

Ultrasensitive Measurement of Both SARS-CoV-2 RNA and Antibodies from Saliva

Dmitry Ter-Ovanesyan,⁺ Tal Gilboa,⁺ Roey Lazarovits,⁺ Alexandra Rosenthal, Xu Yu, Jonathan Z. Li, George M. Church, and David R. Walt^{*}



Cite This: *Anal. Chem.* 2021, 93, 5365–5370



Read Online

ACCESS |



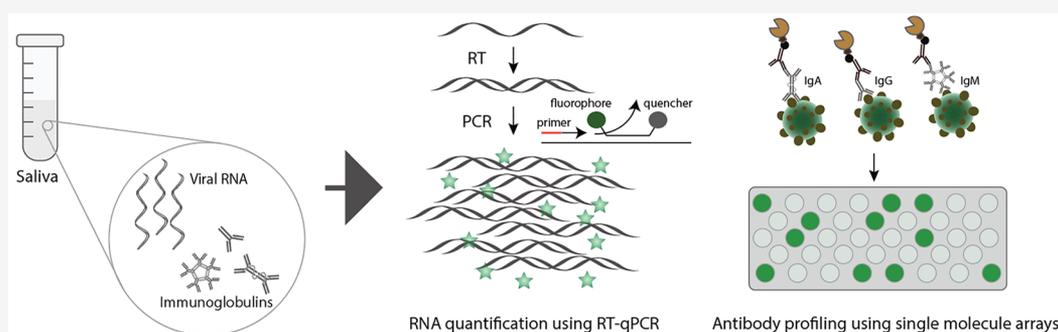
Metrics & More



Article Recommendations



Supporting Information



ABSTRACT: Tests for COVID-19 generally measure SARS-CoV-2 viral RNA from nasal swabs or antibodies against the virus from blood. It has been shown, however, that both viral particles and antibodies against those particles are present in saliva, which is more accessible than both swabs and blood. We present methods for highly sensitive measurements of both viral RNA and antibodies from the same saliva sample. We developed an efficient saliva RNA extraction method and combined it with an ultrasensitive antibody test based on single molecule array (Simoa) technology. We apply our test to the saliva of patients who presented to the hospital with COVID-19 symptoms, some of whom tested positive with a conventional RT-qPCR nasopharyngeal swab test. We demonstrate that combining viral RNA detection by RT-qPCR with antibody detection by Simoa identifies more patients as infected than either method alone. Our results demonstrate the utility of combining viral RNA and antibody testing from saliva, a single easily accessible biofluid.

INTRODUCTION

The two main tests for SARS-CoV-2 infection are molecular tests to detect the presence of the virus (RNA or antigen) and serological tests to detect the presence of antibodies against the virus.^{1–7} Both tests have advantages and disadvantages. RT-qPCR, the main diagnostic test and current gold standard, is a sensitive method for measuring the presence of viral RNA, usually performed from nasopharyngeal (NP) or anterior nasal swabs.^{1–3} However, it has been shown that not all patients who are infected with SARS-CoV-2 test positive for viral RNA.^{1,2,5,8–10} There are several potential reasons for this: low viral load, variability in swabbing, or late swab collection relative to the time of infection.^{2,5} The time at which the swab is performed is important because, after initial infection, levels of virus sharply rise and then drop, providing a relatively narrow window at which viral RNA is present.^{1,5,6,11–19} Serology tests detect antibodies that develop against the virus during infection.^{1,6} These antibodies remain stable for at least several months, widening the time window of testing for SARS-CoV-2 infection.^{20–22} Combining RNA detection with antibody testing has the potential to increase the sensitivity of

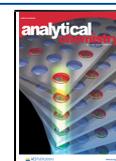
RT-qPCR alone^{6,15,21,23–27} by reducing false negatives in RT-qPCR.

