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A Hemagglutination-Based Semiquantitative Test for Point-of-Care Determination of SARS-CoV-2 Antibody Levels

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ABSTRACT Serologic point-of-care tests to detect antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are an important tool in the COVID-19 pandemic. The majority of current point-of-care antibody tests developed for SARS-CoV-2 rely on lateral flow assays, but these do not offer quantitative information. To address this, we developed a novel antibody test leveraging hemagglutination, employing a dry card format currently used for typing ABO blood groups. Two hundred COVID-19 patient and 200 control plasma samples were reconstituted with O-negative red blood cells (RBCs) to form whole blood and added to dried viral-antibody fusion protein, followed by a stirring step and a tilting step, 3-min incubation, and a second tilting step. The sensitivities of the hemagglutination test, Euroimmun IgG enzyme-linked immunosorbent assay (ELISA), and receptor binding domain (RBD)-based CoronaChek lateral flow assay were 87.0%, 86.5%, and 84.5%, respectively, using samples obtained from recovered COVID-19 individuals. Testing prepandemic samples, the hemagglutination test had a specificity of 95.5%, compared to 97.3% and 98.9% for the ELISA and CoronaChek, respectively. A distribution of agglutination strengths was observed in COVID-19 convalescent-phase plasma samples, with the highest agglutination score (4) exhibiting significantly higher neutralizing antibody titers than weak positives (2) ($P < 0.0001$). Strong agglutinations were observed within 1 min of testing, and this shorter assay time also increased specificity to 98.5%. In conclusion, we developed a novel rapid, point-of-care RBC agglutination test for the detection of SARS-CoV-2 antibodies that can yield semiquantitative information on neutralizing antibody titer in patients. The 5-min test may find use in determination of serostatus prior to vaccination, postvaccination surveillance, and travel screening.

KEYWORDS SARS-CoV-2, antibody, hemagglutination, point of care, red blood cell, serology

The COVID-19 pandemic has impacted nearly all facets of health and society. The scale and speed with which severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection spread introduced myriad challenges. Early on, testing was identified as being critical to contend with the global health crisis. Testing is needed both for diagnosis of those who are actively infected and for monitoring of those who have recovered. The latter is increasingly important for immune surveillance, which, in turn, has a range of applications spanning ascertainment of vaccination uptake to travel. This has led to a surge in the development and marketing of SARS-CoV-2 serology assays to monitor antibody development.

Antibodies confer protection in the overwhelming majority (>90%) of seropositive individuals, with the caveat that the longevity of those antibodies has yet to be

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determined (1). Further, many of the approved SARS-CoV-2 vaccines in use confer high levels of protection against SARS-CoV-2 by provoking a brisk humoral response (2). The presence of antibodies has also been the basis for selected therapeutics such as COVID-19 convalescent-phase plasma (CCP) (3) and monoclonal antibodies against COVID-19 (4). A determination of serostatus is predictive of response to treatment with these therapies. For example, those who are seronegative at diagnosis have been found to have a significant decrease in hospitalization rate following monoclonal antibody therapy; in contrast, seropositive patients do not benefit significantly from monoclonal antibody therapy (4). Similarly, CCP appears to be optimally beneficial when high titer units are provided early in the disease course (5, 6).

Recent studies have shown that individuals who have recovered from COVID-19 may require only a single dose of vaccine for protection comparable to that of naive individuals following receipt of two vaccine doses (7). Modification of existing vaccine policy accordingly could reduce the total amount of vaccine doses needed to achieve herd immunity. Nonetheless, it would require a rapid method to screen individuals for antibodies (i.e., to confirm prior infection). To date, the rapid SARS-CoV-2 antibody tests approved under emergency use authorization are lateral flow assays, whose performance characteristics have been highly variable (8). Furthermore, lateral flow tests do not offer any quantitative data on antibody levels; the latter are important given the wide range of antibody responses, particularly following mild SARS-CoV-2 infection (9).

We sought to develop a point-of-care (POC) test for SARS-CoV-2 antibody detection based on hemagglutination, leveraging a test platform that is already routinely used at the point of care for determination of blood types. Previous work demonstrated proof of concept for hemagglutination-based SARS-CoV-2 antibody detection but required a 1-h incubation time and pipetting in a 96-well plate (10, 11). We also sought to determine if the test could yield a semiquantitative readout of antibody levels among COVID-19 recovered patients within few minutes, which would represent the first rapid SARS-CoV-2 serology test to do so.

