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Characterisation of Dynamiker SARS-Cov-2 Neutralization Antibody (Nabs) Lateral Flow Immunoassay (LFA) and Enzyme Immunoassays (EIAs) DiaSorin Liaison RBD and Abbott IgG Quant in Convalescent Sera of SARS-CoV-2 Patients

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ABSTRACT

The EIAs (enzyme immunoassays) that react with the SARS-CoV-2 RBD protein and Spike protein such as DiaSorin S1/S2 IgG and Abbott II IgG correlate very well with neutralizing antibodies. LFAs (lateral flow assays) are the fastest and most convenient tests usually performed within 10 – 15 minutes. Several LFAs have been commercialized to detect IgG, IgM antibodies. In this short report we characterised the Dynamiker SARS-CoV-2 neutralising antibodies (Nabs) LFA with EIAs (DiaSorin Liaison SARS-CoV-2 RBD antibodies and Abbotts IgG spike antibodies) in the sera of Covid-19 convalescent patients (40 – 93 days) post onset of symptoms. The EIAs (DiaSorin Liaisons SARS-CoV-2 RBD antibodies and Abbotts IgG spike antibodies) were detected in the sera of all the Covid-19 convalescent patients (100%), while the LFA Dynamiker SARS-CoV-2 Nabs were found in the sera of 86% of Covid-19 convalescent patients. In addition, the Dynamiker SARS-CoV-2 Nabs lateral flow immunoassay (LFA) had a positive correlation with the DiaSorin Liaison (RBD) and Abbott IgG Quant (Spike) antibody assays.

INTRODUCTION

In the quest to understand the pathology of Covid-19 Infection, there are numerous serological assays that have been manufactured and commercially available. These assays detect IgG, IgA, IgM and total antibodies to epitopes of SARS-CoV-2, also antibodies are directed at the envelope (E), membrane (M), nucleocapsid (N), spike (S) proteins. There are 5 functional domains on the S protein, the N-terminal domain, and the receptor binding domain (RBD) are responsible for receptor binding whilst the other 3 are responsible for membrane fusion. These assays for these antibodies can be either enzyme linked immunosorbent assays (ELISAs), chemiluminescent

immunoassays (CLIAs) collective called enzyme immunoassays (EIAs) and lateral flow immunoassays (LFAs). The EIAs that react with the spike protein, are likely to have the function of neutralizing antibodies such as DiaSorin S1/S2 IgG and Abbott II IgG are very good RBD and Spike protein assays that correlate very well with neutralizing antibodies Other spike protein EIAs such as Roche Total S, Serion/Virion IgG and the DiaSorin trimeric spike IgG have also shown very good correlation[1,2]. Other EIAs (Abbott, Roche, Siemens) SARS-CoV-2 protein antibody levels have showed a strong positive correlation with a surrogate neutralization test [3]. LFAs are the fastest and most convenient tests usually performed within 10 – 15 minutes. Several LFAs have been commercialized to detect IgG, IgM antibodies. One such LFA highlighted a diverse antibody result profile in the convalescent plasma donor samples. Overall, 88.9% (56/63) of the donors were considered positive. 87.3% (55/63) of convalescent plasma (CP) donors were positive for IgG and 50.8% (32/63) of the donors were positive for IgM[4]. LFAs also have been produced to qualitatively measure neutralising antibodies[5,6]. One of such is the Dynamiker SARS-CoV-2 Neutralising antibody rapid test. There has been very little data with regards correlating LFAs for SARS-CoV-2 Neutralising antibodies and EIAs for RBD and Spike antibodies.

Convalescent plasma of SARS-CoV-2 infected patients can be a great source for characterizing antibody assays. Data from samples taken during the convalescent and recovery phase (after day 28 following onset of fever) are grouped into longer time blocks (29–100 days, 101–200 days, and >201 days), showed that in the first week, all patient samples were negative for neutralizing antibody, then by week 2, 64% became positive with 100% becoming positive by week 3[7]. In addition, neutralizing antibodies have been observed to increase significantly from the time post onset of symptoms reaching a peak at 31 – 35 days[8] and they have been detected up to 10 months post onset of symptoms[9]. In addition, neutralizing antibodies have been detected in 100% of samples within 20 to 53 days since onset[5,6].

