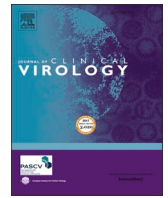




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## Short communication

## Evaluation of SARS-CoV-2 total antibody detection via a lateral flow nanoparticle fluorescence immunoassay

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## ABSTRACT

**Background:** The coronavirus disease 2019 (COVID-19) endgame may benefit from simple, accurate antibody testing to characterize seroprevalence and immunization coverage.

**Objectives:** To evaluate the performance of the lateral flow QIAreach anti-SARS-CoV-2 Total rapid nanoparticle fluorescence immunoassay compared to reference isotype-specific IgG, IgM, and IgA SARS-CoV-2 ELISA using S1 or receptor binding domain (RBD) as antigens.

**Study design:** A diagnostic comparison study was carried out using 154 well-characterized heparin plasma samples. Agreement between assays was assessed by overall, positive, and negative percent agreement and Cohen's kappa coefficient.

**Results:** Overall agreement between the QIAreach anti-SARS-CoV-2 Total and any anti-spike domain (S1 or RBD) antibody isotype was 96.0 % (95 % CI 89.8–98.8), the positive percent agreement was 97.6 % (95 % CI 91.0–99.9), the negative percent agreement was 88.2 % (95 % CI 64.4–98.0). The kappa coefficient was 0.86 (95 % CI 0.72 to 0.99).

**Conclusion:** The QIAreach anti-SARS-CoV-2 Total rapid antibody test provides comparable performance to high-complexity, laboratory-based ELISA.

### 1. Introduction

Efforts to understand and control the coronavirus disease 2019 (COVID-19) pandemic have led to the detailed characterization of the humoral response to SARS-CoV-2 infection. At a median of approximately 2 weeks after onset of symptoms, specific IgM, IgG and IgA antibodies become detectable in blood [1,2]. Antibody titers peak at around 1 month post symptom onset, and then decrease, relatively rapidly for IgM and IgA, and more gradually for IgG [3]. In vaccine licensing studies, SARS-CoV-2 immunization elicits robust antibody responses and at least short-term protection from natural infection [4–6].

SARS-CoV-2 antibody testing is recommended for the evaluation of patients with a high clinical suspicion of infection and repeatedly negative nucleic acid amplification tests, as well as in the assessment of suspected multisystem inflammatory syndrome in children [7,8].

SARS-CoV-2 antibody testing is also a critical public health tool, enabling surveillance efforts to characterize seroprevalence and vaccine coverage.

Methods for SARS-CoV-2 antibody detection target various viral antigens and include laboratory-based testing, such as enzyme-linked immunosorbent assays (ELISA), as well as rapid, lateral flow immunoassays (LFIA) that also may be used at the point-of-care. These rapid assays provide a low-throughput antibody testing option for laboratories with limited resources and are particularly useful for epidemiologic field studies. However, a meta-analysis evaluating the diagnostic accuracy of SARS-CoV-2 serologic testing concluded that LFIAs were consistently less sensitive than ELISA or CLIA methods [9], and subsequent studies have reported a wide range of sensitivities and specificities [10–24]. Nevertheless, the Infectious Diseases Society of America (IDSA) recommends against the use of IgG or IgM antibody combination tests, where

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detecting either antibody isotype is used to define a positive result [7]. Notably, of the LFIA that have obtained FDA emergency use authorization, 87.5 % (14/16) are combination tests [25].

In this study, well-characterized clinical plasma specimens were utilized to evaluate a SARS-CoV-2 total antibody (IgG, IgM, IgA) nanoparticle fluorescence immunoassay (QIAreach anti-SARS-CoV-2 Total) that uses as antigen the spike protein S1 domain, which also contains the angiotensin converting enzyme-2 (ACE2) receptor binding domain (RBD).

## 2. Methods

### 2.1. Patient consent statement

This study was approved by the Stanford Institutional Review board (IRB protocol #48973). Per IRB assessment, informed consent was waived for this study.

### 2.2. Reference ELISA testing

Isotype-specific IgG, IgM, and IgA SARS-CoV-2 S1 and RBD ELISAs were performed manually as previously described, as was a competition ELISA to detect antibodies blocking binding of ACE2 to RBD [1]. Pre-pandemic samples were tested using automated versions of the SARS-CoV-2 RBD IgG and IgM ELISAs on the Quanta-Lyser ESP600 (Innova Diagnostics, Inc. San Diego, CA).

### 2.3. Sample selection

Archived heparin plasma samples (n = 100) collected from fifty-eight SARS-CoV-2 reverse transcription – polymerase chain reaction (RT-PCR) positive patients and tested by isotype-specific IgG, IgM, and IgA SARS-CoV-2 S1 and RBD manual ELISAs as well as the RBD-ACE2 blocking assay, were selected to encompass a range of OD values, patterns of isotype reactivity, and blocking activity. Pre-pandemic heparin plasma samples (n = 42) negative by automated SARS-CoV-2 RBD IgG and IgM ELISAs were used to evaluate specificity. S1 and RBD IgG, IgA, and IgM negative heparin plasma samples (n = 12) with IgM plastic binding activity were also included in specificity experiments.