RT-qPCR tests for SARS-CoV-2 mostly analyze RNA from nasal swabs, while serology tests are generally performed using blood.^{1,2,3,28–30} However, studies have shown that both SARS-CoV-2 viral RNA^{15,31–34} and antibodies^{20,31,35} against the virus are present in saliva. Since saliva is easier to collect than either swabs or blood, its accessibility makes it an ideal biofluid for widespread diagnostic use. Research is still ongoing regarding how well saliva correlates to different types of swabs in terms of sensitivity of viral RNA detection.^{31–33,36–38} This will depend largely on several factors such as patient selection, sample collection, and RNA extraction methodology. Nonetheless, it is clear that SARS-CoV-2 viral RNA can be readily detected in

Received: February 3, 2021

Accepted: March 17, 2021

Published: March 23, 2021



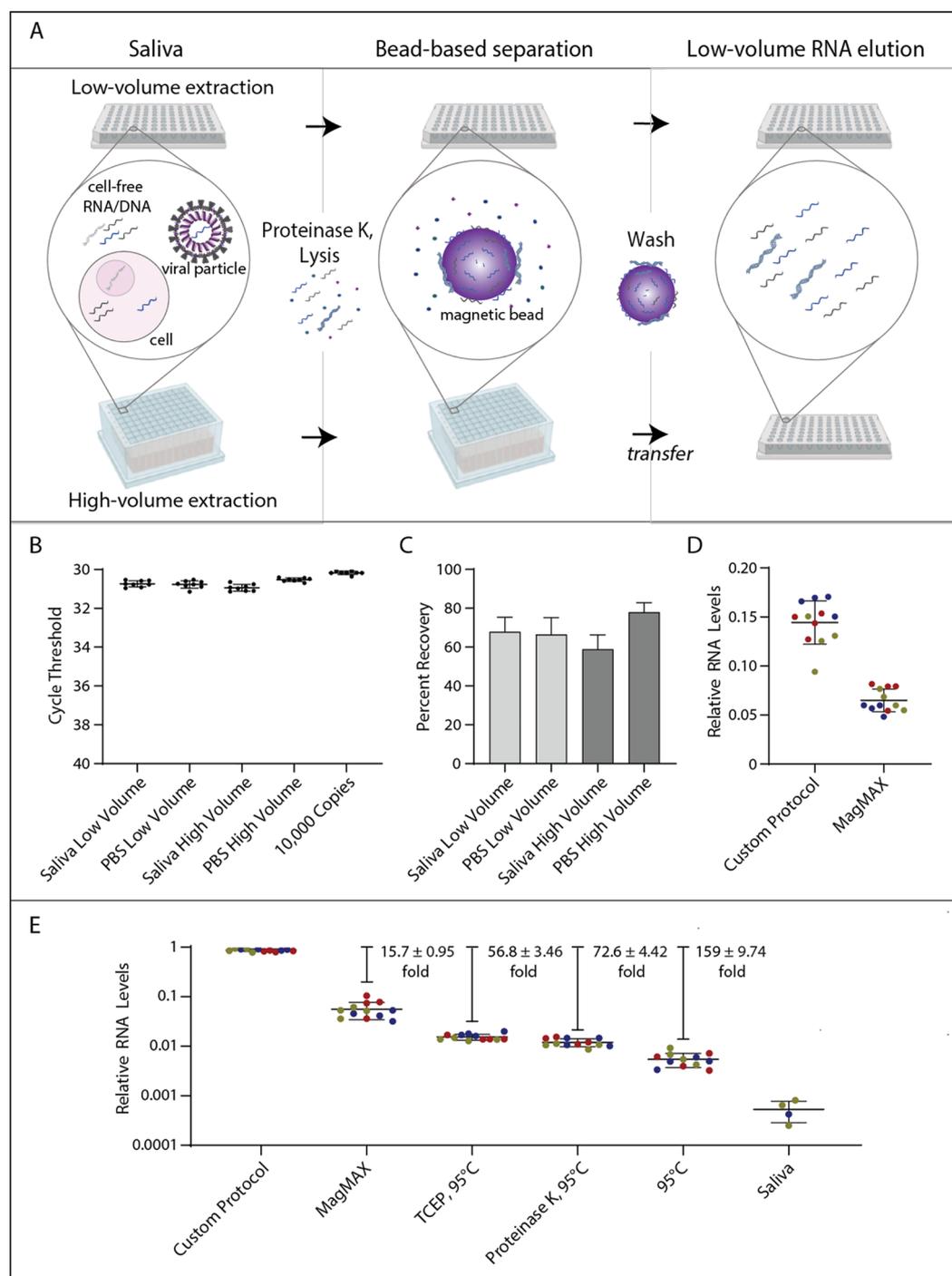


Figure 1. Custom RNA extraction protocol recovers viral RNA with high efficiency. (A) Schematic representation showing our bead-based extraction protocol in low-volume and high-volume versions. (B) High-efficiency viral RNA recovery from PBS and saliva using custom protocol. PBS or saliva (30 μL for low volume or 300 μL for high volume) were spiked with 10 000 copies of synthetic SARS-CoV-2 RNA. Our custom RNA extraction protocol was performed, and extracted RNA was compared to the input amount using RT-qPCR. (C) Recovery of RNA is calculated as the RNA level of extracted SARS-CoV-2 RNA relative to the spike-in amount. (D) Direct comparison of custom protocol with commercial kit using the same input and elution volumes. RNA was extracted from 200 μL of saliva with 50 particles/ μL of heat-inactivated virus and eluted into 50 μL using either our custom protocol or the MagMAX kit and quantified by RT-qPCR. (E) Comparison of extraction and extraction-free methods. For all methods, saliva spiked with 50 viral particles/ μL was used, and the same volume of inactivated saliva or eluted RNA was compared by RT-qPCR. For comparison of RNA extraction methods, maximum input volumes (400 μL for MagMAX vs 300 μL for custom) and minimal elution volumes (50 μL for MagMAX vs 10 μL for custom) were used. Relative RNA levels (log scale) represent each condition relative to spike-in aliquot on the same RT-qPCR plate. Fold-differences relative to custom protocol are indicated above each condition. For the saliva control (no-inactivation), 8/12 replicates had no detectable levels of RNA and were not plotted. Four replicates were repeated across three different days for each condition (different colors represent different days). Error bars for all figures indicate the standard deviation.

saliva.^{2,31–33} Similarly, previous studies have shown a strong correlation between antibodies against SARS-CoV-2 in blood and saliva.^{20,31,35}