Both DiaSorin S1/S2 IgG and Abbott IgG II Quant assays are very good RBD and Spike protein assays that have been demonstrated to correlate very well with neutralizing antibodies[1]. The purpose of this short report was to characterise the neutralizing antibodies with the Dynamiker SARS-CoV-2 neutralising antibodies LFA with EIAs DiaSorin Liaisons SARS-CoV-2 RBD antibodies and Abbotts IgG spike antibodies in the sera of Covid-19 convalescent patients (40 – 93 days) post onset of symptoms.

MATERIALS AND METHODS

Convalescent Donors

1 ml serum samples from twenty-eight SARS-CoV-2 patient donor panel, consisting of 8 males and 20 females, with an average age of 44.6 years (25 – 66 years) were obtained from Cambridge Biosciences, which obtains fresh human blood service in partnership with London-based Research Donors. Research Donors is a HTA licenced and ISO 9001 2015 certified company with Research Ethics (REC) approval as a Research Tissue bank, as well as participating in the UK NEQAS QA scheme. The Covid-19 positivity of the donors was confirmed by positive PCR or MD diagnosis and serum samples were collected after positive test result, the samples were collected on average of 69.5 days (40 – 93 days) from onset of symptoms. Informed consent was obtained from the donors for the use of the serum for research.

Table 1. Demographics of donors

Subject #	Age	Gender	Days from onset of symptoms
1	38	F	73
2	45	F	66
3	44	F	80
4	48	F	49
5	55	F	74
6	65	F	87
7	40	F	76
8	39	M	77
9	34	F	58
10	46	F	72
11	30	M	65
12	47	F	75
13	57	F	80
14	36	M	73
15	30	F	67
16	60	F	66
17	30	M	48
18	50	F	68
19	35	F	80
20	47	F	80
21	45	M	61
22	42	F	48
23	66	M	63
24	53	F	93
25	50	M	73
26	25	F	78
27	63	M	40
28	30	F	76

M-Male, F-Female, C-Caucasian, AA-African American, NA-not available.

Dynamiker SARS-CoV-2 Neutralizing antibody rapid test

The Dynamiker SARS-CoV-2 Neutralizing Antibody Rapid Test is used for in vitro qualitative detection of Covid-19 neutralization antibodies in human serum and plasma:

- Place test cassette on flat and clean bench
- Add 60µL (two drops) of serum/plasma into mixing tube, then add 60µL of dilution solution into the mixing tube
- Press the nozzle cap tightly onto the tube. Shake and mix well
- Add 60µL of the diluted sample into the sample well on the cassette.
- Read and record the results after 10 - 15 minutes
- Result Interpretation:
    - Positive: Presence of two red lines, test line (T) and control line (C). T line is lighter than C line, indicates the presence of neutralization antibodies.

- Strongly Positive: Presence of single control line (C) indicates the presence of neutralization antibodies and a higher concentration.
- Negative: Presence of two red lines, test line (T) and control line (C). The colour and intensity is similar. This indicates the absence of neutralization antibodies.

Abbott SARS-CoV-2 IgG II Quant-test (Abbott S IgG)

The Abbott SARS-CoV-2 IgG II Quant-test (Abbott S IgG) is a chemiluminescence microparticle immunoassay (CMIA). It quantifies IgG-type antibodies against the RBD of the viral S-protein on an Abbott Architect platform (Abbott, Abbott Park, IL, USA) between 21.0 and 40,000.0 AU/ml, with ≥50 AU/ml as a threshold for positivity.

DiaSorin Liaison SARS-CoV-2 S1/2

The DiaSorin Liaison SARS-CoV-2 S1/2 CLIA (DiaSorin S1/2 IgG) detects IgG antibodies against an S1/S2 combination antigen on a DiaSorin Liaison (DiaSorin, Stillwater, OK, USA). The quantification range is between 3.8 and 400.0 AU/ml. The cutoff for positivity is >15 AU/ml, whereby results between 12.0 and 15.0 AU/ml are considered borderline.