MATERIALS AND METHODS

Design and construction of hemagglutination, point-of-care test. Previous studies by our group and a second group have demonstrated the capability of using a fusion protein of an antibody against a red blood cell (RBC) antigen connected to the receptor binding domain (RBD) of SARS-CoV-2 to detect antibodies against RBD in patient plasma (10, 11). We adapted dry hemagglutination cards, which are currently used in countries across the world in a room temperature-stable kit for rapid, point-of-care testing for ABO and Rh blood types. We collaborated with Eldon Biologicals (Gentofte, Denmark), which currently sells cards with dried antibodies (EldonCards) to detect ABO and Rh for blood typing. The hemagglutination card kits comes with components of a lancet to elicit blood, a dropper to add water to the platform, and stirring sticks to develop the assay, which we utilized in our testing (see Fig. S1A in the supplemental material). Each EldonCard test circle has dried antibodies to the target RBC antigen to trigger hemagglutination and typing determination (Fig. S1B). We repurposed these cards for COVID-19 antibody detection by formulating the cards instead with the IH4-RBD fusion protein, previously described (11).

As outlined in Fig. 1, we took a fusion protein from one of the studies, IH4-RBD (11), and dried it onto a hemagglutination card to formulate the test. The IH4-RBD fusion protein was obtained from Absolute Antibody (Oxford, United Kingdom) as a gift of the investigators (Alain Townsend) of the previous study (11). A total of 533.2 ng of IH4-RBD protein was dissolved in a proprietary buffer and placed onto the card. The cards were then heated to leave a dried protein mixture on the card, which is stable at room temperature and can be packaged and shipped. Addition of water solubilizes the fusion protein, and addition of blood containing COVID-19 antibodies facilitates cross-linking of RBCs, which after stirring can be observed macroscopically (Fig. 1).

Clinical specimen tests. The characteristics of the study population and associated clinical specimens have previously been described (8, 12). In brief, the convalescent-phase SARS-CoV-2 samples had been collected from COVID-19 patients who were confirmed positive by reverse transcription-PCR (RT-PCR) at least 28 days prior to specimen collection (mean, 45 days; standard deviation [SD], 7.5). The prepandemic control samples were collected from a prior study of patients who presented to the Johns Hopkins Hospital emergency department with symptoms of an acute respiratory tract infection between January 2016 and June of 2019 as part of the Johns Hopkins Center for Influenza Research and Surveillance study. An analysis of the stored samples and data from those studies was conducted. No additional samples were collected for the current study.

Sample preparation. Type O, Rh-negative packed red blood cells (pRBCs) were obtained from Tennessee Blood Services and provided by Biochemed Services (Winchester, VA). On receipt, the pRBCs were stored at 4°C and used entirely within 28 days of collection. All RBCs were washed with phosphate-