Although RT-qPCR is highly sensitive, there is great variability in the RNA extraction efficiency, which is generally performed using commercial kits. These kits have advantages in terms of ease but are prone to supply chain limitations.³⁹ Furthermore, using kits may lead to incompatibilities with upstream or downstream steps since the components are unknown to the user. Lastly, kits are not optimized for specific biofluids such as saliva, leading to potentially low RNA recovery. Antibody testing in saliva also presents challenges, namely, that concentrations of antibodies in saliva are much lower than they are in blood^{20,31,35} and that different isotypes may be present in the different fluids.

We set out to develop a highly sensitive test to detect both viral RNA and antibodies against the virus from the same saliva sample. We developed an optimized RNA extraction protocol that is highly efficient for saliva. We also adapted a single molecule array⁴⁰ (Simoa)-based ultrasensitive test we previously developed for detecting SARS-CoV-2 antibodies in blood for use in saliva.^{8,41} Combining our RNA extraction protocol for RT-qPCR with this ultrasensitive antibody test, we are able to better classify COVID-19 patients as being actively or previously infected with SARS-CoV-2, demonstrating the utility of this approach for accurate diagnosis of COVID-19.

RESULTS AND DISCUSSION

In order to develop a highly sensitive test for both SARS-CoV-2 RNA and antibodies against the virus in saliva, we first optimized the RNA extraction. We developed two versions: a low-volume version (30 μL of saliva) that can be performed in a 96-well PCR plate and a high-volume version (300 μL of saliva) that can be performed in a deep-well plate. We started with a general method based on binding nucleic acids to carboxylated paramagnetic beads in the presence of a guanidinium thiocyanate lysis buffer^{42,43} (Figure 1a) and optimized each step of the protocol using saliva samples. To evaluate our extraction method, we spiked in known amounts of synthetic SARS-CoV-2 RNA or heat-inactivated SARS-CoV-2 viral particles into PBS or human saliva and then quantified recovery after RNA extraction by RT-qPCR (Figure 1a, Supporting Information Figure S1). We tested a large number of parameters using this approach until we arrived at a protocol that consistently resulted in 50–100% recovery in 30 μL of saliva (Figure 1b,c). To maximize sensitivity, we opted to use more saliva (300 μL) in a deep-well plate format. However, as it is difficult to elute into a small volume using a deep well plate, we included a transfer step in our protocol where we transfer the beads (during a 70% ethanol wash step) from a deep-well plate to a PCR plate, allowing us to elute the RNA in only 10 μL (Figure 1a).

