



BRIEF REPORT

Preliminary Evaluation of QuantiFERON SARS-CoV-2 and QIAreach Anti-SARS-CoV-2 Total Test in Recently Vaccinated Individuals

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ABSTRACT

Introduction: There is an increasing body of evidence surrounding the importance of a T cell-mediated response to SARS-CoV-2 infection and after COVID-19 vaccination. In this internal feasibility study, we evaluated both the total antibody (IgA, IgM, and IgG) and T cell responses in a cohort of COVID-19 convalescents and vaccinated individuals.

Methods: Whole blood specimens were collected weekly from 12 subjects at different time points within/after the COVID-19 mRNA vaccination regimen, and from 4 PCR-confirmed convalescent donors to measure durability of humoral and cell-mediated immune response. T cell and antibody responses were evaluated via the QuantiFERON SARS-CoV-2 research use only (QFN SARS-CoV-2) assay which is an

interferon gamma release assay (IGRA) and QIAreach Anti-SARS-CoV-2 total (Anti-CoV-2) test, respectively.

Results: In a cohort of recently vaccinated individuals, subjects demonstrated robust total antibody and CD4+/CD8+ T cell response to SARS-CoV-2 mRNA vaccines when followed for 2 months post-2nd dose. In most individuals, T cell response declined between the 1st and 2nd doses suggesting a need for a booster or the completion of the 2-dose vaccine series. In a group of convalescent donors tested with QFN SARS-CoV-2 and Anti-CoV-2 tests, all patients had an antibody and T cell response up to 1 year after natural infection.

Conclusion: This small feasibility study demonstrates that the QFN-SARS-CoV-2 test is able to identify CD4+ and CD8+ T cell-mediated responses in SARS-CoV-2-vaccinated subjects and those recovered from COVID-19, alongside a qualitative antibody response detectable via the QIAreach Anti-CoV2 test.

Keywords: Adaptive immunity; Cell-mediated immunity; COVID-19; QuantiFERON; SARS-CoV-2; T cell-mediated response; Vaccination

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Key Summary Points

Why carry out this study?

There is an increasing body of evidence surrounding the importance of a T cell-mediated response to SARS-CoV-2 infection, with evidence suggesting that a T cell response is sustained for several months after infection and potentially lasting longer than the antibody response.

This was an internal feasibility study to evaluate both the total antibody (IgA, IgM, and IgG) response and T cell response in a cohort of mRNA COVID-19-vaccinated individuals and PCR-confirmed convalescent donors.

What was learned from this study?

In this small cohort of recently vaccinated individuals, subjects demonstrated robust total antibody and CD4/CD8 T cell response to SARS-CoV-2 when followed for 2 months post-2nd dose.

In most individuals, T cell response declined between 1st and 2nd doses, suggesting a need for a booster or the completion of the 2-dose mRNA vaccine series.

In a subset of convalescent donors tested with QuantiFERON SARS-CoV-2 RUO tubes and QIAreach Anti-SARS-CoV-2 total antibody, all patients had an antibody and T cell response even 1 year after natural infection.

durable immunity to SARS-CoV-2 are a crucial component of the strategy to combat COVID-19 [2]. To achieve the desired durable immunity, a vaccine should not only evoke a robust production of neutralizing antibodies (nAb) but also evoke a strong CD4+ and CD8+ T cell response [3].

There is an increasing body of evidence surrounding the importance of a T cell-mediated response to SARS-CoV-2 infection. Peripheral T cell lymphopenia appears to correlate with increased COVID-19 disease severity [4], while COVID-19 recovery is often associated with the presence of reactive CD4+ and CD8+ T cells [5, 6]. Robust evidence on long-term immunity is lacking, but it appears likely that a T cell response is sustained for several months after infection and may last longer than a detectable antibody response [7, 8]. Past experience from the 2003 SARS-CoV-1 epidemic supports this, as initial specific immunoglobulin (Ig)G and nAb response to SARS-CoV-1 diminished 2–3 years after infection [9], while T cell memory was detected up to 11 years after infection [10]. Evidence also indicates that the T cell response is less likely to be affected by SARS-CoV-2 variants than the antibody response [11, 12]. In a recent study, Geers et al. demonstrated that the B.1.1.7 and B.1.351 variants of concern were able to partially evade humoral immunity, but that CD4+ T cell activation was unaffected [12].

