Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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A high-throughput Anti-SARS-CoV-2 IgG testing platform for COVID-19

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ABSTRACT

Background: Serology tests for detecting the antibodies to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can identify previous infection and help to confirm the presence of current infection.
Objective: The aim of this study was to evaluate the performances of a newly developed high throughput immunoassay for anti-SARS-CoV-2 IgG antibody detection.
Results: Clinical agreement studies were performed in 107 COVID-19 patient serum samples and 226 negative donor serum/plasma samples. Positive percent agreement (PPA) was 46.15 % (95 % CI: 19.22 % ~74.87 %), 61.54 % (95 % CI: 31.58 % ~86.14 %), and 97.53 % (95 % CI: 91.36 % ~99.70 %) for samples collected on 0–7 days, 8–14 days, and ≥15 days from symptom onset, respectively. Negative Percent Agreement (NPA) was 98.23 % (95 % CI: 95.53 % ~99.52 %). No cross-reactivity was observed to patient samples positive for IgG antibodies against the following pathogens: *HIV, HAV, HBV, RSV, CMV, EBV, Rubella, Influenza A,* and *Influenza B*. Hemoglobin (200 mg/dL), bilirubin (2 mg/dL) and EDTA (10 mM) showed no significant interfering effect on this assay.
Conclusion: An anti-SARS-CoV-2 IgG antibody assay with high sensitivity and specificity has been developed. With the high throughput, this assay will speed up anti-SARS-CoV-2 IgG testing.

1. Introduction

A novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, previously provisionally named 2019 novel coronavirus or 2019-nCoV), has been identified as the source of a pneumonia outbreak that started in Wuhan, China in late 2019 (Zhu et al., 2020) and has since caused a global pandemic (COVID-19). This virus is a single-stranded RNA virus with high sequence overlap to SARS-CoV. It contains nearly 29,900 nucleotides and has at least 14 open reading frames (ORFs): ORF1ab, spike (S), ORF3a, envelope (E), membrane (M), ORF8, and nucleocapsid (N) (Holshue et al., 2020). A recent study demonstrated that both IgM and IgG antibodies were detectable 5 days after onset in all 39 patients with SARS-CoV-2 infection (Loeffelholz and Tang, 2020). The median day of seroconversion for both IgG and IgM were 13 days post symptom onset (Long et al., 2020). Serological testing can help detect PCR-negative COVID-19 cases, especially for cases with high clinical suspicion but more than 7 days post symptom onset (Patel et al., 2020). Other clinical uses include epidemiologic survey of COVID-19 seroprevalence and identifying suitable subjects who are referred for donating convalescent plasma for potential therapeutic use. Serological testing may also help guide return-to-work decisions. Although many commercial testing products have been developed or are under development now, there are unmet needs for the sensitive and high throughput serological testing kits under the current global COVID-19 pandemic situation.

Herein, we reported the performance evaluation of the QuantiVirus™ anti-SARS-CoV-2 IgG test which is a two-step immunoassay using Luminex platform to detect anti-SARS-CoV-2 spike protein 1 (S1) receptor-binding domain (RBD) IgG antibody in human serum or plasma specimens. Validation of the test was conducted using COVID-19 negative and positive samples on both Luminex 200 and MAGPIX® instruments. The test takes approximately 3 h per run with a 96-well plate capable of testing 92 patient samples.

2. Methods

2.1. Instrumentation

According to the guidance issued by Centers from Disease Control (CDC) and the World Health Organization (WHO), all studies were

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conducted in a Biosafety Level 2 (BSL-2) cabinet when handling COVID-19 patient samples. The microplate shaker (PlexBio Co, Taiwan) was used for microplate shaking and incubation. Data acquisition was performed on Luminex 200 and MAGPIX® instruments (Luminex, Austin, TX).

2.2. Reagents and patient samples

The recombinant SARS-CoV-2 Spike protein 1 (RBD)-His containing 330–524 amino acids of Spike protein was produced from HEK293 suspension cells (ProMab Biotechnologies Inc, CA). SARS-CoV-2 Spike S1 Antibody (human chimeric, IgG isotype) was purchased from GenScript Biotech Corporation (Piscataway, NJ). Anti-SARS-CoV-2 Spike RBD monoclonal antibody (IgM isotype) was purchased from Creative Diagnostics (Shirley, NY). PE conjugated anti-human IgG Fc antibody was purchased from BioLegend (San Diego, CA). MagPlex Microsphere and xMAP® Antibody Coupling (AbC) kit were purchased from Luminex (Austin, TX). Hemoglobin (human), bilirubin and EDTA were purchased from Sigma-Aldrich (St. Louis, MO).