To see how our protocol compares to a commonly used commercial kit, we performed a head-to-head comparison with the ThermoFisher Scientific MagMAX Viral/Pathogen Nucleic Acid Isolation kit. We first extracted RNA from equal volumes of saliva spiked with viral particles and eluted the captured RNA into equal volumes (Figure 1d). In this comparison, we found that our custom protocol had more than twice the recovery of MagMAX. We also compared our protocol to MagMAX using the maximum input volume and minimum elution volumes for both protocols using saliva with the same concentration of viral particles. In this case, our protocol

recovered 16 \times more RNA (Figure 1e). We then compared our protocol to three recently developed protocols for measuring RNA from saliva without purification. These protocols, SalivaDirect,⁴⁴ TCEP inactivation,⁴⁵ and 95 $^{\circ}\text{C}$ heating,⁴⁶ all use a small volume of saliva (<10 μL) since the final volume of RT-qPCR reactions is usually 20 μL . We compared our high-volume RNA extraction to these three protocols using saliva containing the same concentration of viral particles. We found that we detected significantly more RNA in extraction-based protocols, largely due to the ability to use larger volumes of saliva (Figure 1e).

After validation of our RNA extraction protocol, we turned to combining RNA detection with antibody detection in clinical saliva samples from COVID-19 patients. We have previously developed single molecule array (Simoa)-based ultrasensitive profiling of IgG, IgM, and IgA antibodies against SARS-CoV-2 nucleocapsid (N), spike (S), S1, and RBD protein targets in blood.⁸ Our assay employs a bead-based, digital ELISA for high-throughput, automated ultrasensitive detection of antibodies in small volumes. Using only 40 μL of saliva per sample (10 μL for antibodies and 30 μL for RNA), we characterized 12 antibody interactions and quantified SARS-CoV-2 RNA across 18 saliva samples. We tested two prepandemic saliva samples from healthy individuals and 16 samples from symptomatic individuals who visited the MGH Respiratory Infection Clinic during the pandemic (see the Supporting Information Table S1 for a clinical characteristics summary). The patients were all tested by RT-qPCR from NP swab samples upon arrival to the clinic, and saliva was collected on the same day.

Combined measurement of SARS-CoV-2 RNA and IgG, IgM, and IgA levels against S1 (Figure 2, Supporting Information Figure S2) revealed that five patients were positive for either RNA, antibodies, or both, in saliva compared to three patients by NP RT-qPCR alone. Two patient samples were positive for antibodies in saliva but negative for SARS-CoV-2 RNA by RT-qPCR (in both NP swabs and saliva). Since these patients displayed severe respiratory illness upon presentation to the hospital, their RT-qPCR results were likely false negatives. RNA was detected in saliva for two of the three patients with positive NP swabs. For the patient with RNA detected in the NP swab but not saliva, antibody levels were above the threshold for IgA and IgM against S1. Since our previous work showed that IgA-S1 displayed the best separation between positives and controls, this sample would be classified as positive in our antibody assay. We also measured saliva from a subset of the patients at six late time points (>9 days after the first positive PCR). We found no RNA was detected but antibody levels were high for most of the immunoglobulin subtypes we measured, as expected (Supporting Information Figure S3). Finally, to see if we could achieve multiplexed detection of viral RNA and antibodies on the same platform from one sample, we developed a Simoa assay for the direct detection of SARS-CoV-2 RNA. Although we found this assay to be less sensitive than RT-qPCR, the Simoa assay detects RNA without amplification. We combined this assay with our antibody assay to detect RNA and antibodies spiked into saliva samples (Supporting Information Figure S4, Table S2), demonstrating multiplexed detection of RNA and antibodies on the Simoa platform.