In addition to the importance of T cell response to natural SARS-CoV-2 infection, there is a significant need to research T cell responses to COVID-19 vaccination. In phase 1 and 2 trials, the mRNA-1273, BNT162b2, ChAdOx1 and Ad26.COVS vaccines were all associated with a specific T cell response to vaccination, at time points ranging from 7 to 43 days after first vaccination dose [13–16]. Kalimuddin recently reported a T cell response measured by IFN- γ secretion in 19/20 subjects 10 days after a single dose of the BNT162b2 vaccine, before a nAb response was detected [17]. This led the authors to conclude that an early T cell response in vaccinated individuals may play a greater role than nAb in generating early protection against COVID-19 [17]. Interestingly, Parry recently reported a detectable T cell response in 12.3%

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic caused by the novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has resulted in significant global morbidity and mortality, with far-reaching health and economic implications [1]. Vaccines that provide

and 30.7% of older donors (≥ 80 years), 5 weeks after a single dose of the BNT162b2 or ChAdOx1 vaccines, respectively [18], suggesting that T cell responses may vary depending on vaccine, and potentially may be reduced in older populations.

Therefore, in this small internal feasibility study, we evaluated both the total antibody (IgA, IgM, and IgG) response and T cell response in a cohort of COVID-19 vaccinated individuals and convalescent donors. We hypothesized that SARS-CoV-2 mRNA-vaccinated subjects will generate specific CD4+ and CD8+ T cell-mediated responses, detectable using the QuantiFERON® SARS-CoV-2 research use only (QFN SARS-CoV-2) assay. The assay is based on the same platform as the QuantiFERON-TB Plus, which is a well-established diagnostic aid for tuberculosis disease [19]. In recent publications, good correlation between cellular responses detected by QFN SARS-CoV-2 and other assays has been demonstrated [20, 21]. Additional hypotheses for this study were that the T cell response will be detectable earlier than the antibody response, as measured using the QIArearch Anti-SARS-CoV-2 total antibody (Anti-CoV2) test, and that higher responses may be seen after a booster dose compared with the initial vaccination dose.

METHODS

Samples

Consenting subjects taking part in a COVID-19 mRNA vaccination regimen provided whole blood specimens, collected weekly in lithium heparin tubes, immediately after the first vaccination dose and until at least 6 weeks following the second vaccination dose. Convalescent plasma was obtained from four donors who recovered from PCR-confirmed COVID-19. All specimens were shared between the QFN SARS-CoV-2 assay and the Anti-CoV2 test.

Antibody response was measured using the Emergency Use Authorization (EUA) granted Anti-CoV2 test, a digital lateral flow serological test using patented nanoparticle fluorescence technology that qualitatively detects total

antibodies to SARS-CoV-2 in human serum and plasma. For this assay, the specimens in lithium heparin tubes were first centrifuged for 15 min at 2500 RCF to separate plasma from cells and facilitate sample harvesting. For each subject specimen, plasma was harvested from the lithium heparin tube and run with Anti-CoV2 according to the instructions for use. Qualitative test results and time-to-result for Anti-CoV2-positive subjects were recorded.

The T cell response was evaluated via the QFN SARS-CoV-2 assay which is an interferon gamma release assay (IGRA). This assay consists of three antigen tubes, SARS-CoV-2 Ag1, Ag2 and Ag3, that use a combination of proprietary antigen peptides specific to SARS-CoV-2 to stimulate lymphocytes involved in cell-mediated immunity in heparinized whole blood. The QFN SARS CoV-2 Ag1 tube contains CD4+ epitopes derived from the S1 subunit (Receptor Binding Domain) of the Spike protein, the Ag2 tube contains CD4+ and CD8+ epitopes from the S1 and S2 subunits of the Spike protein, and the Ag3 tube consists of CD4+ and CD8+ epitopes from S1 and S2, plus immunodominant CD8+ epitopes derived from whole genome. Plasma from the stimulated samples can be used for the detection of IFN- γ using an enzyme-linked immunosorbent assay (ELISA)-based platform. Specimens were processed as per the manufacturer's guidelines [22–25]. Following ELISA, quantitative results (IFN- γ concentration in IU/ml) were recorded and used for analysis. Elevated response was defined as a value at least 0.20 IU/ml greater than the background IU/ml value from the QFN-SARS-CoV-2 Nil tube; the Nil tube value was subtracted to mitigate against background IFN- γ in the sample that was not a result of SARS-CoV-2-specific T cell stimulation. Median (min–max) Nil subtracted IFN- γ responses were plotted, and the median chosen to illustrate the central measurement of the dataset in which biological variation could skew results. Minimum and maximum values were provided to inform on the range of responses in addition to the central (median) value.