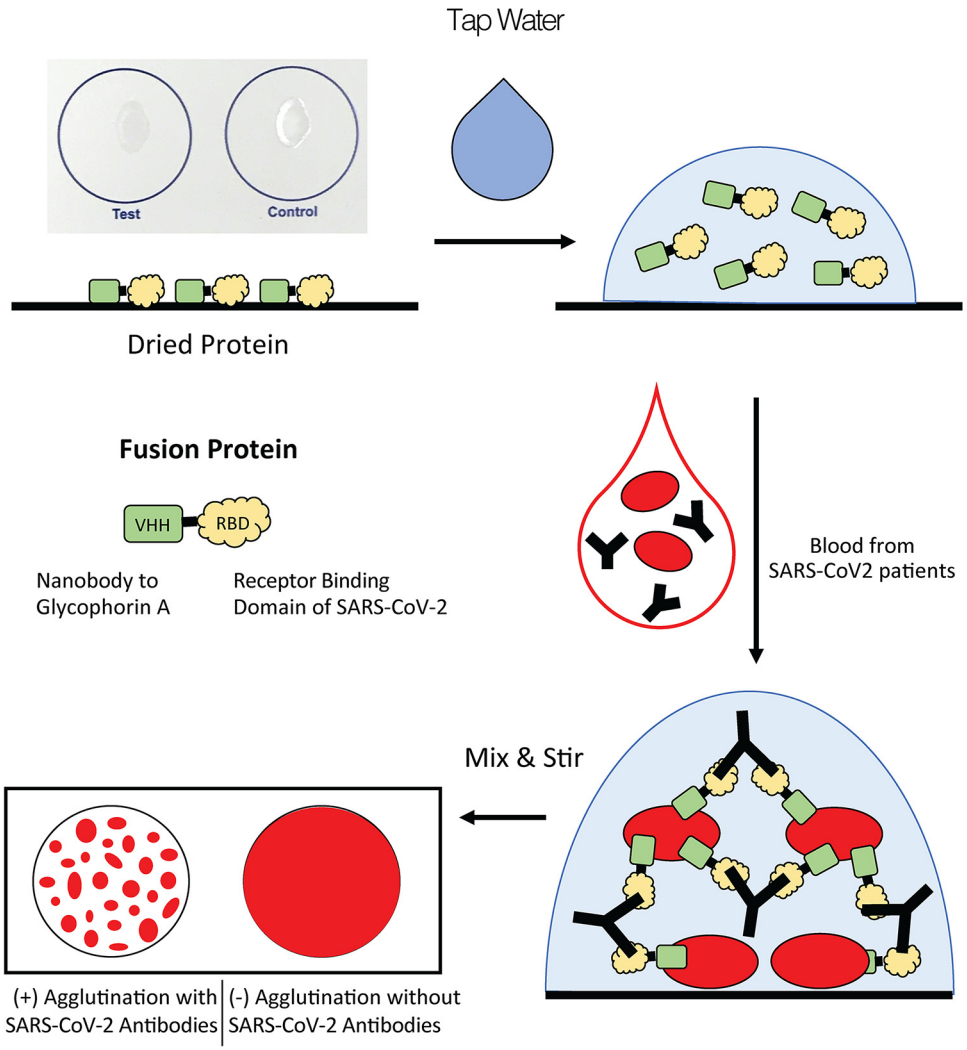

FIG 1 Mechanism of a dry card assay for hemagglutination-based detection of SARS-CoV-2 antibodies. A fusion protein consisting of a nanobody targeting human glycophorin A and the receptor binding domain (RBD) of SARS-CoV-2 was previously described (11). The fusion proteins are dried onto a non-water-absorbent card, remaining stable at room temperature indefinitely in a desiccant pouch. For testing, the fusion proteins are resuspended in a water droplet, followed by the addition of whole blood containing antibodies and RBCs. Stirring facilitates cross-linking of large aggregates of RBCs, which are visible by the naked eye.

buffered saline (PBS) to remove any residual plasma. Washed pRBCs were combined with frozen plasma to reconstitute "whole blood" with ~40% hematocrit after combining pRBCs and frozen plasma.

Testing protocol. For each test, 20 μl of tap water was placed onto the dried protein spot within the test circles on the card to dissolve the protein. Reconstituted whole blood (20 μl) was then added to the spot. The fluid of water and blood was mixed with a plastic Eldon stick, spreading the liquid completely within the circle to make sure that the dissolved protein was mixed well with blood on the card. The card was tilted for 10 s in each 90° direction (4 times in total) and allowed to incubate on a flat surface for 3 min. The card was then tilted again as before in each 90° direction. Demonstration and validation of the test with a vaccinated individual are shown in Supplemental Video S1.

Tests were interpreted according to similar protocols established for scoring hemagglutination in EldonCard blood typing assays. The scoring system revolves around how much red blood cell clumping and clearing of the test circle surface occur to form a white or pink background. The agglutination score 4 represents the most clumping, with large aggregates, with smaller scores demonstrating progressively smaller aggregate sizes. The tests were assigned scores of 4, 3.5, 3, 2.5, 2, 1, and 0. The scores of 1 and 0 were assigned as negative results, and a score of 2 or higher was a positive test result. Tests were interpreted both during tilting of the card and when the card was on a flat horizontal surface, since weak agglutinations could be appreciated in certain cases more easily with the liquid droplet on the side.

Serologic testing using commercial assays. The CoronaChek SARS-CoV-2 lateral flow assay and the Euroimmun IgG spike enzyme-linked immunosorbent assay (ELISA) were performed in accordance with the manufacturers' instructions and test results previously reported on a different study on the same

TABLE 1 Hemagglutination-based SARS-CoV-2 antibody assay performance[a]

Antibody test	% sensitivity (CI)	No./total samples	% specificity (CI)	No./total samples
Hemagglutination test	87.0 (81.5–91.3)	174/200	95.5 (91.6–97.9)	191/200
Euroimmun spike IgG ELISA	86.5 (81.0–90.9)	173/200	97.3 (95.5–98.5)	502/516
CoronaChek lateral flow assay	84.5 (78.7–89.2)	169/200	99.0 (97.78–99.6)	574/580

[a]Sensitivity and specificity are presented for the hemagglutination test using 200 samples of PCR-confirmed COVID-19 patients and 200 prepandemic samples of patients with acute respiratory symptoms. Results of a regulatory-approved Euroimmun IgG ELISA and RBD-based CoronaChek lateral flow test on the same samples are also presented for comparison. Specificity results are presented for an equivalent bank of prepandemic samples, although not all samples overlap between the three groups. Borderline samples on ELISA were called positive, and faint samples on lateral flow assay were called positive. Confidence intervals are presented within parentheses.

specimens (8, 12, 13). Microneutralization assays in VeroE6-TMPRSS2 cells were used to quantify neutralizing antibody titers against 100 50% tissue infectious doses ($TCID_{50}$). Neutralizing antibody titer area under the curve (AUC) was calculated through the exact number of wells protected from infection at every dilution. Half of the lowest measured AUC value was assigned to the samples with no neutralizing activity. Their acquisition has previously been described (12, 14).