Several studies have shown that saliva is suitable for both SARS-CoV-2 viral RNA detection and antibody measurements,

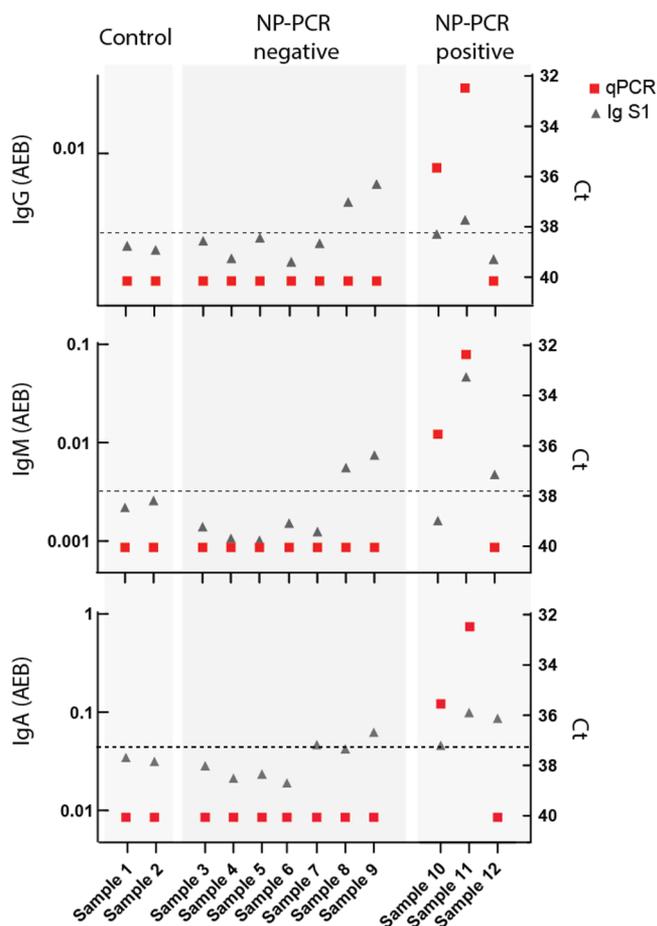


Figure 2. Detection of SARS-CoV-2 RNA and antibodies from the same saliva sample of hospitalized COVID-19 patients on initial day of hospitalization. RT-qPCR Ct levels (red rectangular) and Simoa antibody mean AEB (average enzyme per bead) levels (gray triangles) for IgG, IgM, and IgA against the S1 subunit. The samples were divided into three groups: pre-pandemic control samples (left, $n = 2$), saliva samples from patients who tested negative by RT-qPCR from nasopharyngeal swabs (NP-PCR negative, middle, $n = 7$) and saliva samples from patients who tested positive by RT-qPCR from nasopharyngeal swabs (NP-PCR positive, right, $n = 3$). Conditions for RT-qPCR where signal is undetected are set to Ct = 40. All antibody samples were measured with two technical replicates. Black dotted lines indicate antibody threshold levels, defined as three standard deviations above the highest pre-pandemic controls signals. Samples on or below the dotted line are considered negative.

but these measurements have not been made together using the same sample. In this study, we combined RT-qPCR with antibody measurements on a small number of symptomatic COVID-19 patient saliva samples. We demonstrate that combining these measurements identifies SARS-CoV-2 infection in patients that RT-qPCR alone misses, providing information that can help guide clinical decision making. This is particularly important in patients who present to the hospital with COVID-19 symptoms but have negative RT-qPCR results despite having been infected with SARS-CoV-2 (for example, because the immune system has already cleared the virus).