This study protocol and documentation was approved by Advarra, an independent Institutional Review Board. The trial was performed in

accordance with the Helsinki Declaration of 1964 and its later amendments. All subjects included in the study provided informed consent, and all blood collections and testing were performed by trained professionals at QIAGEN Sciences.

RESULTS

Subjects

In total, 16 subjects were included: 12 subjects who received two doses of mRNA-1273 SARS-CoV-2 vaccine and 4 subjects diagnosed with COVID-19. Among the 12 vaccinated subjects, 2 (16.7%) were male and 10 (83.3%) were female. The median age for vaccinated subjects was 44.5 years. Following vaccination, results were obtained at regular weekly intervals when possible, up to 70 days post-1st vaccination ($n = 3$), 74 days post-1st vaccination ($n = 8$) or 99 days post-1st vaccination ($n = 1$).

Among the 4 convalescent subjects, 3 (75%) were male and 1 (25%) was female. The median age for convalescent subjects was 53 years. The time between PCR-confirmed COVID-19 diagnosis and first plasma samples ranged from 1 to 11 months, and all subjects had confirmed resolution of COVID-19 symptoms prior to donating plasma; one subject reported no symptoms at the time of COVID-19 diagnosis (Table 1).

One negative control was included in the study, a non-vaccinated subject with non-reactive COVID-19 serology test results, which were obtained for this subject at regular intervals between January 26 and March 2, 2021. The

subject received Ad26.COVS.S COVID-19 vaccine (Johnson & Johnson, NJ, USA) on March 6, 2021. Results post-vaccination for this subject were not analyzed in this study.

QFN SARS-CoV-2 Assay in Vaccinated Subjects

An elevated IFN- γ response in at least one Ag tube was observed in the majority of vaccinated subjects (10/12) following first dose, in all subjects after the second dose, and at most subsequent time points (Fig. 1). Most subjects had elevated Ag responses sustained through the study, including one subject with a sample 70 days after a booster dose.

The median (min–max) Nil subtracted IFN- γ response for the 12 vaccinated subjects following initial vaccination dose and immediately prior to the booster dose (median 25 days, range 22–28 days) was 0.13 (–0.02 to 1.76), 0.20 (0.02–1.88) and 0.25 IU/ml (0.01–2.43) for Ag1, Ag2, and Ag3 tubes, respectively. The median (min–max) Nil subtracted Ag1, Ag2 and Ag3 IFN- γ responses at 4 weeks (median 32 days, range 28–32 days) post-second dose for 11 vaccinated subjects were 0.70 (–0.27 to 3.67), 0.90 (0.07–5.17), and 1.15 (0.11–5.75) IU/ml, respectively, compared with 0.00, 0.00, and 0.00 IU/ml in the non-reactive control subject. One of the vaccinated subjects was unavailable for testing at 4 weeks following the booster dose.

Following the initial mRNA-1273 vaccination dose, QFN SARS-CoV-2 responses peaked at 11–14 days, with median Nil subtracted responses of 1.29 (0.04–4.76), 3.46 (0.07–8.40),

Table 1 COVID-19 disease information convalescent subjects providing samples for testing on QFN-SARS-CoV-2

Subject ID	Symptom onset	COVID-19 diagnosis date	Symptom end
1	1/10/2021	1/5/2021	1/12/2021
2	12/28/2020	1/10/2021	1/22/2021
3	NA	3/9/2020	6/30/2020
4	9/25/2020	9/26/2020	10/20/2020

COVID-19 coronavirus disease 2019, QFN-SARS-CoV-2 QuantiFERON[®] SARS CoV-2 research use only assay