RESULTS

The Dynamiker SARS-CoV-2 neutralization antibodies were detected in 86% of the samples analysed, they were strongly positive, positive, and negative in 10/28 (36%), 14/28 (50%) and 4/28 (14%) respectively see table 1 for complete dataset).

The estimated Abbott IgG quant antibodies ranged between 264.7 AU/mL and 20062.7 AU/mL in the sera of the convalescent patients infected with SAR-CoV-2. (table 2). The Abbott IgG quant antibodies were identified in the sera of all the patients with a positivity cut-off of ≥50 AU/ml. When comparing the outcome of the results based on the presence or absence of neutralising antibodies using the Dynamiker LFA:

- The patients with negative neutralising antibodies had Abbott IgG II Quant antibodies ranging from 264.7 AU/mL to 14,942.2 AU/mL, with a mean of 1,946 AU/mL.
- The patients with positive neutralising antibodies had Abbott IgG II Quant antibodies ranging from 699.7 AU/mL to 20,062.7 AU/mL, with a mean of 3,187.1 AU/mL.
- The patients with strongly positive neutralising antibodies had Abbott IgG II Quant antibodies ranging from 2,528 AU/mL to 6,604.3 AU/mL, with a mean of 2,983.7 AU/mL.

The estimations DiaSorin Liaison S1/S2 antibodies ranged between 18.2 AU/mL and 355 AU/mL (table 2). When comparing the outcome of the results based on the presence or absence of neutralising antibodies using the Dynamiker LFA (figure 1):

- The patients with negative neutralising antibodies had DiaSorin Liaison S1/S2 antibodies ranging from 23.8 AU/mL to 355 AU/mL, with a mean of 88.52 AU/mL.
- The patients with positive neutralising antibodies had DiaSorin Liaison S1/S2 antibodies ranging from 18.2 AU/mL to 346 AU/mL, with a mean of 110.28 AU/mL.
- The patients with strongly positive neutralising antibodies had DiaSorin Liaison S1/S2 antibodies ranging from 36.3 AU/mL to 254 AU/mL, with a mean of 141.32 AU/mL.

Table 2. Results of Dynamiker neutralization antibodies (Nabs), DiaSorin Liaison S1/S2 (RBD) antibodies and Abbott IgG quant (Spike) antibodies in sera of 28 SARS-CoV-2 infected patients

Patient	Dynamiker Nabs	DiaSorin Liaison Concentration (AU/mL)	Abbott IgG II (AU/mL)
1	Negative	254.00	5009.5
2	Strongly Positive	23.80	264.7
3	Positive	79.30	1106.5
4	Negative	121.00	6604.3
5	Strongly Positive	29.50	471
6	Positive	116.00	1359.7
7	Positive	108.00	1406
8	Positive	109.00	3784.9
9	Strongly Positive	56.70	689.7
10	Strongly Positive	133.00	332.9
11	Positive	18.20	1718.9
12	Strongly Positive	57.80	941.4
13	Strongly Positive	24.20	230.1
14	Positive	147.00	1846.9
15	Positive	346.00	1440.3
16	Positive	94.00	20062.7
17	Strongly Positive	59.60	831.6
18	Positive	62.90	1938.5
19	Negative	36.30	2528
20	Strongly Positive	120.00	455.6
21	Positive	114.00	4106
22	Positive	38.70	699.7
23	Strongly Positive	25.60	305.4
24	Positive	83.90	1014.4
25	Positive	111.00	2812.5
26	Positive	116.00	1322.7
27	Strongly Positive	355.00	14942.2
28	Negative	154.00	2802.8

