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COMMENTARY

Finding the Middle Ground with the Clinical Laboratory's Role in SARS-CoV-2 Genomic Surveillance

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ABSTRACT Continued replacement of the dominant severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) lineages, and associated surges, highlights the importance of genomic surveillance to identify the next possible threats. Despite concerted efforts between clinical laboratories and public health to generate sequence data, the United States has lagged in percentage of SARS-CoV-2 cases sequenced. A more simple and cost-effective option is needed to allow front-line clinical laboratories to perform high-throughput surveillance and refer important samples for slow and expensive next-generation sequencing (NGS). In this issue of the *Journal of Clinical Microbiology*, A. Babiker, K. Immergluck, S. D. Stampfer, A. Rao, et al. (J Clin Microbiol 59:e01446-21, 2021, https://doi.org/10.1128/JCM.01446-21) describe a rapid and flexible multiplex single-nucleotide polymorphism (SNP) assay targeting mutations associated with Alpha, Beta/Gamma, and, added later, Delta variants. They show 100% accuracy in characterized variant pools and clinical samples confirmed by NGS. Such an approach could be a happy medium in the role of front-line laboratories to assist with critically needed high-throughput genomic surveillance.

More than 18 months into the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, we are still struggling nationally and globally to control continued spread of the virus despite U.S. Food and Drug Administration (FDA) emergency use authorization (EUA) and, in one case approval, of effective vaccines. This is in part due to the waves of SARS-CoV-2 variant viruses, some of which are more transmissible (1, 2) and at least partially evade immune responses, including those induced by vaccines (3, 4). We have seen the replacement of the dominant lineage numerous times in various geographic regions since the initial cases were sequenced (5), with the United States and the world most recently dominated by the World Health Organization-labeled Delta variant (6). Concerns over reduced vaccine efficacy against the Delta variant, especially for those who received a single dose or in whom significant time has passed since the second dose (7, 8), as well as evidence of potential transmission between vaccinated individuals (9), has appropriately renewed focus on infection control measures and vaccination programs, with booster doses currently under review with the FDA (https://www.fda.gov/news-events/press-announcements/joint-statement-hhs-public-health-and-medical-experts-covid-19-booster-shots). This pattern of repeated replacement of the dominant lineage raises the questions of which variant will dominate next and when we might identify a dreaded variant of high consequence. No doubt the key to finding these emerging and potentially dominating variants is routine surveillance, including wide-spread diagnostic testing and genome sequencing.

Next-generation sequencing (NGS)-based virus genotyping directly from SARS-CoV-2 antigen or molecular test-positive patient samples is currently the most comprehensive and informative approach to genomic surveillance. This methodology is highly specialized and not at all standardized across laboratories, despite efforts to share best practices and common protocols initiated by the CDC's SARS-CoV-2 Sequencing for Public Health Emergency Response,

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Epidemiology and Surveillance (SPHERES) program. NGS for SARS-CoV-2 genome analysis is slow, in that it typically requires batching, includes several wet-bench steps that can be automated or performed manually, and, for high-throughput systems, takes at least 24 h until data are generated. Once sequence data are available, the analysis requires a sophisticated workforce that includes bioinformatics expertise. Added to that is a significant cost, not only for the equipment and consumables, but also for the expertise required.

U.S. genomic surveillance of this kind has been primarily a public health program supported by federal and/or state money (10, 11). However, at least in the early days of the pandemic, SARS-CoV-2 genome sequencing was largely supported by academic and private institutions that took it upon themselves to perform SARS-CoV-2 NGS via much of their own preexisting infrastructure, including analysis pipelines and funding sources (12). Some states have organized consortia that include funding support of health system-based or other laboratories outside public health (13), while select academic and private laboratories have received federal funding via the SPHERES program (https://www.cdc.gov/amd/whats-new/cdc-announces-awards-SARS-CoV-2-sequencing-SPHERES-initiative.html). The U.S. Centers for Medicare and Medicaid services have even allowed non-CLIA laboratories to perform SARS-CoV-2 patient sample sequencing only if such results were reported to public health and not back to the patient or physician. Despite these combined efforts, the United States has received criticism for lagging far behind other developed countries in terms of the percentage of SARS-CoV-2 cases sequenced (https://www.gisaid.org/submission-tracker-global/), and there is tremendous variability between the percentages of positive cases sequenced at the state level (14).

