*Clinical Infectious Diseases*

BRIEF REPORT

# Interferon-γ Release Assay for Accurate Detection of Severe Acute Respiratory Syndrome Coronavirus 2 T-Cell Response

**Kanagavel Murugesan,[1] Prasanna Jagannathan,[2] Tho D. Pham,[1,3] Suchitra Pandey,[1,3] Hector F. Bonilla,[2] Karen Jacobson,[2] Julie Parsonnet,[2] Jason R. Andrews,[2] Daniela Weiskopf,[4] Alessandro Sette,[4,5] Benjamin A. Pinsky,[1,2,6] Upinder Singh,[2] and Niaz Banaei[1,2,7]**

[1]Department of Pathology, Stanford University School of Medicine, Stanford, California, USA, [2]Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, California, USA, [3]Stanford Blood Center, Stanford Health Care, Stanford, California, USA, [4]Center for Infectious Disease and Vaccine Research, La Jolla Institute for Immunology, La Jolla, California, USA, [5]Department of Medicine, Division of Infectious Diseases and Global Public Health, University of California, San Diego, La Jolla, California, USA, [6]Clinical Virology Laboratory, Stanford Health Care, Stanford, California, USA, and [7]Clinical Microbiology Laboratory, Stanford Health Care, Stanford, California, USA

We investigated feasibility and accuracy of an interferon-γ release assay (IGRA) for detection of T-cell responses to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Whole blood IGRA accurately distinguished between convalescent and uninfected healthy blood donors with a predominantly $CD4^+$ T-cell response. SARS-CoV-2 IGRA may serve as a useful diagnostic tool in managing the coronavirus disease 2019 pandemic.

**Keywords.** interferon-γ release assay; SARS-CoV-2; T cells; IGRA; immune response.

Recent studies have revealed a durable memory T-cell immune response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) following coronavirus disease 2019 (COVID-19) or seasonal coronavirus respiratory infection that is a more sensitive marker of past infection than humoral response and postulated to represent a correlate of protective immunity to SARS-CoV-2 [1–4]. To better evaluate the immune status of individuals and populations and assess emerging vaccines, novel immunodiagnostics that measure cellular immune response to SARS-CoV-2 are needed. Although much progress has been made in development of immunoassays for detection of antibody responses to SARS-CoV-2, clinical assays for detection of cellular immune response have lagged. The interferon gamma (IFN-γ) release assay (IGRA) is an in vitro blood diagnostic used in clinical laboratories to measure IFN-γ released by antigen-specific T cells after overnight stimulation with pathogen-specific peptides. Here, we describe clinical performance and kinetics of a whole blood IGRA (Figure 1A) for simple and high-throughput detection of cellular immune response to SARS-CoV-2.

All participants provided written informed consent. Freshly collected blood in lithium heparin tube was (1) left unstimulated as negative control; stimulated with (2) a single SARS-CoV-2 peptide pool for $CD4^+$ T cells and (3) 2 SARS-CoV-2 peptide pools for $CD8^+$ T cells; and (4) stimulated with mitogen as positive control. A single $CD4^+$ T-cell megapool ($CD4^+$ pool) consisted of 221 predicted HLA class II $CD4^+$ T-cell epitope peptides covering the entire viral proteome except for the spike protein, which was covered with 253 15-mer peptides overlapping by 10 residues and 2 $CD8^+$ T-cell megapools ($CD8^+$ pools A and B) together consisting of 628 predicted HLA class I $CD8^+$ T-cell epitopes from the entire SARS-CoV-2 proteome [5, 6]. The IFN-γ concentration in the plasma fraction was measured with an automated enzyme-linked immunosorbent assay (ELISA) instrument in international units per milliliter (IU/mL). IFN-γ response was defined as peptide stimulated minus unstimulated. The Mann-Whitney *U* test was used to compare median IFN-γ responses between groups. The Wilcoxon signed-rank test of medians was used to compare differences between paired results. The receiver operating characteristic curve was used to derive an IFN-γ response cutoff at the Youden maximum index value, which assigns equal weight to sensitivity and specificity.

In total, 82 adult COVID-19 convalescent patients including 64 from an outpatient COVID-19 treatment trial of interferon lambda (IλT) and 18 from Stanford Blood Center convalescent plasma donors (CPDs), and 48 uninfected healthy adult blood donors with negative ELISAs for SARS-CoV-2 immunoglobulin G, were tested with the IGRA. IFN-λ has been shown to not have a negative impact on adaptive response to SARS-CoV-2 [7]. Among all convalescents, the median age was 38 years (range, 21–65 years) and 34% were female. Nearly all cases had mild outpatient COVID-19. CPDs were tested 45–137 days (median, 83 days) post–reverse-transcription polymerase chain reaction (RT-PCR) positivity, and IλT participants were tested on days 17 and 31 post–RT-PCR positivity.