### 2.4. Lateral flow nanoparticle fluorescence immunoassay

QIAreach anti-SARS-CoV-2 Total Test (Qiagen, Germantown, MD) was performed according to the manufacturer's instructions. Briefly, the Access eHub was connected to a power source via USB. Next, a Processing Tube and eStick were inserted into the eHub. 300 µL of Diluent Buffer was transferred into the Processing Tube, followed by 50 µL of the heparin plasma sample. Using a pipette set to 150 µL, the sample was mixed at least 4 times in the Processing Tube, and then 150 µL of the mixture was added to the eStick. A result displayed within 3–10 min (180–600 s). Time to result in seconds was recorded.

### 2.5. Statistics

Overall percent agreement, positive percent agreement (PPA), negative percent agreement (NPA), and Cohen's kappa coefficient with associated 95 % confidence intervals (95 % CI) were calculated using GraphPad online. Cohen's kappa values were interpreted according to Landis and Koch [26].

## 3. Results

The specificity of the QIAreach Total was evaluated using 54 clinical samples, including 42 pre-pandemic samples, as well as 12 post-pandemic samples negative for S1- or RBD-binding IgG, IgM or IgA, but with IgM plastic-binding activity. False positive QIAreach Total

results were detected in 4.8 % (2/42) of pre-pandemic samples and 8.3 % (1/12) of post-pandemic samples, for an overall specificity of 94.4 % (51/54) (95 % confidence interval 84.3–98.7). The QIAreach time to result for each of these three false positive samples was 600 s (10 min).

The QIAreach Total was further evaluated using 100 archived plasma specimens from 58 SARS-CoV-2 RT-PCR positive patients. These included 83 samples positive for any S1 or RBD antibody isotype and 17 samples negative for S1 or RBD antibodies. 56.6 % (47/83) of the positive samples had RBD-ACE2 receptor blocking activity with a median of 21 % [interquartile range (IQR) 4–57] blocking. The median number of days since onset of symptoms was 13 days, IQR 8–21, and overall range 0–150 days (Table 1).

Overall percent agreement between the QIAreach Total and ELISAs detecting any anti-S1 antibody isotype was 91.0 % (95 % CI 83.6–95.4). The PPA was 98.7 % (95 % CI 92.2–100.0) and the NPA was 66.7 % (95 % CI 46.6–82.2). The kappa coefficient was 0.73 (95 % CI 0.56 to 0.89), indicating substantial agreement (Table 2). When the QIAreach Total was compared with specific anti-S1 antibody isotypes (IgG, IgM, IgA) the NPA ranged from 29.8–57.1%. Similar performance was observed when the QIAreach Total was compared to the anti-RBD ELISAs (Table 2).

The performance of the QIAreach Total was then assessed using detection of any anti-spike domain (S1 or RBD) antibody isotype as reference. The overall percent agreement was 96.0 % (95 % CI 89.8–98.8), the PPA was 97.6 % (95 % CI 91.0–99.9), and the NPA was 88.2 % (95 % CI 64.4–98.0). The kappa coefficient was 0.86 (95 % CI 0.72 to 0.99) indicating near perfect agreement. Of the concordant positive samples, the median QIAreach Total time to result was 190 s (IQR 185–215). Time to result was not linearly related to ELISA OD values (Figure S1).

This analysis revealed four discrepant results (Table S1). Two of the samples were reproducibly QIAreach Total negative / ELISA positive. For these patients, samples collected six and two days later, respectively, were positive by both QIAreach and ELISA, suggesting that the original samples were falsely QIAreach Total negative. The two other discrepant samples were reproducibly QIAreach Total positive / ELISA negative. Similarly, for one of these patients, a sample collected two days later was positive by both QIAreach Total and ELISA, revealing that the original sample was likely falsely ELISA negative. The other QIAreach Total positive / ELISA negative sample was collected two days after the reported onset of symptoms. The interpretation here is challenging, because subsequent samples were not available to help resolve these discrepant results. Typically, antibodies do not develop at this early timepoint, however, the reported day of symptom onset may be inaccurately reported, or symptom onset might be accurate, but unusually late in the true course of infection.

## 4. Discussion

In this work, the QIAreach anti-SARS-CoV-2 Total antibody lateral flow nanoparticle fluorescence immunoassay demonstrated comparable

**Table 1**  
Demographic characteristics of 58 SARS-CoV-2 RT-PCR positive individuals.

|  | Overall (n = 58) |
|--|------------------|
| Age, median (IQR)                                  | 59 (41–72)       |
| Sex, no. (%)                                       |                  |
| Male   | 28 (48.3)        |
| Female   | 30 (51.7)        |
| Outpatients, no. (%)                               | 6 (10.3)         |
| Admitted, non-ICU, no. (%)                         | 22 (37.9)        |
| Admitted, ICU, no. (%)                             | 30 (51.7)        |
| Deceased, no. (%) <sup>*</sup>                     | 16 (27.6)        |
| Number of samples tested per patient, median (IQR) | 2 (1–2)          |
| Days of symptoms, median (IQR)                     | 13 (8–23)        |

IQR, interquartile range; ICU, intensive care unit.