As these genomic surveillance efforts are critical to controlling the current pandemic, more emphasis needs to be placed on reasonable approaches to include front-line clinical laboratories in this process, since they have immediate access to patient specimens. There remains a gap between what is financially feasible for front-line clinical laboratories and what, if any, direct impacts there might be on patient care. As mentioned, SARS-CoV-2 NGS is expensive, slow, and requires expertise (15) that is not accessible for most clinical laboratories, except those in which NGS is already employed for research and/or clinical purposes, making it impractical for use in routine coronavirus disease 2019 (COVID-19) patient care. As an alternative to SARS-CoV-2 NGS, several studies have investigated PCR-based detection of relevant single-nucleotide polymorphisms (SNPs) that may correlate with specific variants or lineages and virus phenotypes. These PCR-based SNP detection approaches employ either melt analyses or probe dropout to determine base identity, similar to the spike gene dropout analysis approaches first used to identify the Alpha variant via commercial SARS-CoV-2 detection assays (16). SNP detection approaches are faster, less expensive, and require less technical expertise than NGS-based genotyping approaches (17).

Babiker et al. describe their version of an SNP detection approach to SARS-CoV-2 genotype inference using an initial set of multiplexed locked nucleic acid (LNA) probes targeting relevant SNPs after amplification of the spike gene (18). At the time of assay design, targeted SNPs were most commonly associated with the Beta and Gamma variants (484K); Alpha, Beta, and Gamma variants (501Y); and a smart partial positive control probe targeting the K417 position that is deleted in Beta and Gamma variants but appears to be present in all other lineages sequenced to date (https://www.gisaid.org/hcov19-variants/). Thus, a Beta or Gamma variant would be expected to test positive for the 484K and 501Y probes, but negative for the K417 probe. They applied this initial multiplex assay to dilution pools of characterized and quantified variants, showing 100% SNP detection accuracy for samples with at least 1,000 genome equivalents per milliliter, which was comparable to limit of detection (LOD) analysis using Alpha and Iota variants that demonstrated an LOD of 1 or 2 genome equivalents per reaction. Thus, the sensitivity of this approach appears ever so slightly less than those of many of the commercially available molecular detection assays (19–21). They next applied their assay to clinical samples obtained between January and March 2021, when the Alpha variant was not yet dominant in the United States. Perhaps not surprisingly they found that the majority of samples for which they had a spike SNP detection (n = 238, 91%), SNP profiles were consistent with prevariant lineages (91%). They

did detect 9 samples (3.8%) with Alpha lineage-consistent SNP profiles and 12 samples (5%) with Beta/Gamma-consistent SNP profiles at a time when Beta/Gamma variants were rarely reported in the United States. (https://www.gisaid.org/hcov19-variants/). The authors confirmed a subset of their detections with NGS, including most of the Alpha and Beta/Gamma detections, showing 100% accuracy. The assay did miss detections at high threshold cycle (C_T) values, consistent with their LOD analyses, but still showed superior sensitivity compared to that of NGS-based approaches (22). To adapt to the ever-changing variant situation, toward the end of the study the authors, added an unmodified (non-LNA) probe targeting the 452R mutation consistent with the Delta variant or lineage. While they did not extensively test this addition to their multiplex assay, it appeared not to impact specificity in previously tested variant pools and detected the SNP in appropriate variant pools and clinical samples, though the latter data are not shown.

The approach of Babiker et al. is promising as a rapid, simple, and flexible alternative to NGS-based genotyping. However, the authors point out several appropriate limitations to SNP-based genotyping, including the shared SNPs between several emerging lineages. For example, the 452R SNP is shared between Delta and Lambda, lineages while the 484K SNP is now shared between Beta, Gamma, Mu, Eta, and Iota lineages. As expected, SNP detection provides lower resolution than NGS-based genotyping; however, it does have advantages beyond those already mentioned, in that it appears to be more analytically sensitive and, as the authors point out, could be useful in settings where viral titers are lower.