Stimulation of whole blood with the $CD4^+$ T-cell peptide pool showed a significantly higher median IFN-γ response in 82 convalescents compared with 48 healthy blood donors

Received 7 August 2020; editorial decision 1 October 2020; published online 9 October 2020.
Correspondence: N. Banaei, Stanford University School of Medicine, 3375 Hillview Ave, Rm 1602, Palo Alto, CA 94304 (nbanaei@stanford.edu).

**Clinical Infectious Diseases® 2021;73(9):e3130–2**

DOI: 10.1093/cid/ciaa1537

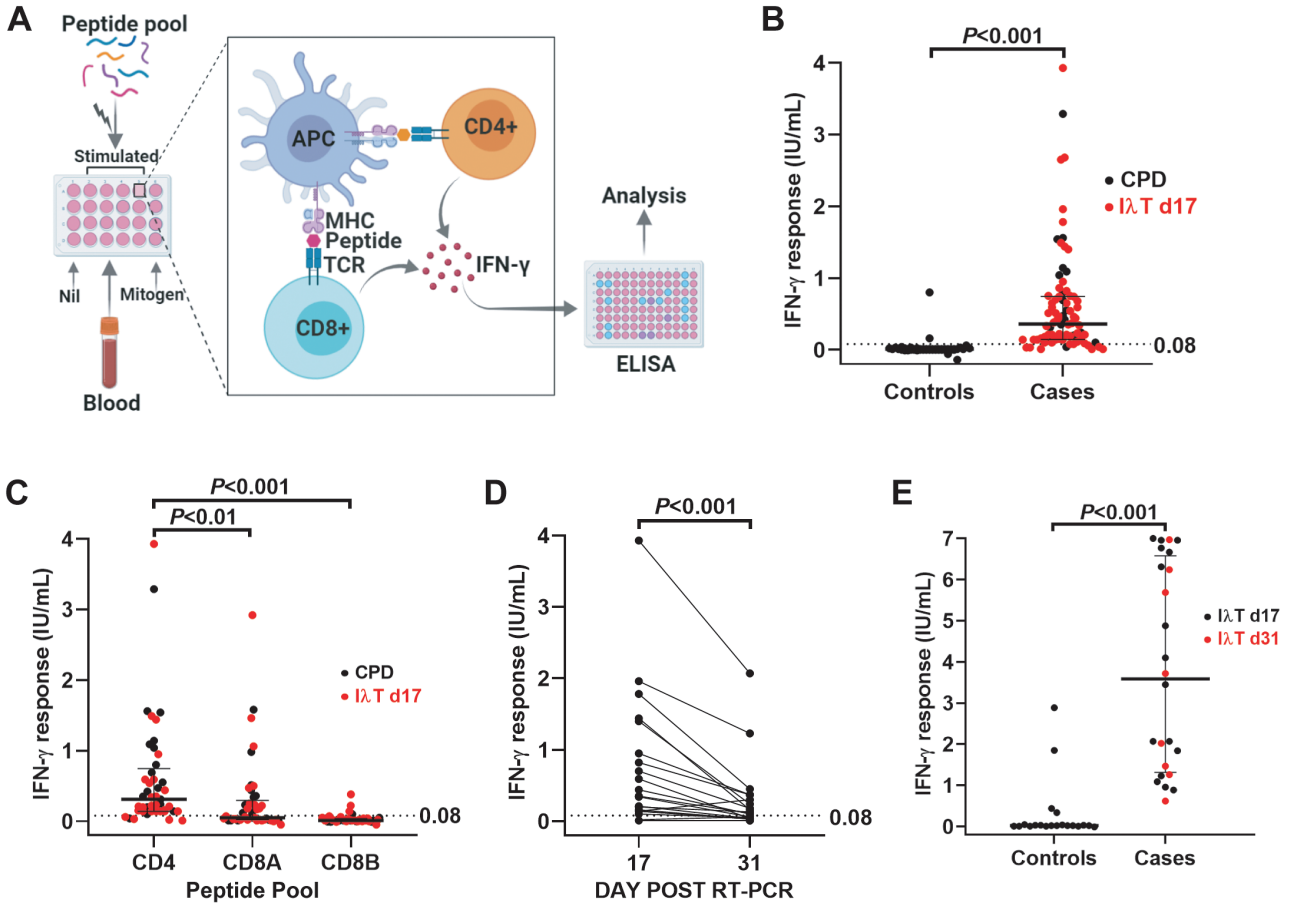


**Figure 1.** Performance of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) interferon gamma (IFN-γ) release assay (IGRA). *A*, Schematic showing methodology of whole blood IGRA for detection of cellular immune response to SARS-CoV-2. *B*, IFN-γ response of whole blood stimulated with $CD4^+$ T-cell peptide pool in healthy blood donors with negative SARS-CoV-2 immunoglobulin G (controls) and convalescent cases with mild coronavirus disease 2019 (COVID-19) (convalescent plasma donors [CPDs] and participants of an interferon lambda [IFN-λ] therapy trial [IλT] on day 17 post–reverse-transcription polymerase chain reaction [RT-PCR] positivity). Median IFN-γ response was 0.36 (interquartile range {IQR}, 0.14–0.74) IU/mL in cases vs 0.01 (IQR, 0–0.01) IU/mL in controls. SARS-CoV-2 IGRA showed sensitivity of 90% (95% confidence interval [CI], 82%–95%) and specificity of 96% (95% CI, 86%–99%) at a cutoff of 0.08 IU/mL (dotted line). *C*, IFN-γ responses for peptide pools stimulating $CD4^+$ and $CD8^+$ (pools A and B) T cells. Median IFN-γ response was 0.31 (IQR, 0.14–0.75) IU/mL to $CD4^+$ T-cell peptide pool vs 0.05 (IQR, 0.02–0.30) IU/mL to $CD8^+$ T-cell peptide pool A and 0.01 (IQR, 0–0.04) IU/mL to $CD8^+$ T-cell peptide pool B. *D*, IFN-γ response to $CD4^+$ T-cell peptide pool on days 17 and 31 post–RT-PCR positivity in convalescent IλT participants. Median IFN-γ response was 0.35 (IQR, 0.14–1.06) IU/mL on day 14 vs 0.11 (IQR, 0.04–0.32) IU/mL on day 28. *E*, IFN-γ response in 20 controls and 24 IλT convalescents (16 on day 17 and 8 on day 31) using a commercial