Statistical analyses. Sensitivity of the assays was calculated among COVID-19 convalescent individuals, and specificity was calculated among prepandemic population. Ninety-five percent Clopper-Pearson confidence intervals (CI) of sensitivity and specificity were also computed. Statistical comparisons made between agglutination scores and ELISA optical density and neutralizing antibody titers or AUC were made using nonparametric Mann-Whitney tests. Statistical significance was set at a P value of <0.05. All statistical analyses were performed using GraphPad Prism 9.0.0 for Mac (GraphPad Software, San Diego, CA).

Human subjects. The parent studies of the collected patient samples were approved by The Johns Hopkins University School of Medicine institutional review board (IRB00247886, IRB00250798, and IRB00091667). All samples were deidentified prior to testing in the current study, and the original studies were conducted according to the ethical standards of the Helsinki Declaration of the World Medical Association.

RESULTS

Hemagglutination test performance against clinical samples. Plasma samples obtained from COVID-19 convalescent (n = 200) and prepandemic individuals (n = 200) were reconstituted with O-negative blood to a hematocrit of ~40%, approximating whole blood for the hemagglutination test. The testing protocol matched the protocol that is currently used in ABO typing with the EldonCard, with results within 5 min. The sensitivity for detecting antibodies was 87.0% (CI, 81.5% to 91.3%). In comparison, the FDA-approved Euroimmun spike IgG ELISA showed a sensitivity of 86.5% (CI, 81.0% to 90.9%), while a high-performing RBD-based lateral flow assay, CoronaChek (15), had a sensitivity of 84.5% (CI, 78.7% to 89.2%) on the same 200 samples (Table 1). Specificity for the hemagglutination test was calculated at 95.5% (CI, 91.6% to 97.9%), which was not statistically lower than for the Euroimmun ELISA, 97.3% (CI, 95.5% to 98.5%), and CoronaChek, 99.0% (97.8% to 99.6%). An additional cohort of 16 lateral flow assays was also compared for sensitivity to the hemagglutination test on a smaller set of samples, with the hemagglutination test showing similar sensitivity (data not shown) (8).

We next scored all agglutinations observed on the card across 200 samples tested, building off the previous scoring system of hemagglutination developed by Eldon for ABO typing. As shown in Fig. 2, the highest agglutination was scored at 4, wherein large clumps of RBCs were seen with few residual free cells, to 0, wherein no reaction was observed. The agglutination scores of 0 and 1 were termed negative, while any score at 2 or above was positive. Scoring is presented as the cards resting on a horizontal surface in Fig. 2, as well as slanted after final mixing in Fig. S2. In the latter scenario, it can often be easier to see small agglutinations without the large liquid droplet of unagglutinated RBCs obscuring the view, as well as the kinetic movement of these agglutinations across the test circle. In an agglutination score 1 field, there can be a small number of agglutinations observed, but these are usually very few and often fixed to the card and do not move like most agglutinations in a 2 score.

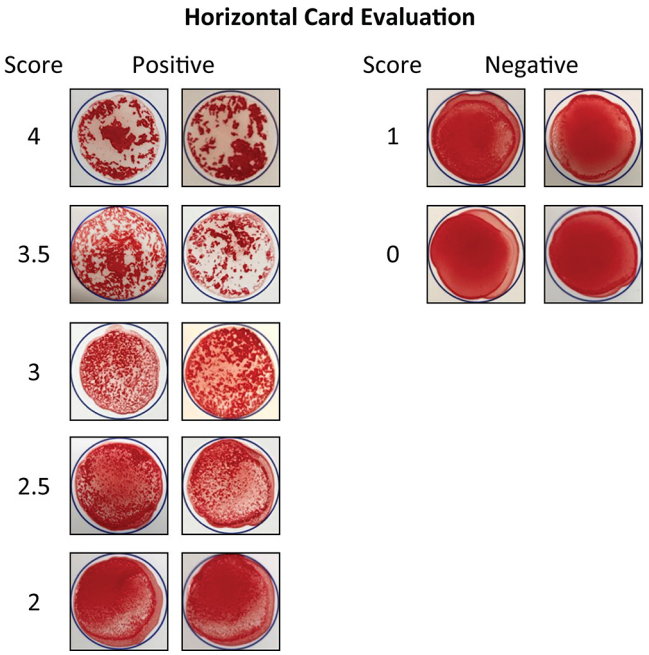

FIG 2 Hemagglutination can be scored for reaction strength. Scores were developed to quantify the degree of agglutination observed across COVID-19 convalescent-phase samples. Cards are depicted horizontal on a table surface after testing. Strong agglutinations (for example, with a score of 4) quantified the majority of red blood cells sticking together, with a white background without unbound cells. Weaker reactions (for example, with a score of 2) had smaller but frequent agglutinations. The scores of 0 and 1 were deemed negative for the purpose of the test.