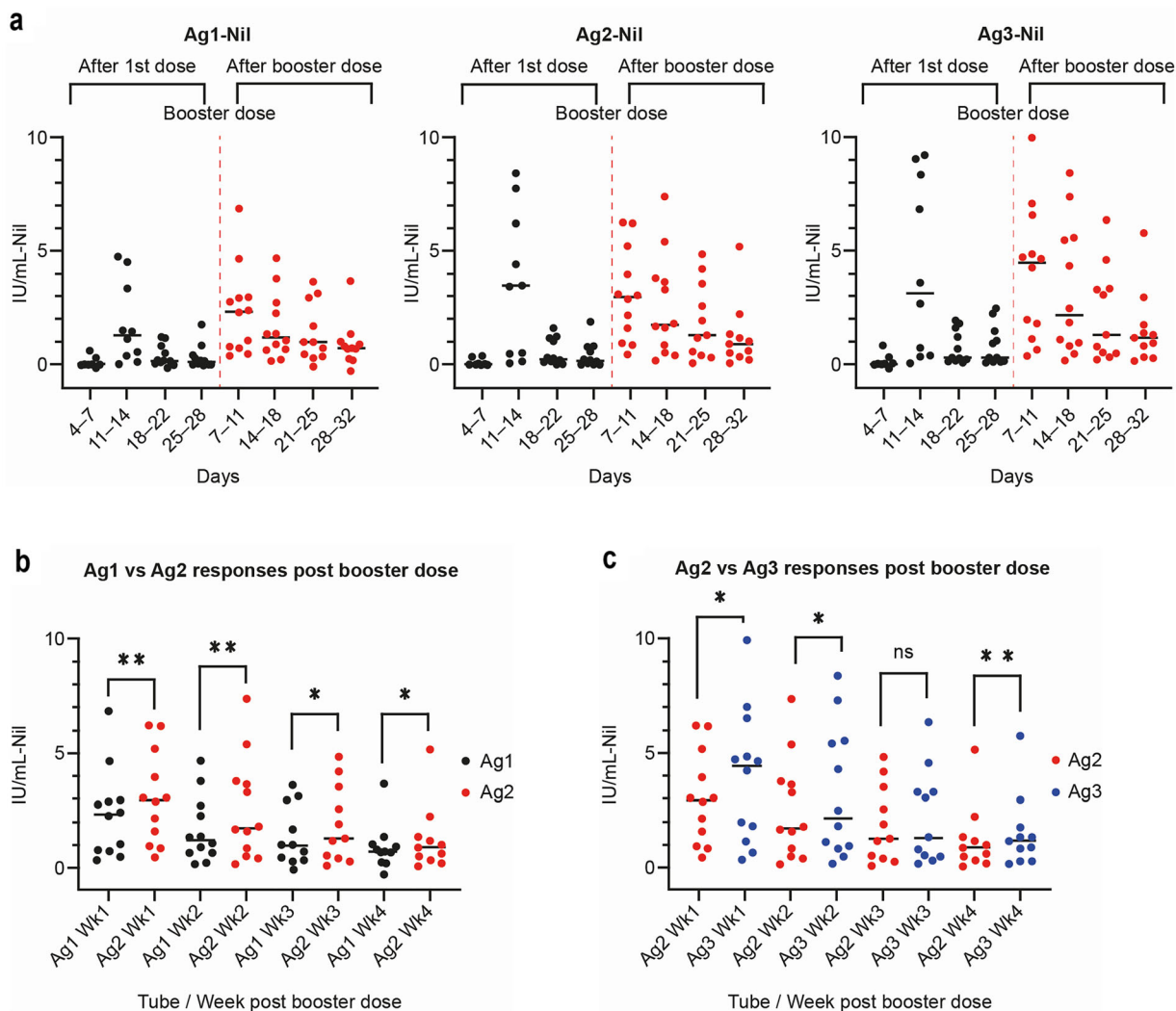


Fig. 1 Comparison of **a** longitudinal QFN-SARS-CoV-2 antigen tube (Ag minus Nil) response in subjects receiving both doses of the mRNA-1273 SARS-CoV-2 vaccine. Each date range contains a scatter plot with horizontal lines to represent median response. Number of subjects: 4–7 days (8), 11–14 days (10), 18–22 days (11), 25–28 days (11), 7–11 days (12), 14–18 days (12), 21–25 days (11), and 28–32 days (11). **b** QFN-SARS-CoV-2 Ag1-Nil and Ag2-Nil responses and **c** QFN-SARS-CoV-2 Ag2-Nil and Ag3-Nil responses in subjects vaccinated with the mRNA-1273 SARS-CoV-2 vaccine for four consecutive weeks post-booster dose. Week 1: 12 subjects, median 11 days (7–11), week 2: 12 subjects, median 18 days (14–18), week 3: 11 subjects, median 25 days (21–25), and