Other studies have described similar approaches with equivalent success (23–26). One other recent study targeted 3 of the same SNPs (484K, 501Y, and 452R) using dually labeled probes while specifically amplifying the spike regions of interest, rather than the one-amplicon approach of Babiker et al. In their study, Wang et al. found 100% agreement between SNP detection and NGS results, though they intentionally excluded samples with low viral titers (23). Taken together, these studies support the high sensitivity and specificity of SNP-based genotyping approaches.

What remains to be demonstrated is the impact of SARS-CoV-2 SNP detection on public health surveillance and/or infection control programs. A recently published study performed in Canada applying prospective detection of SNPs associated with the Alpha and Beta/Gamma lineages rapidly detected the emergence of the Gamma lineage (27). After observation of a set of atypical melt curves and sequence confirmation via NGS, the authors were able to identify a discrete transmission cluster. This study highlights a potential role for the combined use of SNP detection as a screening assay followed by NGS for select cases of interest. One could envision a coordinated network in which front line clinical laboratories would refer samples of interest based on SNP findings to a central hub, such as an academic medical center or state public health laboratory, for NGS interrogation. The funding mechanisms of such a network would need to be carefully considered, as clinical laboratory budgets revolve around testing that impacts patient care and is ideally reimbursable. Further consideration would need to be given to the rotation of relevant SNPs included in multiplex assays. As variants become dominant, it may no longer be useful to screen for them. For example, at the end of August 2021, >98% of all U.S. SARS-CoV-2 specimens sequenced belonged to the Delta lineage harboring the 452R SNP (https://covid.cdc.gov/covid-data-tracker/#variant-proportions). Thus, further screening for this SNP seems unnecessary, and focus may need to shift to SNPs associated with emerging variants of interest, such as 346K, which is associated with the Mu lineage.

The role of SNP detection or, for that matter, that of NGS-based genotyping in direct patient care has yet to be established. Most studies cite the implications for monoclonal antibody susceptibility, yet no studies have applied a companion diagnostic approach in which SNP detection or NGS genotyping has been used to guide administration of bamlanivimab and etesevimab. Similarly, identification of 484K and 501Y comutations implies reduced sensitivity of the virus to neutralizing antibodies (28), yet no studies have used detection of such mutations to guide administration of convalescent plasma. Detection of reinfection could be determined by SNP detection or by NGS-based genotyping, but there is currently no guidance as to how identification of reinfection alters patient management, such as administration

of additional vaccine doses or specific therapeutics. As previously mentioned, in order to be realistically adopted, clinical laboratory testing is ideally reimbursed by insurance, and in order to receive reimbursement, clinical utility must be demonstrated. Finally, while SNP detection and NGS-based genotyping assays are not currently regulated by the FDA, there may come a time at which emergency use authorization is required for reporting such results in the patient's medical record. This could limit the availability and feasibility of the application of such assays.

In summary, SARS-CoV-2 SNP detection assays show promise as a "middle ground" alternative to costly and time-consuming NGS-based genotyping assays. Their speed and simplicity could allow widespread adoption and high-throughput surveillance for SARS-CoV-2 variants in front-line clinical laboratories. Partnership with hub centers to perform higher-resolution NGS analysis on select samples could speed coordinated surveillance and infection control efforts. In order to achieve such a surveillance partnership effort, financial support would need to be established, since there is currently no direct or reimbursable impact to patient care.