Across the 200 recovered COVID-19 patients, a range of different agglutination scores were observed (Fig. 3A). Interestingly, relatively few patients achieved the highest levels of agglutination, 4 and 3.5, while 46.5% of patients had borderline studies (2 to 2.5). We sought to confirm that these differences in agglutination were related to the antibody concentration

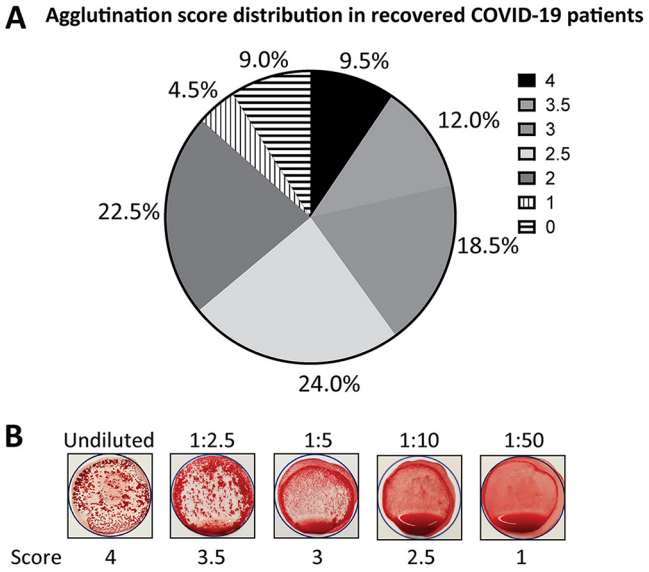

FIG 3 COVID-19 recovered patients exhibit a distribution of agglutination scores that correlate with the dilution of COVID-19 convalescent-phase plasma. (A) Agglutination scores for 200 patient samples were tabulated, and percentages for each agglutination score are provided. Agglutination scores of 1 and 0 were deemed negative and are stripped for distinction from the positive test results in solid color. (B) A sample with a strong agglutination (4) was obtained, and the plasma was progressively diluted with prepandemic plasma. The same amount of RBCs was added for all conditions. Agglutinations are depicted in the tilted card position and were clearly seen down to 1:10, while 1:50 only had very weak agglutinations below the assay cutoff.

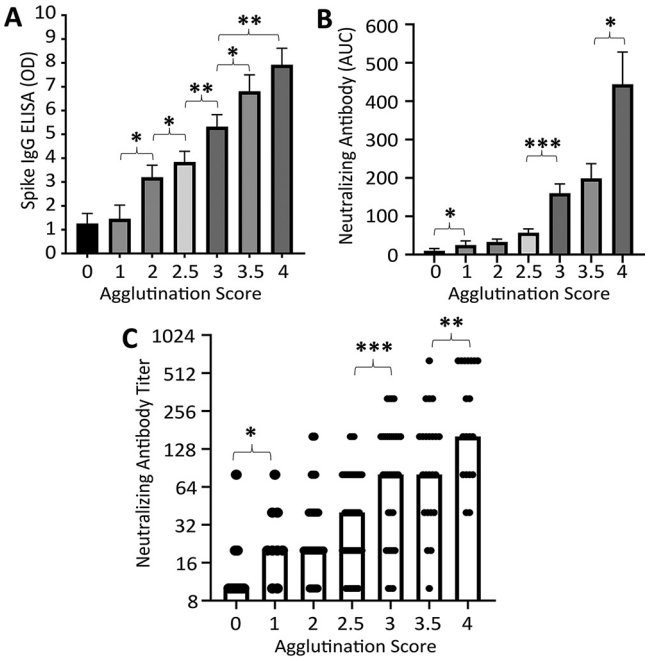

FIG 4 Agglutination scores correlate with ELISA and neutralizing antibody titer assays. (A) The optical density (OD) of the Euroimmun spike IgG ELISA (1:101 dilution) was categorized for each agglutination score. (B) Neutralizing antibody AUC (area under the curve) was quantified for each specimen and plotted against the respective agglutination score. (C) A plot of neutralizing antibody titers at different agglutination score is presented, wherein each dot represents a single sample and each bar represents the median among the samples. Statistical analysis was performed using nonparametric, one-tailed Mann-Whitney U tests. Statistical significance is shown with asterisks. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

in the plasma, which would influence the amount of RBC cross-linking observed. We took a patient sample that scored a 4 in agglutination and performed a serial dilution in order to assess if agglutination decreased. A decline in agglutination correlated progressively with more dilute samples (Fig. 3B), with a 1:10 dilution scoring a clear but weak reaction, while a 1:50 dilution did not show a clear reaction after 5 min. Thus, the agglutination results correlate with antibody concentration.

