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Journal of Virological Methods 302 (2022) 114472

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Accuracy of interferon gamma release assays for the COVID-19 immunity assessment

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ARTICLE INFO

Keywords:
SARS-CoV-2
IGRA
T-cell immunity

ABSTRACT

Emerging evidence suggests that T-cells play a significant role in COVID-19 immunity both in the context of natural infection and vaccination. Easy to use IGRA assays including QFN SARS are considered attractive alternatives to more "traditional" but laborious methods for detection of SARS-CoV-2-specific T-cell responses.

In our Letter we are proposing explanations to an apparently lower than expected T-cell responses (44 % reactive individuals) reported by Krüttgen et al in a small cohort of healthy double vaccinated individuals. These results could have been affected by reporting raw optical density values instead of calculated Interferon-ɣ concentrations which is supported by unexpectedly low mitogen responses in healthy individuals.

This study highlights an importance of adhering to good laboratory practice principles as well as overall importance of accurate T-cell immunity assessment using IGRA assays.

Dear Editor

We read with interest the paper by Krüttgen et al., *Evaluation of the QuantiFERON SARS-CoV-2 interferon-ɣ release assay in mRNA-1273 vaccinated health care workers,* (Krüttgen et al., 2021) evaluating cellular and humoral immune responses in a cohort of 18 healthy healthcare workers tested within 4 weeks after two doses of mRNA-1273 vaccine. Krüttgen et al. found that all vaccinees have successfully mounted humoral immune responses (assessed using binding anti-Spike IgG and neutralizing antibody assays) after mRNA-1273 vaccination while only 44 % of subjects returned a QuantiFERON SARS-CoV-2 RUO (QFN SARS) Ag1 or Ag2 response > 0.15 IU/mL.

Emerging evidence suggests that T-cells play a significant role in COVID-19 immunity both in the context of natural infection and vaccination (Tarke et al., 2021; Goletti et al., 2021; Moor et al., 2021). Cellular immunity has attracted more attention recently in light of the emergence of new Variants of Concern (VoC) and its potential role in protection of individuals with significantly impaired B cell immunity (Moor et al., 2021; Geers et al., 2021). Simple laboratory tests including interferon-ɣ release assays (IGRAs) have shown promise in evaluating the magnitude, durability, and other characteristics of T-cell responses essential for better understanding of the protection offered by COVID-19 vaccination especially in vulnerable populations (Goletti et al., 2021; de Vries et al., 2021).

In the Krüttgen et al. study, T-cell response was substantially lower than in multiple similar studies measuring T-cell responses to mRNA-1273 vaccination in healthy individuals using the QFN SARS assay that demonstrated consistently high (>90 %) proportions of reactive subjects (Jaganathan et al., 2021; Sablerolles et al., 2021; Martinez-Gallo et al., 2021). Importantly, even in clinically vulnerable populations, including patients with chronic kidney disease, those on hemodialysis and nursing home residents, proportions of reactive subjects as well as the magnitude of T-cell responses were higher compared to those in healthy individuals reported by Krüttgen et al. Of note, Tychala et al. reported 100 % T-cell reactivity in a small cohort of healthy HCW with high anti-Spike IgG titers while cellular responses were lower in those with low (<4000 AU/mL) humoral responses (Tychala et al., 2021). Review of the available data from Krüttgen et al. raises questions around interpretation of the QFN SARS test data.

Per the authors, the study enrolled 18 healthcare workers with a median age 46.9 years to measure immune responses to SARS-CoV-2 vaccination. From the demographic information provided, there is no mention that any of the subjects were immunocompromised. Yet when

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https://doi.org/10.1016/j.jviromet.2022.114472
Received 23 December 2021; Accepted 17 January 2022
Available online 20 January 2022

reviewing the results of the Mitogen positive control tube, a mean response of 3.27 IU/mL ± 0.068 IU/mL was reported (Figure 2 in (Krüttgen et al., 2021)).

Mitogen responses are non-specific to the antigen being evaluated and are included to confirm that the proper blood handling techniques were performed prior to 37 °C incubation of whole blood, as well as patient immunocompetency. The Mitogen tube used in QFN SARS is the same tube included with the QuantiFERON-TB Gold Plus test and expected results for Mitogen responses in healthy individuals are overwhelmingly higher than 3.27 IU/mL and typically > 10 IU/mL, beyond the measurable range of the QuantiFERON ELISA (Anon, 2022; Powell et al., 2011).

Reporting raw ELISA OD values prior to the IU/mL calculation using the ELISA IFN-γ standard curve could be among possible explanations for significantly lower than expected Mitogen and Ag1 and Ag2 responses reported by Krüttgen et al. Following the ELISA, raw OD values should be transformed to IU/mL values using the standard curve which is calculated via the QuantiFERON software or with a basic statistics programming tool. Most plate readers will not report OD values substantially higher than 3 absorbance units as this is beyond the linear measuring range of the instrument. Krüttgen et al. reported negligible Mitogen value distribution of only ± 0.068 IU/mL for 18 healthy donors, strongly suggesting that the plate reader signal has plateaued for IU/mL values > 10 IU/mL in the context of the standard curve, which would then align with expected Mitogen responses in a healthy patient cohort.

Since the Mitogen tube values are on the same ELISA plate as QFN SARS Ag1 and Ag2 samples and are interpreted using the same standard curve, we would expect this rationale to also apply to all samples, that is, we predict a larger percentage of the HCW cohort would report Ag1 or Ag2 values > 0.15 IU/mL and would agree with findings in the aforementioned published studies.

Easy to use IGRA assays including QFN SARS are considered attractive alternatives to more “traditional” but laborious methods for detection of SARS-CoV-2-specific T-cell responses for (Goletti et al., 2021; de Vries et al., 2021). In this context, accurate assessment of T-cell responses is of utmost importance as many studies including pharmaceutical and vaccine trials are more likely to rely on results of rapid screening assays including IGRA.

We are encouraged to see the QuantiFERON SARS-CoV-2 RUO product used in clinical settings to provide additional valuable insight to the complementary roles of humoral and cell mediated immune responses to SARS-CoV-2 infection, and we are grateful to both the research team at University Hospital RWTH Aachen and to *Journal of Virological Methods* for inclusion of QuantiFERON SARS-CoV-2 RUO in their publication.

Funding

Study was internally funded by QIAGEN.

Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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