Healthy donor EDTA K2 plasma samples were purchased from Golden West Biosolutions (Temecula, CA) in 2019 prior to the outbreak of COVID-19. COVID-19 negative EDTA K2 plasma samples were also obtained from University of Florida Department of Radiation Oncology in 2017. Healthy donor serum samples were purchased from Innovative Research, LLC (Plymouth, MN). COVID-19 patient serum samples were acquired from ProMedDx (Norton, MA) and University of California and VA Healthcare System.

Patient serum samples positive for IgG to *HBV/HCV/HIV/RSV* were purchased from Antibody Systems, Inc (Hurst, TX). Patient serum samples positive for IgG to *HAV/CMV/EBV/Rubella/Influenza B* were purchased from ProMedDx (Norton, MA). Patient serum samples positive for IgG to *Influenza A* were purchased from Dx Biosamples, LLC (San Diego, CA).

2.3. Assay procedure

The assay principle is shown in Fig. 1. Recombinant spike protein 1 (S1) RBD was covalently coupled to the surface of MagPlex® Microspheres (magnetic beads) via a carbodiimide linkage using xMAP® Antibody Coupling (AbC) kit. S1 RBD protein coated magnetic beads and human specimens were mixed and incubated at room temperature for 1 h. The IgG antibodies present in human specimens against S1 RBD protein (the antigen) will bind to the coated magnetic beads. After washing, PE conjugated anti-human IgG antibody was added to the reaction mixture and incubated at room temperature for 0.5 h. After washing, PE fluorescence of each well in a 96-well microplate was measured on Luminex 200 or MAGPIX® instrument for Median Fluorescence Intensity (MFI). Interpretation of the testing results was performed by calculating the MFI ratio of each sample to the average MFI of two blank wells.

2.4. Performance evaluation

To evaluate the clinical performance of the QuantiVirus™ Anti-SARS-CoV-2 IgG Test, 226 COVID-19 negative samples and 107 COVID-19 positive samples were tested and evaluated for NPA and PPA.

Cross-reactivity was evaluated using serum or plasma samples which are positive for IgG antibodies to the following pathogens: *HIV, HAV, HBV, RSV, CMV EBV, Rubella, Influenza A*, and *Influenza B*.

Class specificity was evaluated by spiking anti-SARS-CoV-2 RBD antibody IgG isotype and IgM isotype into a negative serum sample, respectively.

Within-run precision (repeatability) was evaluated by testing negative and positive samples in 21 or 24 replicates. Between-run precision was evaluated by testing negative and positive samples on five separate runs (3∼4 replicates per run).

For interference testing, hemoglobin (200 mg/dL), bilirubin (2 mg/dL) and EDTA (10 mM) were spiked into four serum samples, respectively, and the MFI was compared with the control samples.

2.5. Statistical analysis

For precision evaluations, coefficient of variation (CV) was calculated as the ratio of the standard deviation (SD) to the mean. For interference testing, the samples spiked with hemoglobin or EDTA were compared with the control samples by paired Student's *t*-test with $p \leq$