The relationship between agglutination score and traditional ELISA and neutralizing antibody assays against SARS-CoV-2 was next determined. There was a correlation between the optical density (OD) of the spike IgG ELISA and agglutination score, despite the hemagglutination assay containing only RBD, which is a small portion of the spike protein (Fig. 4A). The RBD is a major target of neutralizing antibodies, so we examined agglutination score versus neutralizing antibody levels. There was a general correlation with increasing agglutination score and higher neutralizing antibody levels for both the AUC (Fig. 4B) and endpoint dilution titer (Fig. 4C) against the virus. Notably, agglutination scores of 1 and 2 had no difference in neutralizing antibodies, while strong agglutination scores 3 or higher were clearly defined by higher neutralizing antibody levels.

Analysis of false-positive and false-negative samples. We next sought to determine how the assay time influenced the observed test performance, given our experience that many strong agglutination reactions were observed after just the first series of card tilting and blood mixing. In a smaller cohort of 73 tests within the 200 samples, we scored the test after initial tilting as well as after the complete assay time. We found that the sensitivity of the assay decreased to 60.3% if only 1 min of assay time was leveraged (Fig. 5A). The additional incubation time led to another 23.3% of samples becoming positive, although the agglutination scores of these tests were almost all 2 (94.1%) (Fig. 5B). Interestingly, the neutralizing antibody titers of these weak reactions requiring additional incubation time were all very low and were significantly lower

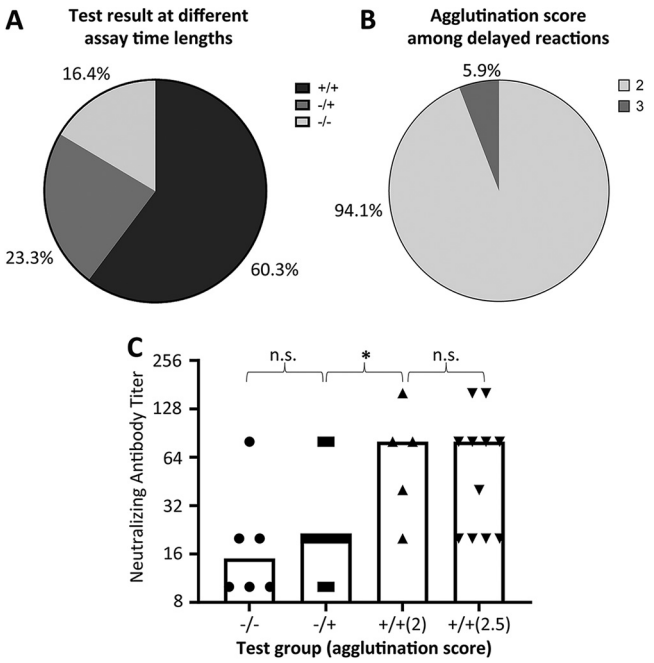

FIG 5 Specimens requiring longer assay time to yield agglutination have low agglutination scores and neutralizing antibody titer. A subset of convalescent-phase COVID-19 samples (n = 73) was scored after the first round of card tilting, followed by a 3-min incubation and a second round of card tilting for a second score. (A) The distribution of samples that were positive already at the first round (+/+), positive after the second round (−/+), and negative after the complete assay (−/−) is presented. (B) The agglutination scores of −/+ samples are provided, demonstrating that almost all had a 2 agglutination score. (C) The neutralizing antibody titers for negative (−/−), delayed agglutination (−/+), and fast (+/+) samples with low agglutination scores, 2 and 2.5, are presented. The latter would correspond to the ultimate agglutination scores observed for −/+ samples. Each point represents a single sample, and each bar represents the median of the group. Statistical analysis was determined using a nonparametric Mann-Whitney U test. *, $P < 0.05$. n.s., nonsignificant.

than the neutralizing antibody titers for similarly weak reactions (2 to 2.5) that were initially visible after 1 min (Fig. 5C). Together, these findings indicate increased sensitivity but lower functional utility in increasing assay time.

The false-positive samples in the prepandemic samples were next interrogated. Among the 9 false positives, 6 required the additional incubation time to become positive (Fig. 6A). The agglutination scores among the false positives were also weak, with only 2 out of 9 registering a score of 3 (Fig. 6B). If the assays were limited to just interpretations after 1 min, the specificity would increase to 98.5% (197/200). The false negatives among the ELISA, hemagglutination, and CoronaChek assays were next compared. While there were no statistical differences between tests, the lateral flow test had a trend toward higher levels of neutralizing antibodies completely missed (Fig. 6C). Interestingly, one sample measured 1:320 neutralizing antibody titer, a score of 4 by agglutination, but was negative on the CoronaChek test.

DISCUSSION

In this study, we established a new point-of-care SARS-CoV-2 antibody testing platform that distinguishes itself from the commonly used lateral flow assays given its being semiquantitative for detecting antibodies. We found that this hemagglutination-based assay has a more rapid turnaround time than lateral flow assays (5 min versus 15 min, respectively) and ELISAs (24- to 48-h turnaround time) and can achieve similar sensitivities. Moreover, a technically equivalent test is sold commercially by Eldon Biologicals for blood typing, which should aid in the manufacturing and regulatory approval.