REFERENCES

1. Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, Hengartner N, Giorgi EE, Bhattacharya T, Foley B, Hastie KM, Parker MD, Partridge DG, Evans CM, Freeman TM, de Silva TI, Sheffield C-GG, McDanal C, Perez LG, Tang H, Moon-Walker A, Whelan SP, LaBranche CC, Saphire EO, Montefiori DC, Sheffield COVID-19 Genomics Group. 2020. Tracking changes in SARS-CoV-2 spike: evidence that D614G increases infectivity of the COVID-19 virus. Cell 182:812–827.e19. https://doi.org/10.1016/j.cell.2020.06.043.
2. Liu Y, Rocklov J. 2021. The reproductive number of the Delta variant of SARS-CoV-2 is far higher compared to the ancestral SARS-CoV-2 virus. J Travel Med https://doi.org/10.1093/jtm/taab124.
3. Chen RE, Zhang X, Case JB, Winkler ES, Liu Y, VanBlargan LA, Liu J, Errico JM, Xie X, Suryadevara N, Gilchuk P, Zost SJ, Tahan S, Droit L, Turner JS, Kim W, Schmitz AJ, Thapa M, Wang D, Boon ACM, Presti RM, O'Halloran JA, Kim AHJ, Deepak P, Pinto D, Fremont DH, Crowe JE, Jr, Corti D, Virgin HW, Ellebedy AH, Shi PY, Diamond MS. 2021. Resistance of SARS-CoV-2 variants to neutralization by monoclonal and serum-derived polyclonal antibodies. Nat Med 27:717–726. https://doi.org/10.1038/s41591-021-01294-w.
4. Planas D, Veyer D, Baidaliuk A, Staropoli I, Guivel-Benhassine F, Rajah MM, Planchais C, Porrot F, Robillard N, Puech J, Prot M, Gallais F, Gantner P, Velay A, Le Guen J, Kassis-Chikhani N, Edriss D, Belec L, Seve A, Courtellemont L, Pere H, Hocqueloux L, Fafi-Kremer S, Prazuck T, Mouquet H, Bruel T, Simon-Loriere E, Rey FA, Schwartz O. 2021. Reduced sensitivity of SARS-CoV-2 variant Delta to antibody neutralization. Nature 596:276–280. https://doi.org/10.1038/s41586-021-03777-9.
5. Du Plessis L, McCrone JT, Zarebski AE, Hill V, Ruis C, Gutierrez B, Raghwani J, Ashworth J, Colquhoun R, Connor TR, Faria NR, Jackson B, Loman NJ, O'Toole A, Nicholls SM, Parag KV, Scher E, Vasylyeva TI, Volz EM, Watts A, Bogoch II, Khan K, Consortium C-GU, Aanensen DM, Kraemer MUG, Rambaut A, Pybus OG, COVID-19 Genomics UK (COG-UK) Consortium. 2021. Establishment and lineage dynamics of the SARS-CoV-2 epidemic in the UK. Science 371:708–712. https://doi.org/10.1126/science.abf2946.
6. Konings F, Perkins MD, Kuhn JH, Pallen MJ, Alm EJ, Archer BN, Barakat A, Bedford T, Bhiman JN, Caly L, Carter LL, Cullinane A, de Oliveira T, Druce J, El Masry I, Evans R, Gao GF, Gorbalenya AE, Hamblion E, Herring BL, Hodcroft E, Holmes EC, Kakkar M, Khare S, Koopmans MPG, Korber B, Leite J, MacCannell D, Marklewitz M, Maurer-Stroh S, Rico JAM, Munster VJ, Neher R, Munnink BO, Pavlin BI, Peiris M, Poon L, Pybus O, Rambaut A, Resende P, Subissi L, Thiel V, Tong S, van der Werf S, von Gottberg A, Ziebuhr J, Van Kerkhove MD. 2021. SARS-CoV-2 variants of interest and concern naming scheme conducive for global discourse. Nat Microbiol 6:821–823. https://doi.org/10.1038/s41564-021-00932-w.
7. Pouwels KBP, Matthews PC, Stoesser N, Eyre DW, Vihta K-D, House T, Hay J, Bell JI, Newton JN, Farrar J, Crook D, Cook D, Rourke E, Studley R, Peto T, Diamond I, Walker AS, the COVID-19 Infection Survey Team. 2021. Impact of Delta on viral burden and vaccine effectiveness against new SARS-CoV-2 infections in the UK. medRxiv https://doi.org/10.1101/2021.08.18.21262237.
8. Scobie HMJ, Suthar AB, Severson R, Alden NB, Balter S, et al. 2021. Monitoring incidence of COVID-19 cases, hospitalizations, and deaths, by vaccination status—13 U.S. jurisdictions, April 4–July 17, 2021. MMWR Morb Mortal Wkly Rep 70:1284–1290. https://doi.org/10.15585/mmwr.mm7037e1.