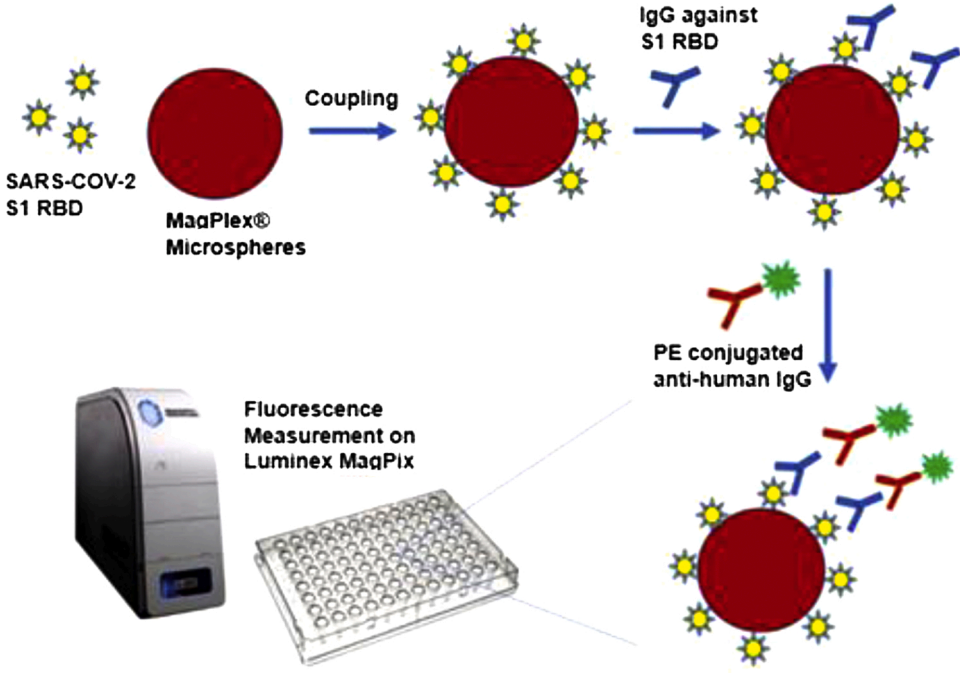

Fig. 1. The high throughput Immunoassay for anti-SARS-CoV-2 IgG detection.

0.05 defined as significantly different.

3. Results

3.1. Comparison between Luminex 200 and MAGPIX®

Unlike the Luminex 200, the MAGPIX system is not based on flow cytometry, but instead uses light-emitting diodes (LEDs) for excitation and a CCD camera for detection. Despite the difference in signal detection, the performance of the MAGPIX instrument has been shown to be comparable to the Luminex 200 instrument (Anon, 2020a). To confirm this, we tested 5 COVID-19 negative samples and 4 COVID-19 positive samples with QuantiVirus™ Anti-SARS-CoV-2 IgG Test and performed data acquisition on both Luminex 200 and the MAGPIX® instrument. As shown in Table 1, there was no significant difference in MFI values between Luminex 200 and MAGPIX (paired t-test, $p = 0.39$), and the average concordance was 98 % indicating high consistency between these two instruments.

3.2. Clinical performance

One hundred and seven (107) serum samples collected at different times from individuals who tested positive with a RT-PCR method for SARS-CoV-2 infection were used in the evaluation of positive percent agreement (PPA). Two hundred and twenty-six (226) serum or EDTA plasma samples collected from healthy donors prior to the outbreak of COVID-19 were used in the evaluation of Negative Percent Agreement (NPA). As shown in Table 2, PPA was 46.15 % (95 % CI: 19.22 % ~74.87 %), 61.54 % (95 % CI: 31.58 % ~86.14 %), and 97.53 % (95 % CI: 91.36 % ~99.70 %) for samples collected on 0−7 days, 8−14 days, and ≥15 days from symptom onset, respectively, and NPA was 98.23 % (95 % CI: 95.53 % ~99.52 %).

Thirty (30) serum samples were also further evaluated by comparing to Abbott SARS-CoV-2 IgG antibody test which has been approved by FDA EUA. The results showed 100 % concordance between the two assays (Supplementary Table 1).

In addition, 5 pairs of matched serum and EDTA plasma samples (i.e. collected from the same COVID-19 patients) were tested with QuantiVirus™ Anti-SARS-CoV-2 IgG Test and 100 % concordance was observed. It indicates that serum and EDTA plasma is comparable for this test (Supplementary Table 2).

3.3. Cross-reactivity

Cross-reactivity of the QuantiVirus™ Anti-SARS-CoV-2 IgG Test was evaluated by using serum or plasma samples which are positive for IgG antibodies to pathogens such as Influenza A or B (listed in Table 3). The result indicates that no cross-reactivity was found in any of the samples tested.

Table 1
Fluorescence Signal (MFI) Comparison of Luminex 200 and MAGPIX.

Sample name	MAGPIX®	Luminex 200	Concordance
Negative Serum_26	53	51	96.23 %
Negative Serum_27	62	63	101.61 %
Negative Serum_28	46	42	91.30 %
Negative Serum_29	33	34	103.03 %
Negative Serum_30	151	143	94.70 %
Positive serum_1	4793	4512	94.14 %
Positive serum_2	8083	8254	102.12 %
Positive serum_3	1738	1835	105.58 %
Positive serum_4	3319	3244	97.74 %
Average Concordance (%)			98.48 %

3.4. Interfering substance

Hemoglobin (200 mg/dL) was spiked into four serum samples to test the potential interfering effect of high-level hemoglobin which might be present in hemolysis and other conditions. Bilirubin (2 mg/dL) was spiked into four serum samples to test the potential interfering effect of high-level bilirubin in the blood which might be caused by liver dysfunction such as hepatitis and cirrhosis. Lastly, EDTA (10 mM) was spiked into four serum samples to test the potential interfering effect of EDTA which is the anticoagulant used in EDTA blood collection tubes.