and 3.08 (0.03–9.17) IU/ml for Ag1, Ag2, and Ag3, respectively, then steadily decreased until reaching 0.10 (–0.02 to 1.76), 0.17 (0.02–1.88),

week 4: 11 subjects, median 32 days (28–32). Responses are shown in a scatter plot with horizontal lines to represent median response. Results from a Wilcoxon matched-pairs signed rank test are shown for each week, with ns signifying a *P* value > 0.05, ** a *P* value < 0.01, and * a *P* value between 0.01 and 0.05. **c** Longitudinal QFN-SARS-CoV-2 antigen tube (Ag minus Nil) response in subjects receiving both doses of the mRNA-1273 SARS-CoV-2 vaccine. Each date range contains a box plot with min–max range bars. Number of subjects: 4–7 days (8), 11–14 days (10), 18–22 days (11), 25–28 days (11), 7–11 days (12), 14–18 days (12), 21–25 days (11), and 28–32 days (11)

and 0.26 (0.01–2.43) IU/ml at 25–28 days immediately prior to, or just after, receiving the booster dose (Fig. 1a). QFN SARS-CoV-2

responses spiked again 7–11 days after the vaccine booster dose, with median Nil subtracted responses of 2.34 (0.38–6.84), 2.96 (0.46–6.22), and 4.42 (0.33–9.93) IU/ml for Ag1, Ag 2, and Ag3 tubes, respectively, then trended downwards to 0.70 (–0.27 to 3.67), 0.90 (0.07–5.17), and 1.15 (0.11–5.75) IU/ml at 28–32 days following the booster dose.

Across all vaccinated subjects, higher responses were observed in Ag3 tubes than in Ag2 tubes, and in Ag2 tubes than in Ag1 tubes. In a non-parametric Wilcoxon signed rank test comparison between paired Ag1-Nil and Ag2-Nil responses performed separately for each of 4 weeks following the vaccine booster dose, Ag2-Nil responses were consistently higher than Ag1-Nil responses (Fig. 1b). Likewise, for the same comparison between paired Ag3-Nil and Ag2-Nil responses, Ag3-Nil responses were higher for 3 of 4 weeks of testing the post-booster dose (Fig. 1c).

Longitudinally, no elevated responses were recorded on day 4 after the first dose, but all subjects had elevated responses by day 11 (Fig. 1a). Among two subjects with samples at

day 7 post-first dose, both had elevated (Nil subtracted) responses: 0.27/0.61, 0.39/0.33, and 0.29/0.80 IU/ml for the Ag1, Ag2, and Ag3 tubes, respectively.

QIAreach Anti-CoV2 Test in Vaccinated Subjects

All subjects had detectable SARS-CoV-2 antibodies, as measured in their last sample in the study; average time-to-result (TTR) was slower in earlier samples post-first dose (Fig. 2). Among seven subjects with consecutive day 4 and 11 samples post-first dose, there were no positive results at day 4, whereas all day 11 samples were subsequently positive; median TTR was 3.4 min. No antibody response was detected in the two subjects tested at day 7 post-first dose; both subjects had positive samples in their subsequent day 14 samples.

Convalescent Subjects

All samples provided by convalescent subjects were serologically positive by the QIAreach

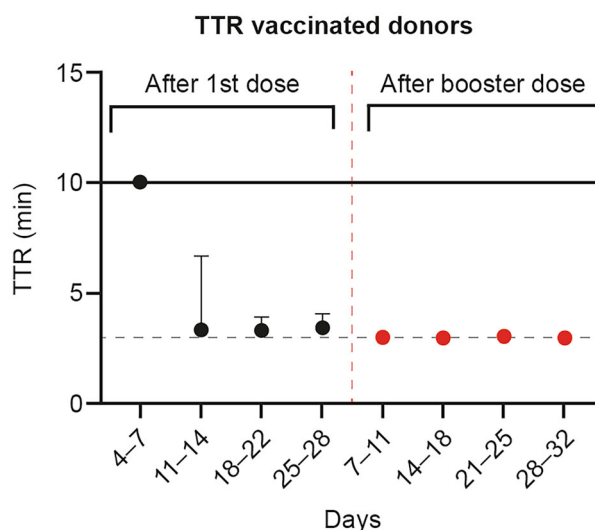


Fig. 2 QIAreach Anti-SARS-CoV-2 Total Test (Anti-CoV2) time-to-result (TTR) in subjects vaccinated with mRNA-1273 SARS-CoV-2 vaccine ($n = 12$). For Anti-CoV2 testing, a result will be determined between 3–10 min. All negative tests will have a 10-min TTR. Positive antibody test results (detecting a total antibody

response) will have a TTR between 3 and 10 min, with a lower TTR for higher levels of antibody detected by the device. All results following the 28–32 days post-vaccination are from serology samples collected following the vaccine booster dose. DOT represents median response with bars showing the interquartile range