Our results found 87.0% sensitivity among COVID-19 recovered patients, which compared well to leading ELISA (86.5%) and lateral flow assays (84.5%) already in use. Our

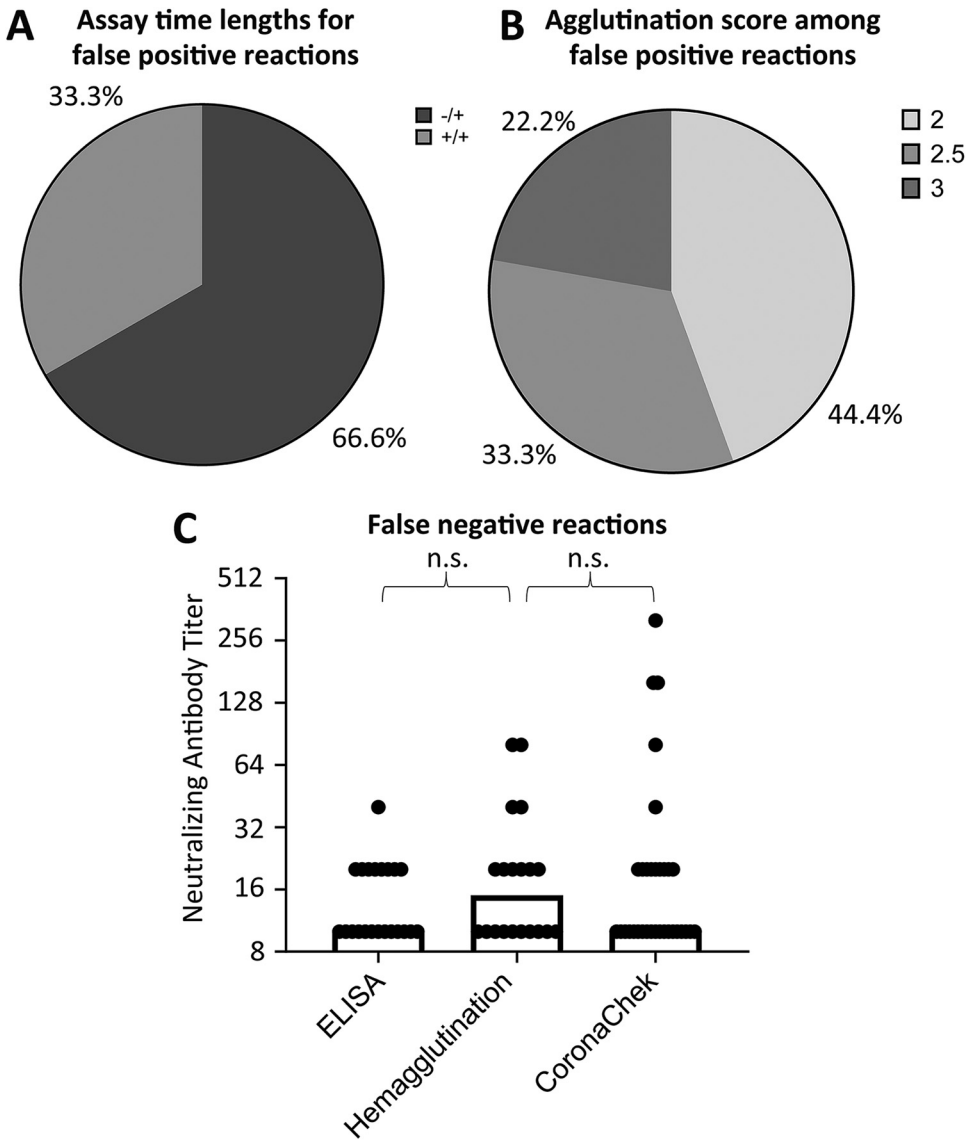

FIG 6 False-positive samples on hemagglutination test yield weak agglutinations, while high-titer false negative agglutinations are rare. Nine samples that were false positives among the prepandemic emergency department samples were further analyzed. (A) The distribution of samples that were positive already at the first round (+/+) versus positive after the second round (−/+) is presented. (B) The distribution of agglutination scores among false-positive samples is also demonstrated, generally showing very weak agglutination. (C) Neutralizing antibody titers are presented for false-negative samples among the hemagglutination-based test, Euroimmun spike IgG ELISA, and the CoronaChek lateral flow assay. Each point represents a single sample, and each bar represents the median of the group. Statistical analysis was performed using a nonparametric Mann-Whitney U test.