<sup>\*</sup> Deceased patients comprised 3 admitted, non-ICU, and 13 admitted, ICU patients.

**Table 2**  
Agreement of QIAreacH Total with Spike S1 and Receptor Binding Domain Isotype-Specific ELISA.

| ELISA                 | QIAreacH Total  |                 |                | PPA        | PPA 95 % CI    | NPA       | NPA 95 % CI         | Cohen's Kappa (95 % CI) |
|-----------------------|-----------------|-----------------|----------------|------------|----------------|-----------|---------------------|-------------------------|
|                       | Overall         | Overall 95 % CI | PPA            |            |                |           |                     |                         |
| S1 IgG                | 87.0 % (87/100) | 78.9–92.4       | 98.6% (71/72)  | 91.8–100.0 | 57.1 % (16/28) | 39.0–73.5 | 0.63 (0.46 to 0.81) |                         |
| S1 IgM                | 60.0 % (60/100) | 50.2 to 69.1    | 100.0%(43/43)  | 90.2–100.0 | 29.8% (17/57)  | 19.5–42.7 | 0.27 (0.15 to 0.39) |                         |
| S1 IgA                | 64.0 % (64/100) | 54.2 to 72.7    | 100.0%(47/47)  | 91.0–100.0 | 32.1% (17/53)  | 21.0–45.5 | 0.31 (0.18 to 0.44) |                         |
| Any S1 Isotype        | 91.0 % (91/100) | 83.6–95.4       | 98.7 % (75/76) | 92.2–100.0 | 66.7 % (16/24) | 46.6–82.2 | 0.73 (0.56 to 0.89) |                         |
| RBD IgG               | 85.0 % (85/100) | 76.6–90.8       | 98.6% (69/70)  | 91.6–100.0 | 53.3% (16/30)  | 36.1–70.0 | 0.59 (0.42 to 0.77) |                         |
| RBD IgM               | 71.0 % (71/100) | 61.4 to 79.0    | 100.0%(54/54)  | 92.1–100.0 | 37.0% (17/46)  | 24.5–51.4 | 0.39 (0.24 to 0.54) |                         |
| RBD IgA               | 79.0 % (79/100) | 70.0–85.9       | 98.4% (63/64)  | 90.9–100.0 | 44.4% (16/36)  | 29.5–60.4 | 0.49 (0.31 to 0.66) |                         |
| Any RBD Isotype       | 95.0 % (95/100) | 88.5–98.1       | 97.6 % (80/82) | 91.0–99.9  | 83.3% (15/18)  | 60.0–95.0 | 0.83 (0.68 to 0.97) |                         |
| Any S1 or RBD Isotype | 96.0 % (96/100) | 89.8–98.8       | 97.6 % (81/83) | 91.1–99.9  | 88.2 % (15/17) | 64.4–98.0 | 0.86 (0.72 to 0.99) |                         |

CI, confidence interval; PPA, positive percent agreement; NPA, negative percent agreement; S1, spike protein S1 domain; RBD, spike protein receptor binding domain.

performance to ELISA using a set of extensively characterized plasma specimens from individuals of known COVID-19 status.

The high level of agreement observed in this study is consistent with the performance reported by the manufacturer [27], and significantly expands upon the number of clinical specimens used in the previous evaluation. In contrast, the pooled sensitivity calculated in a meta-analysis of other anti-SARS-CoV-2 LFIA was 66.0 % (95 % CI: 49.3–79.3) [9]. Future work will be required to directly compare the QIAreacH Total to other LFIAs, and to assess its performance at the point-of-care with finger-stick blood specimens.

The transition to point-of-care testing should be straightforward as the QIAreacH Total is simple to perform, with minimal hands-on-time. Whereas conventional LFIAs demonstrate faint banding that may be difficult to interpret and is subject to low inter-rater reliability [14,21,23], QIAreacH Total provides digital qualitative results without the requirement for visual interpretation of band reactivity. The results are also available rapidly; the QIAreacH Total exhibited a median time to result of 3 min and 10 s. The time to result, however, is not linearly associated with ELISA OD values and the test, in its current design, should not be used in a semi-quantitative manner.

The major strength of this study is the use of a sample set comprised of a range of ELISA OD values, patterns of isotype reactivity, and RBD-ACE2 receptor blocking activity from patients with differing levels of disease severity. Limitations include its single-center, retrospective design, the relatively small number of samples collected more than 30 days after the onset of symptoms, and the use of archived plasma rather than finger-stick blood specimens.

In summary, the QIAreacH SARS-CoV-2 Total assay demonstrates ELISA-level performance with the simplicity of an LFIA. It is suitable for IDSA-recommended clinical indications as well as public health surveillance.

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## Declaration of Competing Interest

Qiagen provided the QIAreacH anti-SARS-CoV-2 Total Tests and the Access eHub used in this study (B.A.P.). Qiagen had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## 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.jcv.2021.104818>.

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