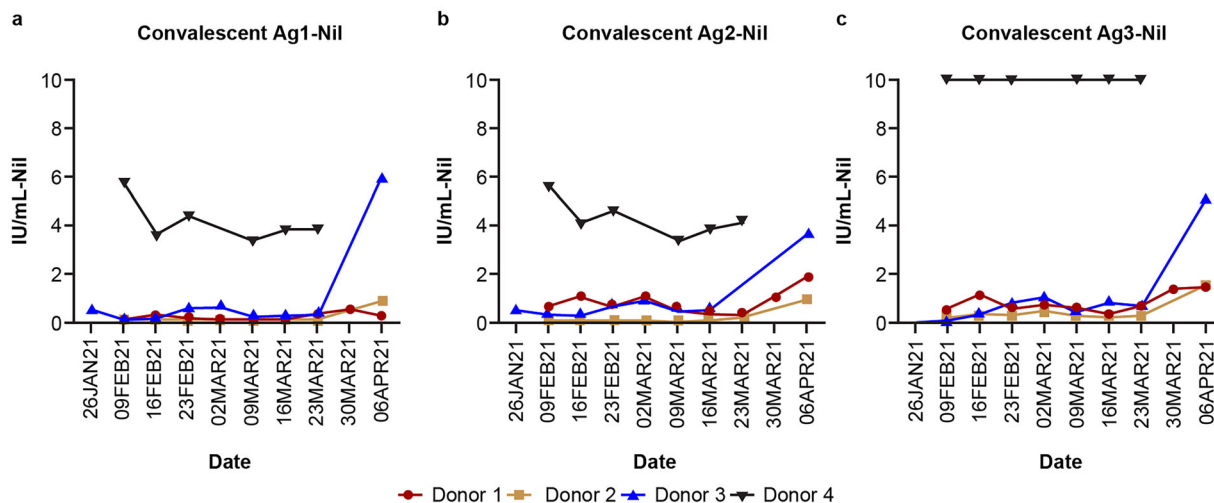


Fig. 3 QFN-SARS-CoV-2 responses (Ag minus Nil) in Ag1 (a), Ag2 (b) and Ag3 (c) antigen tubes from convalescent subjects with prior COVID-19 diagnosis ($n = 4$)

Anti-CoV2 Test at all test points, including initial baseline testing that occurred at least 1 month following COVID-19 diagnosis. Elevated QFN SARS-CoV-2 Ag1 and Ag2 IFN- γ responses were detected throughout sampling in 3/4 subjects, equating to approximately 1, 5, and 11 months post-COVID-19 diagnosis (see Table 1 for dates of COVID-19 diagnosis). IFN- γ responses over time were broadly level (Fig. 3). In one subject who had no detectable responses at the start of sampling, responses were elevated in Ag1 and Ag2 in the last two samples, following vaccination. All of the subject samples had elevated IFN- γ responses in Ag3.

DISCUSSION

In this small internal feasibility study, QFN SARS-CoV-2 assay detected both CD4+ and CD8+ T cell-mediated responses in whole blood samples from subjects vaccinated with mRNA-1273 SARS-CoV-2 vaccine. Elevated IFN- γ were observed in all three of the antigen tubes, suggesting that all are able to detect a T cell-mediated response. In a cohort of convalescent plasma subjects, 3/4 subjects had elevated IFN- γ responses.

In the majority of subjects, detectable antibody and elevated T cell response to vaccination occurred simultaneously. As TTR is directly

proportional to the amount of SARS-CoV-2 antibodies present (minimum TTR of 3 min for high antibody responses and 10 min for a low concentration antibody response), the median time to antibody results of 3.4 min at day 11 implies an elevated humoral response that occurred alongside the elevated T cell responses also detected on that day. However, antibody test results were negative for the two subjects tested at day 7, while Ag1, Ag2, and Ag3 responses were detected in both. This suggests that a T cell-mediated response to vaccination may occur prior to an antibody response, between day 5 and day 7. Phase 1 trials of the Moderna vaccine only assessed T cell response at days 1, 29, and 43 after initial vaccination [26], likely obtaining results several weeks after the T cell response was initiated.