sensitivity was similar to that in the prior study using the IH4-RBD fusion in a 96-well plate with 1-h incubation, which was 90% (11). The slight differences in sensitivity between the two assays may result from the longer incubation time and gravity in a 96-well, U-bottom plate facilitating RBC aggregation. While many ELISA and lateral flow assays use the whole spike protein, we chose to use the spike RBD for this test, since it is the main target of neutralizing antibodies, which should provide protective immunity. RBD has been employed as the target antigen in ELISA (16) and lateral flow tests (8), respectively. Some results from RBD-based ELISAs have found sensitivity of 98% and specificity of 100% (17), sensitivity of 96% and specificity of 99.3% (18), and sensitivity of 88% and specificity of 98% (19). The original CoronaChek RBD-based lateral flow assay

description reported a sensitivity of 88.66% and specificity of 90.63%, differing markedly from the values in this publication (15). The differences between all these studies are likely driven by variable patient symptoms and emphasize the need for comparison of tests on the same set of samples.

A key advantage of our test is the semiquantitative readout of antibody levels, which is unique among all point-of-care COVID-19 serology assays on the market today. We observed a correlation between agglutination score and neutralizing antibody titer, which has also been seen in RBD-based ELISAs where a correlation with neutralizing antibody titer was found (17). The ability to interpret an RBC agglutination pattern for semiquantitative determination was previously used in the SimpliRED test, evaluating D-dimer levels at the point of care (20). Importantly, the correlation between neutralizing antibody levels and agglutination can also help refine use cases for the test in the future, such that scores lower than 3 are determined to lack substantial immunity. Of note, preliminary testing of vaccinated individuals with our hemagglutination test has shown agglutinations of 3.5 or 4, matching the uniformly high levels observed in clinical trials.

The wide distribution of agglutination scores we observed matches the variability in antibody responses among COVID-19 patients, wherein not every patient recovered from COVID-19 produces antibodies at high levels or in some cases at all, particularly among nonhospitalized patients with mild to no symptoms (17), while hospitalized patients are noted to have significantly higher antibody and neutralization titers (21). The test performance reported is strongly dictated by antibody levels in the specimens, which are, in turn, dictated by the patient population, with severe-disease patients having higher antibody levels than mild cases (22).

This study had limitations. The specificity in our assay (95.5%) was lower than the 99% reported using the same fusion protein previously and also lower than the aforementioned RBD-based ELISAs (specificity of 100% [17] and 99.3% [18]). The reason for lower specificity is uncertain but is likely multifactorial. The manufacturing of a dried protein on the card may yield fusion protein clumping not seen with dissolved protein solution in the prior study (11). Another consideration is that the prior study (11) tested healthy donors as a control, while all negative-control samples in our assay were patients with acute respiratory illness, including a subset with active seasonal coronavirus infection. While the sequence identity between the viruses is only ~20% (23), it is possible that even weakly cross-reactive antibodies could achieve binding at high concentrations. Cross-reactivity has been suggested as a reason for significantly worse specificity of SARS-CoV-2 antibody ELISAs (90 to 94% against spike protein) (24) and CoronaChek lateral flow assay (96.5%) (13) in African populations. An important distinction is that ELISA cutoff values for optical density can be optimized for maximal specificity (25), whereas the hemagglutination test relies on visual interpretation with more limited nuance. Soluble ACE2 and white blood cells expressing ACE2 may also contribute to false positives via binding to RBD fusion protein. Of note, specificity could be increased up to 98.5% if assay time was reduced to the initial 1 min of tilting, suggesting that these false positives could be eliminated with further modification of the assay. Moreover, nonreacting tests at 1 min did not have high levels of neutralizing antibodies, meaning that those individuals may have decreased protection anyway.

Among other limitations, our hemagglutination test on a dry card is also limited by the inability to distinguish between IgG, IgM, and IgA, though it may be useful to take into all antibody isotypes when assessing total neutralizing antibody levels. A further limitation of our approach is that it requires visual interpretation by the user, which may be variable. To solve this, automated visual algorithms leveraging cameras on phones could be used. One study has explored the potential for interpreting strong and weak lines from lateral flow assays to correlate with antibody levels (26), but such a strategy has not been translated into commercial instructions and is visually more subtle than the agglutination results presented here.

In summary, we have developed a new platform for SARS-CoV-2 antibody detection that is faster than current point-of-care devices and offers semiquantitative information.

The simplicity and low cost of the assay could enable its widespread use and a range of applications that include testing in low-resource settings, determination of serostatus prior to vaccination, postvaccination surveillance, and travel screening.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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R.L.K, Y.H., and Z.Z.W. are coinventors on a patent application related to the fusion protein detection agent for SARS-CoV-2. We recruited Eldon Biologicals for the collaboration, but the data and conclusions here are fully the authors' and independent from Eldon Biologicals. Beyond testing cards, no formal sponsored research funding was received from Eldon Biologicals.

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