To maximize sensitivity of RNA detection, we developed and optimized a high-efficiency saliva RNA extraction protocol without the use of kits. We found our protocol yields much higher levels of RNA from saliva than a widely used commercial isolation kit when performed manually. We also

directly compared our RNA extraction method to several recently published no-extraction methods for SARS-CoV-2 detection by RT-qPCR in saliva. Although these methods greatly increase ease and throughput of RT-qPCR testing, this comes at a cost of sensitivity. Our high-volume RNA extraction protocol allows for a 30-fold concentration of the saliva sample in addition to the inactivation and removal of enzymatic inhibitors present in saliva. In a comparison utilizing saliva spiked with virus, our high-volume RNA extraction protocol led to the detection of at least 56 times more viral RNA than the use of inactivated saliva as input.

To maximize the sensitivity of our antibody measurements, we employed assays that we recently developed using single molecule array (Simoa) technology.⁸ The high sensitivity of these assays is particularly advantageous in saliva, where antibody levels are known to be low.^{20,31,35} We have also demonstrated that antibody measurements can be combined with direct, amplification-free detection of RNA in saliva samples, which expands on previous work applying the Simoa platform for multianalyte detection.^{47–49} We envision that the sensitivity of the RNA assay can be further improved, for example, by adding Cas13a-based detection.⁵⁰ Future studies may also incorporate additional protein biomarkers in saliva to measure inflammation (cytokines, etc.) or other aspects of host response to increase the utility of multiplexed saliva diagnostics for COVID-19.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.analchem.1c00515>.

Detailed experimental methods; Figure S1, RNA extraction optimizations; Table S1, clinical characteristics of COVID-19+ saliva samples; Figure S2, detection of SARS-CoV-2 RNA and antibodies from saliva samples of hospitalized COVID-19 patients over time; Figure S3, antibody profiles in saliva samples; Figure S4, direct detection of SARS-CoV-2 RNA using Simoa; and Table S2, spike and recovery in control saliva samples (PDF)

■ AUTHOR INFORMATION

Corresponding Author

David R. Walt – Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, Massachusetts 02115, United States; Department of Pathology, Harvard Medical School, Brigham and Women's Hospital, Boston, Massachusetts 02115, United States; orcid.org/0000-0002-5524-7348; Email: dwalt@bwh.harvard.edu

Authors

Dmitry Ter-Ovanesyan – Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, Massachusetts 02115, United States; orcid.org/0000-0002-1134-0073

Tal Gilboa – Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, Massachusetts 02115, United States; Department of Pathology, Harvard Medical School, Brigham and Women's Hospital, Boston, Massachusetts 02115, United States

Roey Lazarovits – Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, Massachusetts 02115, United States

Alexandra Rosenthal – Infectious Disease Division, Harvard Medical School, Brigham and Women's Hospital, Boston, Massachusetts 02115, United States

Xu Yu – Infectious Disease Division, Harvard Medical School, Brigham and Women's Hospital, Boston, Massachusetts 02115, United States; Ragon Institute of MGH, MIT and Harvard, Cambridge, Massachusetts 02139, United States

Jonathan Z. Li – Infectious Disease Division, Harvard Medical School, Brigham and Women's Hospital, Boston, Massachusetts 02115, United States

George M. Church – Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, Massachusetts 02115, United States; Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, United States; orcid.org/0000-0003-3535-2076

Complete contact information is available at:

<https://pubs.acs.org/10.1021/acs.analchem.1c00515>

Author Contributions

[†]D.T.-O., T.G. and R.L. contributed equally.

Notes

The authors declare the following competing financial interest(s): D.R.W. has a financial interest in Quanterix Corporation, a company that develops an ultrasensitive digital immunoassay platform. He is an inventor of the Simoa technology, is a founder of the company, and also serves on its Board of Directors. His interests were reviewed and are managed by BWH and Partners HealthCare in accordance with their conflict of interest policies. G.M.C. commercial interests: <http://arep.med.harvard.edu/gmc/tech.html>.

ACKNOWLEDGMENTS

The authors acknowledge funding from the Bill and Melinda Gates Foundation for this work. The MGH/MassCPR COVID-19 biorepository was supported by a gift from Ms. Enid Schwartz, by the Mark and Lisa Schwartz Foundation, the Massachusetts Consortium for Pathogen Readiness, and the Ragon Institute of MGH, MIT and Harvard.