Longitudinally, the QFN SARS-CoV-2 assay detected a decline in T cell-mediated response following the first vaccine dose, followed by a subsequent increase following second-dose vaccination. In multiple subjects, second-dose vaccination elicited an augmented Ag1, Ag2 and Ag3 response compared with the response to initial dose. Furthermore, an augmented response was seen in many of the Ag2 tubes compared with Ag1 tubes, indicating that both CD4+ and CD8+ T cells contributed to the T cell response detected. Similarly, the BNT162b2

vaccine has been shown to elicit strong CD4+ and CD8+ responses [14]. The Ag3 tube, which consisted of a selection of immunodominant peptides to the whole SARS-CoV-2 genome, demonstrated a slightly higher response compared to Ag1 or Ag2 in some individuals. However, this may be due to the selection of peptides included in the Ag3 tubes, as all subjects received Spike-based vaccines. In terms of a long-term humoral response, the consistently low TTR implies a strong and sustained total antibody response occurred throughout the study.

The results of this study are consistent with other studies investigating the immune response to SARS-CoV-2 infection. Several studies have demonstrated a T cell-mediated response to natural SARS-CoV-2 infection 3–5 days after the onset of symptoms [27, 28], which was estimated to be roughly 7 days after infection [29]. It has also been observed that, in some patients, particularly those with asymptomatic or mild SARS-CoV-2 infection, a T cell-specific response is detectable even in the absence of a detectable antibody response [28, 30, 31]. In addition, numerous vaccine studies have demonstrated a selective T cell response in patients vaccinated against SARS-CoV-2 [14, 15, 26]. Thus, Ewer reported that a T cell-mediated response was also detected as early as day 7, earlier than a serological response, in patients receiving the ChAdOx1 nCov-19 vaccine [15]. In a real-world study of care home residents and staff, 87% of COVID-19 naïve staff ($n = 15$) demonstrated a Th1 IFN- γ response 4 weeks after first vaccination with BNT162b2 [32]. These results were achieved using a cutoff point of 0.15 IU/ml to define a Th1 IFN- γ response, slightly lower than the 0.20 IU/ml used in this study to define an elevated T cell response. However, the T cell response observed in vaccinated, COVID-19-naïve residents was significantly lower than that observed in the staff (48%, $P < 0.001$). This suggests that older recipients of the BNT162b2 vaccine may generate a lower or delayed immunological response to initial vaccination, which warrants further investigation in future studies.

Other vaccine studies have also demonstrated that the T cell response following a booster vaccine dose is consistently higher than the response observed after initial vaccination [26]. In a Phase 1 trial of mRNA-1273 SARS-CoV-2 vaccine, 45 patients were vaccinated on day 1 and received a booster dose 28 days later, on day 29. The T cell response detected on day 43 was frequently higher than the response detected on day 29 [26].

In our current study, T cell response was observable for the duration of the study, including, in one subject, 70 days post-second dose of vaccine. Similarly, sustained T cell responses were observed in convalescent plasma from subjects up to 11 months post-infection. Long-term data on T cell responses post-vaccination are currently lacking. A recent study demonstrated that T cell responses detected in previously-infected individuals after one vaccine dose was equivalent to naïve individuals receiving two vaccine doses [33], suggesting that a long-lasting T cell response occurs from natural infection. This would appear to match the results of our current study, where a prolonged T cell response was observed following booster vaccination in vaccinated subjects.

Hypothetically, assessing the speed and strength of T cell responses to a SARS-CoV-2 vaccine, using diagnostic tools such as the QFN SARS-CoV-2 could potentially provide more insight into vaccine efficacy. Identifying patient populations who demonstrate a reduced T cell response to vaccination or infection may help to predict which patients are most at risk of severe disease. Conversely, detection of T cell response could be particularly useful in selected populations of patients known to have impaired nAb/B cell functionality. For example, Soresina et al. described two patients with X-linked agammaglobulinemia, who, despite presenting with pneumonia after contracting COVID-19, did not require oxygen supplementation [34], indicating that cellular response may limit disease severity. Similarly, a study of patients with impaired humoral immunity due to hematologic cancer found that a CD8+ T cell count was associated with increased survival and lower viral load [35]. In vulnerable patients such as these, identifying specific T cell responses to

vaccination could improve our understanding of vaccine efficacy and aid in stratifying patients by risk of severe disease.