9. Brown CM, Vostok J, Johnson H, Burns M, Gharpure R, Sami S, Sabo RT, Hall N, Foreman A, Schubert PL, Gallagher GR, Fink T, Madoff LC, Gabriel SB, MacInnis B, Park DJ, Siddle KJ, Harik V, Arvidson D, Brock-Fisher T, Dunn M, Kearns A, Laney AS. 2021. Outbreak of SARS-CoV-2 infections, including COVID-19 vaccine breakthrough infections, associated with large public gatherings—Barnstable County, Massachusetts, July 2021. MMWR Morb Mortal Wkly Rep 70:1059–1062. https://doi.org/10.15585/mmwr.mm7031e2.
10. Duras E, Irdem A, Ozkaya O. 2017. Long QT syndrome diagnosed in two sisters with propionic acidemia: a case report. J Pediatr Endocrinol Metab 30:1133–1136. https://doi.org/10.1515/jpem-2016-0469.
11. Blanton L, Wentworth DE, Alabi N, Azziz-Baumgartner E, Barnes J, Brammer L, Burns E, Davis CT, Dugan VG, Fry AM, Garten R, Grohskopf LA, Gubareva L, Kniss K, Lindstrom S, Mustaquim D, Olsen SJ, Roguski K, Taylor C, Trock S, Xu X, Katz J, Jernigan D. 2017. Update: influenza activity—United States and worldwide, May 21–September 23, 2017. MMWR Morb Mortal Wkly Rep 66:1043–1051. https://doi.org/10.15585/mmwr.mm6639a3.
12. Bedford T, Greninger AL, Roychoudhury P, Starita LM, Famulare M, Huang M-L, Nalla A, Pepper G, Reinhardt A, Xie H, Shrestha L, Nguyen TN, Adler A, Brandstetter E, Cho S, Giroux D, Han PD, Fay K, Frazar CD, Ilcisin M, Lacombe K, Lee J, Kiavand A, Richardson M, Sibley TR, Truong M, Wolf CR, Nickerson DA, Rieder MJ, Englund JA, Hadfield J, Hodcroft EB, Huddleston J, Moncla LH, Müller NF, Neher RA, Deng X, Gu W, Federman S, Chiu C, Duchin JS, Gautom R, Melly G, Hiatt B, Dykema P, Lindquist S, Queen K, Tao Y, Uehara A, Tong S, Seattle Flu Study Investigators, et al. 2020. Cryptic transmission of SARS-CoV-2 in Washington state. Science 370:571–575. https://doi.org/10.1126/science.abc0523.
13. Ladner JT, Larsen BB, Bowers JR, Hepp CM, Bolyen E, Folkerts M, Sheridan K, Pfeiffer A, Yaglom H, Lemmer D, Sahl JW, Kaelin EA, Maqsood R, Bokulich NA, Quirk G, Watts TD, Komatsu KK, Waddell V, Lim ES, Caporaso JG, Engelthaler DM, Worobey M, Keim P. 2020. An early pandemic analysis of SARS-CoV-2 population structure and dynamics in Arizona. mBio 11:e02107-20. https://doi.org/10.1128/mBio.02107-20.
14. Crawford DC, Williams SM. 2021. Global variation in sequencing impedes SARS-CoV-2 surveillance. PLoS Genet 17:e1009620. https://doi.org/10.1371/journal.pgen.1009620.
15. Jacot D, Pillonel T, Greub G, Bertelli C. 2021. Assessment of SARS-CoV-2 genome sequencing: quality criteria and low frequency variants. J Clin Microbiol 59:e00944-21. https://doi.org/10.1128/JCM.00944-21.
16. Firestone MJ, Lorentz AJ, Wang X, Como-Sabetti K, Vetter S, Smith K, Holzbauer S, Meyer S, Ehresmann K, Danila R, Lynfield R. 2021. First identified cases of SARS-CoV-2 variant B.1.1.7 in Minnesota—December 2020–January 2021. MMWR Morb Mortal Wkly Rep 70:278–279. https://doi.org/10.15585/mmwr.mm7008e1.
17. Harper H, Burridge A, Winfield M, Finn A, Davidson A, Matthews D, Hutchings S, Vipond B, Jain N, Consortium C-GU, Edwards K, Barker G, The COVID-19 Genomics UK (COG-UK) Consortium. 2021. Detecting SARS-CoV-2 variants with SNP genotyping. PLoS One 16:e0243185. https://doi.org/10.1371/journal.pone.0243185.
18. Babiker A, Immergluck K, Stampfer SD, Rao A, Bassit L, Su M, Nguyen V, Stittleburg V, Ingersoll JM, Bradley HL, Mavigner M, Schoof N, Kraft CS, Chahroudi A, Schinazi RF, Martin GS, Piantadosi A, Lam WA, Waggoner JJ.