As shown in Supplementary Table 3, the difference in fluorescence signal (MFI) between the control samples and the samples spiked with hemoglobin, bilirubin or EDTA was all ≤ 11.5 % which is acceptable for the test, and no false negative or false positive results were observed. Therefore, hemoglobin, bilirubin and EDTA do not have significant interfering effect on QuantiVirus™ Anti-SARS-CoV-2 IgG Test at the tested concentrations.

3.5. Precision

Within-run precision (repeatability) was evaluated by testing negative and positive samples in 21 or 24 replicates. Between-run precision was evaluated by testing negative and positive samples on five separate runs (3~4 replicates per run). As shown in Supplementary Tables 4 and 5, the average CV % of within-run precision and between-run precision was 8.25 % (from 4.71 % to 11.74 %) and 10.47 % (from 5.0 % to 13.4 %), respectively.

3.6. Class specificity

A study was performed to evaluate class specificity for QuantiVirus™ Anti-SARS-CoV-2 IgG Test. The human anti-SARS-CoV-2 Spike RBD antibody (IgG and IgM isotypes) was spiked into a negative serum sample respectively, and then tested by QuantiVirus™ Anti-SARS-CoV-2 IgG Test. A strong binding was observed for IgG isotype while no binding interaction was observed between the anti-human IgG antibody used in the QuantiVirus™ Anti-SARS-CoV-2 IgG Test and human IgM isotype, demonstrating class-specific reactivity only to human IgG isotype (Table 4).

3.7. Serum heat-inactivation

Heat-inactivation is an effective means of destroying many types of viruses and is used to protect the safety of laboratory workers exposed to blood and other bodily fluids while performing their jobs. Therefore, we tested if heat-inactivation will affect the results of QuantiVirus™ anti-SARS-CoV-2 IgG Test. As shown in Supplementary Table 6, heat-inactivation significantly increased the MFI of all the samples tested and one negative plasma sample became false positive, indicating that heat-inactivation is not suitable for the QuantiVirus™ anti-SARS-CoV-2 IgG Test.

4. Discussion

The RT-PCR tests designed to detect SARS-CoV-2 RNA have been the mainstay of testing for COVID-19 diagnosis and follow-up. However, serological testing should be helpful for analyzing RT-PCR negative COVID-19 cases as well as asymptomatic infections (Long et al., 2020). IgM can be an indicator of early stage infection, and IgG can be an indicator of current or prior infection. Besides epidemiological prevalence survey, IgG seropositivity can also be used to suggest the presence of post-infection immunity (Carter et al., 2020). Therefore, it is important to develop sensitive and specific serological testing for COVID-19.

Various methodologies for detecting anti-SARS-CoV-2 antibodies detection have been developed, including the traditional enzyme-linked immunosorbent assay (ELISA), immunochromatographic lateral flow

Table 2
Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA).

Category	Days from Symptom Onset	Number of Samples	IgG Positive	IgG Negative	PPA and NPA (95 % CI)
COVID-19 Positive	0–7 days	13	6	7	PPA: 46.15 % (19.22 % ~74.87 %)
	8–14 days	13	8	5	PPA: 61.54 % (31.58 % ~86.14 %)
	≥15 days	81	79	2	PPA: 97.53 % (91.36 % ~99.70 %)
COVID-19 Negative	n/a	226	4	222	NPA: 98.23 % (95.53 %~99.52 %)

n/a: not applicable.

Table 3
Cross-reactivity Evaluation.

Category	Number of Samples Tested	Positive	Negative
Human immunodeficiency virus (HIV)	4	0	4
Hepatitis A Virus (HAV)	7	0	7
Hepatitis B Virus (HBV)	4	0	4
Hepatitis C Virus (HCV)	4	0	4
Respiratory Syncytial Virus (RSV)	5	0	5
Influenza A	5	0	5
Influenza B	13	0	13
Cytomegalovirus (CMV)	16	0	16
Epstein-Barr Virus (EBV)	13	0	13
Rubella	17	0	17

Table 4
Class-specificity Test.

Sample	MFI-1	MFI-2	Average
Negative serum sample	19	23	21
Negative serum sample with spiked human IgM isotype (5 μg/mL)	18	18	18
Negative serum sample with spiked human IgM isotype (0.5 μg/mL)	17	18	17.5
Negative serum sample with spiked human IgG isotype (2.5 μg/mL)	824	840	832

assay, neutralization bioassay, and specific chemosensors (Carter et al., 2020). Development of high-throughput serology tests has also been a major focus of large diagnostics companies (Anon, 2020b). Due to the specificity challenges associated with high false-positive rates, IgM may not play the primary role in COVID-19 antibody testing (Marie Louise Landry, 2016; Bohn et al., 2020). Therefore, we have developed the QuantiVirus™ anti-SARS-CoV-2 IgG test which can be run on Luminex platform for testing 92 samples per run within 3 h. This high throughput assay has a sensitivity of 97.53 % after 14 days from onset of symptoms and specificity of 98.23 %. It can help clinical laboratories to further ramp up COVID-19 diagnostics.

