgM and 7/13 IgG with the presence of NAbs against SARS-CoV2 virus in the serology positive samples. The neutralizing antibody titers were observed in the range between 1:20 (# 11 & # 17) and 1:320 (# 9). However, as expected, we did not observe any Luminex IgM/IgG response or neutralization antibodies in healthy/negative participant sera (Table I). This part of the study validates the Luminex assay with a similar set of samples and provides scope for expansion on a larger platform.

In summary, the identification of both IgM and IgG may provide details on the timing of the virus infection. The fast identification of both IgM and IgG antibodies would bring importance to COVID-19 disease diagnosis and care. Hence our approach for the COVID-19 serology problem with an alternative method that enables detecting viral antigen-specific antibodies with enhanced specificity and sensitivity in a simple, safe, fast and inexpensive manner might resolve this long-standing question for the faster diagnosis of SARS-CoV2. A correlative study with the early production of neutralizing antibodies in the samples of patients tested by Luminex assay was also documented. The developed assay after a systematic validation with an adequate sample size could be utilized for large scale community screening, screening for the potential plasma donors, and evaluating the efficacy of the future vaccine candidate trials.

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Table 1: Serum neutralization titers of patients samples

Patient No.	Samples Id	RT-PCR	Duration of days of symptoms	Luminex assay IgM RBD	Luminex assay IgM Spike	Luminex as IgG RBD
Cut off				484.93	465.01	32.66
# 1	C3020001120201	Negative	NA	469.75	310	**62.75**
# 2	C3020002120201	Negative	22	423.75	345.75	14
# 3	C3020003120201	Negative	2	364.75	223.25	26.25
# 4	C3020004120201	Positive	0	470	**849.5**	27
# 5	C3020005110201	Negative	4	164.75	206.25	20.75
# 6	C3020006110201	Negative	16	284	242.5	31
# 7	C3020007110201	Negative	9	28.75	24	19.5
# 8	C3030001110201	Positive	10	405.5	**5667**	24.5
# 9	C3030002110201	Positive	NA	**717**	**6133.25**	**113.5**
# 10	C3030003110201	Positive	12	**995.75**	**5692.5**	**91.25**
# 11	C3030004110201	Positive	14	**503.75**	**1505**	27
# 12	C3030005110201	Positive	18	269.25	**1132.25**	20.5
# 13	C3030006110201	Positive	12	363.25	**2553.5**	**33**
# 14	C3030007110201	Positive	11	208.75	**1147.25**	24
# 15	C3030008110201	Positive	11	167	**1045.5**	19.5
# 16	C3030009110201	Positive	12	143.75	394.75	21.5
# 17	C3030010110201	Positive	13	238	**2209.75**	27.5
# 18	C3030011110201	Positive	7	290.75	**1112.75**	23.5
# 19	C3040001110201	Negative	7	380.25	214	27
# 20	C3040002110201	Negative	7	145.25	251.5	20
# 21	P3027501130901	PN	NA	197	135	17.5
# 22	P3029511130901	PN	NA	278	209	21
# 23	P3004391130901	PN	NA	141	107.25	21
# 24	P3024531130901	PN	NA	105	86.5	16
# 25	P3013361130901	PN	NA	249.5	216	23
# 26	P3048921130901	PN	NA	182	313	19
# 27	P3027501130901	PN	NA	286.25	148	24.5
# 28	P3030431130901	PN	NA	200	170	19.5
# 29	P3047941130901	PN	NA	188	121	16.5

Patient No.	Samples Id	RT-PCR	Duration of days of symptoms	Luminex assay	Luminex assay	Luminex as
# 30	P3036761130901	PN	NA	147.75	131.5	19.5

NA- Not available, PN- Presumed negative (pre-pandemic serum)

Figure 1: IgM and IgG antibody response against the spike protein of the SARS-CoV2 virus.

30 serum samples were tested in duplicates and mean fluorescent intensity (MFI) was plotted for each sample. 5 X SD + Mean was used to set up the cut off (red dashed line-IgM and blue dashed line- IgG) which was based on the known healthy/COVID-19 negative serum samples. Samples with no neutralization titer was defined as negative (-); with neutralization titer at serum dilution of 1:20, 1:40–1:80 and 1:160–1:320 as (+), (++) and (+++), respectively.

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