REFERENCES

- (1) Ravi, N.; Cortade, D. L.; Ng, E.; Wang, S. X. *Biosens. Bioelectron.* **2020**, *165*, 112454.
- (2) Feng, W.; Newbigging, A. M.; Le, C.; et al. *Anal. Chem.* **2020**, *92* (15), 10196–10209.
- (3) Venter, M.; Richter, K. J. *Clin. Pathol.* **2020**, *73* (7), 370–377.
- (4) Tang, Y.-W.; Schmitz, J. E.; Persing, D. H.; Stratton, C. W. *J. Clin. Microbiol.* **2020**, *58* (6), e00512-20.
- (5) Wiersinga, W. J.; Rhodes, A.; Cheng, A. C.; Peacock, S. J.; Prescott, H. C. *JAMA* **2020**, *324* (8), 782–793.
- (6) Sethuraman, N.; Jeremiah, S. S.; Ryo, A. *JAMA* **2020**, *323* (22), 2249–2251.
- (7) Udugama, B.; Kadhiresan, P.; Kozlowski, H. N.; et al. *ACS Nano* **2020**, *14* (4), 3822–3835.
- (8) Norman, M.; Gilboa, T.; Ogata, A. F. *Nat. Biomed Eng.* **2020**, *4* (12), 1180–1187.
- (9) Li, Y.; Xia, L. *AJR, Am. J. Roentgenol.* **2020**, *214* (6), 1280–1286.
- (10) Wang, Y.; Kang, H.; Liu, X.; Tong, Z. *J. Med. Virol.* **2020**, *92* (6), 538–539.
- (11) Arons, M. M.; Hatfield, K. M.; Reddy, S. C.; et al. *N. Engl. J. Med.* **2020**, *382* (22), 2081–2090.
- (12) Bi, Q.; Wu, Y.; Mei, S.; et al. *Lancet Infect. Dis.* **2020**, *20* (8), 911–919.
- (13) Savvides, C.; Siegel, R. *medRxiv* **2020**, DOI: 10.1101/2020.06.11.20129072.
- (14) He, X.; Lau, E. H. Y.; Wu, P.; et al. *Nat. Med.* **2020**, *26* (5), 672–675.
- (15) To, KK-W; Tsang, OT-Y; Leung, W.-S.; et al. *Lancet Infect. Dis.* **2020**, *20* (5), 565–574.
- (16) Zou, L.; Ruan, F.; Huang, M.; et al. *N. Engl. J. Med.* **2020**, *382* (12), 1177–1179.
- (17) Wölfel, R.; Corman, V. M.; Guggemos, W.; et al. *Nature* **2020**, *581* (7809), 465–469.
- (18) Yongchen, Z.; Shen, H.; Wang, X.; et al. *Emerging Microbes Infect.* **2020**, *9* (1), 833–836.
- (19) Lauer, S. A.; Grantz, K. H.; Bi, Q.; et al. *Ann. Intern. Med.* **2020**, *172* (9), 577–582.
- (20) Isho, B.; Abe, K. T.; Zuo, M. *Sci. Immunol.* **2020**, *5* (52), eabe5511.
- (21) Wajnberg, A.; Amanat, F.; Firpo, A. *Science* **2020**, *370*, 1227–1230.
- (22) Iyer, A. S.; Jones, F. K.; Nodoushani, A.; et al. *Sci. Immunol.* **2020**, *5* (52), No. eabe0367.
- (23) Rongqing, Z.; Li, M.; Song, H.; et al. *Clin. Infect. Dis.* **2020**, *71* (16), 2066–2072.
- (24) Lee, Y.-L.; Liao, C.-H.; Liu, P.-Y.; et al. *J. Infect.* **2020**, *81* (2), e55–e58.
- (25) Guo, L.; Ren, L.; Yang, S.; et al. *Clin. Infect. Dis.* **2020**, *71* (15), 778–785.
- (26) Zhao, J.; Yuan, Q.; Wang, H.; et al. *Clin. Infect. Dis.* **2020**, *71* (16), 2027–2034.
- (27) Long, Q.-X.; Liu, B.-Z.; Deng, H.-J.; et al. *Nat. Med.* **2020**, *26* (6), 845–848.
- (28) Amanat, F.; Stadlbauer, D.; Strohmeier, S.; et al. *Nat. Med.* **2020**, *26* (7), 1033–1036.