Recently, Cochrane Infectious Diseases Group assessed the diagnostic accuracy of anti-SARS-CoV-2 antibody tests using the Cochrane COVID-19 Study Register and the COVID-19 Living Evidence Database from the University of Bern (Deeks et al., 2020). They found that the sensitivity of anti-SARS-CoV-2 IgG antibody test was highly variable with CLIA (94.6 %), CGIA (87.3 %) and ELISA (85.8 %) all outperforming the lateral flow assay tests (76.0 %). Panagiota et al. showed similar findings (Kontou et al., 2020). Sensitivity also varies significantly with the time since symptom onset with an average sensitivity of 30.1 % in the first week and 72.2 % in the second week post-symptom onset (Deeks et al., 2020).

FDA EUA approved Abbott SARS-CoV-2 IgG test on Abbott Architect platform reportedly has 100 % sensitivity after 17 days from onset of symptoms and 100 % specificity (Bryan et al., 2020). The 100 % concordance rate between the Abbott IgG test and the QuantiVirus™ anti-SARS-CoV-2 IgG test (Supplementary Table 1) indicates that the performance of our assay is comparable to that of the FDA EUA approved Abbott product.

Hettegger et al. demonstrated that IgG profiles in plasma and saliva are highly similar for each individual and found the anti-HBV IgG antibody from saliva (Hettegger et al., 2019). We therefore tested saliva and serum from a COVID-19 patient. Although patient serum was detected for IgG, there was no IgG signal from saliva (Supplementary Table 1, sample UCSF H). Since this was one patient sample testing, we cannot exclude the possibility of saliva as a sample type for SARS-CoV-2 antibody detection. Further development effort is ongoing.

Heat-inactivation is an effective means of destroying many types of viruses to protect the safety of laboratory workers. It is also a standard procedure in diagnostic laboratories to conduct neutralization tests for the purpose of inactivation of complement. Previous other virus studies suggest that serum heat inactivation and optimal dilution enhance WNV E-MIA sensitivity by eliminating the complement interference, thereby detecting low-titer anti-WNV antibodies during early and late phases of infection (Namekar et al., 2012). Recently, two groups have reported that heat-inactivation of blood samples at 56 ℃ for 30 min does not obviously affect the results of immunochromatography and chemiluminescent immunoassay for detection of SARS-COV-2 antibodies (Xue et al., 2020; Hu et al., 2020). However, heat-inactivation cannot be used in fluorescence immunochromatography for SARS-CoV-2 antibody detection because negative samples became positive after heat-inactivation (Xue et al., 2020), which is consistent with our results. We found that heat-inactivation significantly increased the MFI for all samples tested, and some negative plasma samples became a false positive.

We also evaluated the precision of the QuantiVirus™ anti-SARS-CoV-2 IgG test which shows the average CV % of within-run precision and between-run precision being 8.25 % (from 4.71 % to 11.74 %) and 10.47 % (from 5.0 % to 13.4 %), respectively. In an early report by Garrigan C et al., the investigators evaluated a Luminex platform based assay for measuring serum procalcitonin levels (BioPlex Pro™ Human Acute Phase Multiplex Assay), and they found that the CV % of two tested samples (17 replicates for each) was 16.24 % and 14.55 %, respectively (Garrigan et al., 2016). Both our data and the findings of Garrigan C et al. showed that Luminex beads-based assays have CV % greater than 5.0 %, indicating that this suboptimal precision might be an inherent feature of the beads-based Luminex platform since each bead creates an individual signal, and these signals could have a higher CV of mean value.

In conclusion, we have successfully developed a reliable high-throughput immunoassay for qualitative detection of anti-SARS-CoV-2 IgG antibody. The assay was validated with COVID-19 positive samples as well as negative samples obtained from healthy donors on both Luminex 200 and MAGPIX® instruments. We believe that this assay will help to determine the infection status of COVID-19 and the true scope of the pandemic. It may be used to guide return-to-work decisions.

Author contributions

J. Du, E. Chu and D. Zhang conducted the experiments. J. Du and M. Sha wrote the draft of the manuscript. M. Sha, CM. Lu and A. Zhang reviewed and edited the manuscript. All the authors read and approved the final manuscript.

Declaration of Competing Interest

The authors reported no declarations of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jviromet.2020.114009.

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