peptide pool consisting of spike, S1, nucleocapsid, and membrane proteins from Miltenyi Biotec (Bergisch Gladbach, Germany). Median IFN-γ response was 0.02 (IQR, 0.01–0.05) IU/mL in controls and 3.59 (IQR, 1.31–6.58) IU/mL in convalescents. Bars show median IFN-γ response and whiskers show IQR. Abbreviations: APC, antigen presenting cell; CPD, convalescent plasma donor; ELISA, enzyme-linked immunosorbent assay; IFN-γ, interferon gamma; MHC, major histocompatibility complex; RT-PCR, reverse-transcription polymerase chain reaction; TCR, T-cell receptor.

(0.36 [interquartile range {IQR}, 0.14–0.74] IU/mL vs 0.01 [IQR, 0–0.01] IU/mL; $P < .001$) (Figure 1B). There was no difference in the median IFN-γ level in unstimulated blood in convalescents and healthy blood donors (0.04 [IQR, 0.03–0.05] IU/mL vs 0.03 [IQR, 0.02–0.04] IU/mL; $P > .05$). Using Youden maximum index value, the sensitivity and specificity were 90% (95% confidence interval [CI], 82%–95%) and 96% (95% CI, 86%–99%), respectively, at a cutoff value of 0.08 IU/mL. The receiver operating characteristic curve is shown in Supplementary Figure 1. The median IFN-γ responses to 2 $CD8^+$ T-cell peptide pools in 45 convalescents (27 IλT and 18 CPD) with sufficient blood to stimulate with all 3 peptide pools were significantly lower compared with the $CD4^+$ T-cell peptide pool (pool A: 0.05 [IQR, 0.02–0.30] IU/mL vs 0.31 [IQR, 0.14–0.75] IU/mL, $P < .01$; pool B: 0.01 [IQR, 0–0.04] IU/mL vs 0.31 [IQR, 0.14–0.75] IU/mL, $P < .001$) (Figure 1C). This finding is consistent with prior studies showing greater $CD4^+$ vs $CD8^+$ T-cell response in inpatients and convalescent COVID-19 [6, 8]. Within-person comparison of IFN-γ response to the $CD4^+$ T-cell pool on day 17 and day 31 post–RT-PCR positivity in IλT convalescents showed a higher median IFN-γ response on day 17 than day 31 (0.35 [IQR, 0.14–1.06] IU/mL vs 0.11 [IQR, 0.04–0.32] IU/mL; $P < .001$) (Figure 1D). This finding is novel and consistent with a rapid antibody decay reported for convalescent mild COVID-19 [9], although IGRA remained positive in 94% (95% CI, 74%–100%)