Both antibody and T cell responses decline after initial peaks following infection and vaccination [29], leaving uncertainty over the duration of immunity that vaccines provide. In the months following vaccination, QFN SARS-CoV-2 could be used to detect T cell responses even when antibody levels are low or undetectable. As it appears likely that prior COVID-19 protects against reinfection, even in the absence of detectable antibodies [36], testing for T cell response to vaccination could provide clearer information on vaccine-generated immunity, as opposed to testing antibody response alone. Furthermore, over a year into the COVID-19 pandemic, emerging vaccine-resistant variants are a concern, and will likely require the adaptation of existing vaccines or the development of new vaccines [37, 38]. It is possible that T cell response is less likely to be affected by virus variants than antibody response [11, 12], highlighting the potential utility of measuring T cell responses in identifying promising vaccine candidates for the future.

Comparison of the QFN SARS-CoV-2 platform with other T cell assays was outside the scope of this study. However, good correlation between cellular responses detected by QFN SARS-CoV-2 and other platforms has been observed in other recent publications: one study using two different readout assays (ELISA and CLIA) [20] and another which measured by alternative by alternative antibody assay [21]. At the time of writing, and to the best of our knowledge, the only commercially available assay is the T detect assay, which is not authorized for determination of adaptive immunity post-vaccination.

This study is limited by its small testing cohort, frequency of sample collection, and relatively short duration. While the study does demonstrate the ability of QFN SARS-CoV-2 to generate an elevated T cell response, the small number of subjects makes it difficult to draw strong conclusions based on these results. Furthermore, only one negative control subject was included, showing no response in Ag1/Ag2/Ag3.

However, in a study of healthcare workers prior to vaccination, all had values < 0.12 IE/ml, which would not have been classed as elevated responses in our study [20]. Another limitation of our study is the assumption that increased Ag2 responses compared with Ag1 were exclusively due to CD8+ T cells, when S2 also stimulates Ag2. However, S2 has been found to stimulate both CD4+ and CD8+ T cells [39], providing confidence that CD8+ T cell responses were elicited independently of S2. Additionally, one of the key questions surrounding SARS-CoV-2 vaccines is the duration of the immunity generated. While our results suggest that QFN SARS-CoV-2 is a viable method of quantifying T cell response, even in those whose antibody response may have waned over time, further studies to evaluate T cell responses several months after vaccination are warranted. Finally, this study only investigated T cell responses to one mRNA SARS-CoV-2 vaccine (mRNA-1273), meaning that conclusions cannot be drawn more widely for other vaccines.

CONCLUSIONS

This small feasibility study demonstrates that QFN SARS-CoV-2 test is able to identify CD4+ and CD8+ T cell-mediated responses in SARS-CoV-2-vaccinated subjects, alongside a qualitative antibody response detectable via the QIAreach Anti-CoV2 Test. While the T cell and antibody response occurred simultaneously in most subjects, this study suggests that T cell responses may occur prior to an antibody response in some vaccinated subjects. The importance of a T cell-mediated response to vaccination has recently received more recognition and QFN SARS-CoV-2 offers an easy to use, viable method to assess this in vaccinated patients.

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Authorship Contributions. All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Souyma Jaganathan, Francis Stieber, Nadia Allen and Jenny Howard. The first draft of the manuscript was written by Sonia Rao and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Disclosures. Souyma Jaganathan is a former employee of QIAGEN Sciences Inc, Nadia Allen is an employee of QIAGEN Sciences Inc, Francis Stieber is an employee of QIAGEN Sciences Inc, Sonia N. Rao is an employee of QIAGEN Inc, Vladyslav Nikolayevskyy is an employee of QIAGEN Manchester Ltd, Davide Manissero is an employee of QIAGEN Manchester Ltd, Jeff Boyle is a former employee of QIAGEN Sciences Inc, Germantown, MD and Jenny Howard is an employee of QIAGEN Sciences Inc.

Compliance with Ethics Guidelines. This study protocol and documentation was approved by Advarra, an independent Institutional Review Board. The trial was performed in accordance with the Helsinki Declaration of

1964 and its later amendments. All subjects provided informed consent to participate in the study.

Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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