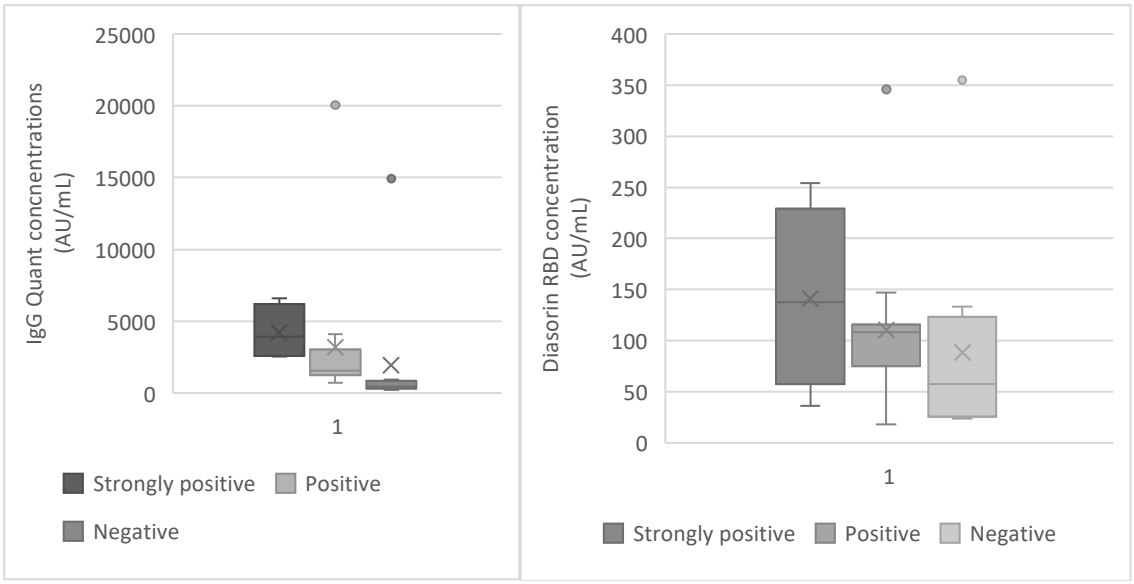

Figure 1. Showing the whisker plot of the number of LFA test (negative, positive, and strong positive) related to Abbott IgG II quant (Spike) antibodies (left) and DiaSorin Liaison S1/S2 (RBD) antibodies (right) in patients with SARS-CoV-2 infection

DISCUSSION

This is the first study evaluating the performance of a LFA for SARS-CoV-2 neutralizing antibodies and EIAs for SARS-CoV-2 Spike and RBD antibodies. In this study, the Dynamiker LFA neutralizing antibody was detected in 86% of the sera of convalescent Covid-19 patients compared to the EIAs for SARS-CoV-2 Spike and RBD antibodies that were detected in the 100% of the sera of convalescent Covid-19 patients. The data on the presence of neutralizing antibodies in this study is comparable with that seen in another study where 88.9% of convalescent plasma donors. Although, the sensitivity of the neutralizing antibody EIAs in the study ranged from 76.4% to 93.9%, with specificity ranging from 87.0% to 99.6%, and therefore may not necessarily have the diagnostic accuracy to detect high neutralizing antibody titers[10]. Contrastingly in some other studies, neutralizing antibodies have been seen in all (100%) of the sera of convalescent patient by week 3 after onset of fever[7]. Neutralizing antibodies usually cross-react with SARS-associated CoV-2 spike-binding antibodies targeting S1, receptor binding domain and S2 regions[11]. Quantitative SARS-CoV-2 protein antibody EIAs (Abbott, Roche, Siemens) showed a strong positive correlation with a surrogate neutralization test[12]. Further studies using EIAs (Roche, Abbott, EUROIMMUN) targeting viral spike or nucleocapsid protein associated with SARS-CoV-2 neutralization also have shown positive correlation, although there was poor negative percent agreement[12]. In this study the Dynamiker LFA neutralizing antibody test correlated positively with the DiaSorin S1/S2 IgG (RBD) and Abbott II IgG (Spike) protein assays.

In conclusion the Dynamiker LFA neutralizing antibody test correlated positively with the DiaSorin S1/S2 IgG (RBD) and Abbott II IgG (Spike) protein assays but does not appear to be always detected in the sera of convalescent patients.

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