of 18 CPD convalescents 83 days post–RT-PCR positivity. However, the sensitivity was 70% (95% CI, 56%–81%) in 50 IλT convalescents on day 31 (Supplementary Figure 2). Stimulating T cells with a commercially available peptide pool consisting of spike, S1, nucleocapsid, and membrane proteins (Miltenyi Biotec, Bergisch Gladbach, Germany) in 20 healthy blood donors and 24 IλT convalescents (16 on day 17 and 8 on day 31), the median IFN-γ response was 0.02 (IQR, 0.01–0.05) IU/mL in healthy blood donors and 3.59 (IQR, 1.31–6.58) IU/mL in convalescents (Figure 1E). The IFN-γ response in 23 convalescents was 19-fold higher with the Miltenyi peptide pool than with the $CD4^+$ T-cell peptide pool described above (3.45 [IQR, 1.26–6.67] IU/mL vs 0.18 [IQR, 0.09–0.42] IU/mL in 23 paired blood samples, $P < .001$; data not shown). The sensitivity and specificity for IGRA with the Miltenyi peptide pool were 100% (95% CI, 86%–100%) and 90% (95% CI, 70%–98%), respectively, at a cutoff value of 0.53 IU/mL. This finding suggests that by using a peptide pool that increases the magnitude of IFN-γ response, it may be possible to maintain SARS-CoV-2 IGRA sensitivity during the follow-up period.

In summary, using a simple and deeply studied IGRA method used for diagnosis of latent *Mycobacterium tuberculosis* infection [10, 11], we show that whole blood stimulation with a SARS-CoV-2 peptide pool can sensitively detect a cellular immune response to SARS-CoV-2 and accurately distinguish between convalescents and uninfected healthy blood donors. Given the relative ease of performing whole blood IGRA and the widespread use of IGRA, SARS-CoV-2 IGRA can be implemented in clinical laboratories to identify individuals with reactive T cells to SARS-CoV-2. The assay may be further enhanced by using more sensitive and commercially available peptide pools with defined specificity for SARS-CoV-2 vs seasonal coronavirus [1, 3]. SARS-CoV-2 IGRA may prove useful in identifying individuals with cellular immunity and thus serve as a powerful diagnostic tool in managing the COVID-19 pandemic.

## Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

## Notes

***Financial support.*** This work was funded by the National Institutes of Health (contract number 75N9301900065 to A. S. and D. W.).

***Potential conflicts of interest.*** A. S. is listed as inventor on patent application number 63/012902, submitted by La Jolla Institute for Immunology on 12 February 2020, that covers the use of the megapools and peptides thereof for therapeutic and diagnostic purposes. A. S. also reports consultancy for Gritstone, Avalia, Flow Pharma, and Merck, outside the submitted work. All other authors report no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

## References

1. Braun J, Loyal L, Frentsch M, et al. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. Nature **2020**. doi:10.1038/s41586-020-2598-9.
2. Gallais F, Velay A, Wendling M, et al. Intrafamilial exposure to SARS-CoV-2 induces cellular immune response without seroconversion. medRxiv [Preprint]. June 22, **2020**. doi:10.1101/2020.06.21.20132449.
3. Mateus J, Grifoni A, Tarke A, et al. Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans. Science **2020**; 370:89–94.
4. Sekine T, Perez-Potti A, Rivera-Ballesteros O, et al. Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. Cell **2020**; 183:158–68.e14.
5. Grifoni A, Sidney J, Zhang Y, Scheuermann RH, Peters B, Sette A. A sequence homology and bioinformatic approach can predict candidate targets for immune responses to SARS-CoV-2. Cell Host Microbe **2020**; 27:671–80.e2.
6. Grifoni A, Weiskopf D, Ramirez SI, et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. Cell **2020**; 181:1489–501.e15.
7. Andreakos E, Tsiodras S. COVID-19: lambda interferon against viral load and hyperinflammation. EMBO Mol Med **2020**; 12:e12465.
8. Weiskopf D, Schmitz KS, Raadsen MP, et al. Phenotype and kinetics of SARS-CoV-2-specific T cells in COVID-19 patients with acute respiratory distress syndrome. Sci Immunol **2020**; 5:abd2071.
9. Ibarrondo FJ, Fulcher JA, Goodman-Meza D, et al. Rapid decay of anti-SARS-CoV-2 antibodies in persons with mild Covid-19. N Engl J Med **2020**; 383:e74.
10. Pai M, Denkinger CM, Kik SV, et al. Gamma interferon release assays for detection of *Mycobacterium tuberculosis* infection. Clin Microbiol Rev **2014**; 27:3–20.
11. Shafeque A, Bigio J, Hogan CA, Pai M, Banaei N. Fourth generation QuantiFERON-TB Gold-Plus: what is the evidence? J Clin Microbiol **2020**; 58:e01950-19.