- (29) Padoan, A.; Sciacovelli, L.; Basso, D.; et al. *Clin. Chim. Acta* **2020**, *507*, 164–166.
- (30) Padoan, A.; Cosma, C.; Sciacovelli, L.; Faggian, D.; Plebani, M. *Clin. Chem. Lab. Med.* **2020**, *58* (7), 1081–1088.
- (31) Ceron, J. J.; Lamy, E.; Martinez-Subiela, S.; et al. *J. Clin. Med.* **2020**, *9* (5), 1491.
- (32) To, KK-W; Tsang, OT-Y; Yip, CC-Y; et al. *Clin. Infect. Dis.* **2020**, *71* (15), 841–843.
- (33) Wyllie, A. L.; Fournier, J.; Casanovas-Massana, A.; et al. *N. Engl. J. Med.* **2020**, *383* (13), 1283–1286.
- (34) Williams, E.; Bond, K.; Zhang, B.; Putland, M.; Williamson, D. A. *J. Clin. Microbiol.* **2020**, *58* (8), No. e00776-20.
- (35) Pisanic, N.; Randad, P. R.; Kruczynski, K.; et al. *J. Clin. Microbiol.* **2020**, *59*, e02204-20.
- (36) Medeiros da Silva, R. C.; Nogueira Marinho, L. C.; de Araujo Silva, D. N.; Costa de Lima, K.; Pirihi, F. Q.; Luz de Aquino Martins, A. R. *Travel Med. Infect. Dis.* **2020**, *38*, 101920.
- (37) Sui, Z.; Zhang, Y.; Tu, S. *J. Infect.* **2021**, *82*, e38–e40.
- (38) Sakanashi, D.; Asai, N.; Nakamura, A.; et al. *J. Infect. Chemother.* **2021**, *27* (1), 126–129.
- (39) Tromberg, B. J.; Schwetz, T. A.; Pérez-Stable, E. J.; et al. *N. Engl. J. Med.* **2020**, *383* (11), 1071–1077.
- (40) Rissin, D. M.; Kan, C. W.; Campbell, T. G.; et al. *Nat. Biotechnol.* **2010**, *28* (6), 595–599.
- (41) Ogata, A. F.; Maley, A. M.; Wu, C. *Clin. Chem.* **2020**, *66*, 1562.
- (42) Boom, R.; Sol, C. J.; Salimans, M. M.; Jansen, C. L.; Wertheim-Van Dillen, P. M.; van der Noordaa, J. *J. Clin. Microbiol.* **1990**, *28* (3), 495–503.
- (43) Oberacker, P.; Stepper, P.; Bond, D. M.; et al. *PLoS Biol.* **2019**, *17* (1), No. e3000107.
- (44) Vogels, C. B. F.; Watkins, A. E.; Harden, C. A. *Med* **2021**, *2* (3), 263-280.E6.
- (45) Rabe, B. A.; Cepko, C. *Proc. Natl. Acad. Sci. U. S. A.* **2020**, *117* (39), 24450–24458.
- (46) Smyrlaki, I.; Ekman, M.; Lentini, A.; et al. *Nat. Commun.* **2020**, *11* (1), 4812.
- (47) Gilboa, T.; Maley, A. M.; Ogata, A. F.; Wu, C.; Walt, D. R. *Adv. Healthcare Mater.* **2021**, *10* (4), 2001111.
- (48) Wang, X.; Walt, D. R. *Chem. Sci.* **2020**, *11* (30), 7896–7903.

(49) Cohen, L.; Hartman, M. R.; Amardey-Wellington, A.; Walt, D. R. *Nucleic Acids Res.* **2017**, *45* (14), No. e137.

(50) Fozouni, P.; Son, S.; Díaz de León Derby, M. *Cell* **2021**, *184* (2), 323–333.e9.

■ NOTE ADDED AFTER ASAP PUBLICATION

This paper was published on March 23, 2021, with an incorrect version of the Abstract and Table of Contents graphics. The corrected version was reposted on March 25, 2021.