2021. Single-amplicon multiplex real-time reverse transcription-PCR with tiled probes to detect SARS-CoV-2 *spike* mutations associated with variants of concern. J Clin Microbiol 59:e01446-21. https://doi.org/10.1128/JCM.01446-21.
19. Jian MJ, Chung HY, Chang CK, Lin JC, Yeh KM, Chen CW, Li SY, Hsieh SS, Liu MT, Yang JR, Tang SH, Perng CL, Chang FY, Shang HS. 2021. Clinical comparison of three sample-to-answer systems for detecting SARS-CoV-2 in B.1.1.7 lineage emergence. Infect Drug Resist 14:3255–3261. https://doi.org/10.2147/IDR.S328327.
20. Perchetti GA, Pepper G, Shrestha L, LaTurner K, Yae Kim D, Huang ML, Jerome KR, Greninger AL. 2021. Performance characteristics of the Abbott Alinity m SARS-CoV-2 assay. J Clin Virol 140:104869. https://doi.org/10.1016/j.jcv.2021.104869.
21. Norz D, Frontzek A, Eigner U, Oestereich L, Wichmann D, Kluge S, Fischer N, Aepfelbacher M, Pfefferle S, Lutgehetmann M. 2020. Pushing beyond specifications: evaluation of linearity and clinical performance of the cobas 6800/8800 SARS-CoV-2 RT-PCR assay for reliable quantification in blood and other materials outside recommendations. J Clin Virol 132:104650. https://doi.org/10.1016/j.jcv.2020.104650.
22. Zhang W, Govindavari JP, Davis BD, Chen SS, Kim JT, Song J, Lopategui J, Plummer JT, Vail E. 2020. Analysis of genomic characteristics and transmission routes of patients with confirmed SARS-CoV-2 in Southern California during the early stage of the US COVID-19 pandemic. JAMA Netw Open 3:e2024191. https://doi.org/10.1001/jamanetworkopen.2020.24191.
23. Wang H, Miller JA, Verghese M, Sibai M, Solis D, Mfuh KO, Jiang B, Iwai N, Mar M, Huang C, Yamamoto F, Sahoo MK, Zehnder J, Pinsky BA. 2021. Multiplex SARS-CoV-2 genotyping reverse transcriptase PCR for population-level variant screening and epidemiologic surveillance. J Clin Microbiol 59:e00859-21. https://doi.org/10.1128/JCM.00859-21.
24. Perchetti GA, Zhu H, Mills MG, Shrestha L, Wagner C, Bakhash SM, Lin MJ, Xie H, Huang ML, Mathias P, Bedford T, Jerome KR, Greninger AL, Roychoudhury P. 2021. Specific allelic discrimination of N501Y and other SARS-CoV-2 mutations by ddPCR detects B.1.1.7 lineage in Washington State. J Med Virol 93:5931–5941. https://doi.org/10.1002/jmv.27155.
25. Vega-Magana N, Sanchez-Sanchez R, Hernandez-Bello J, Venancio-Landeros AA, Pena-Rodriguez M, Vega-Zepeda RA, Galindo-Ornelas B, Diaz-Sanchez M, Garcia-Chagollan M, Macedo-Ojeda G, Garcia-Gonzalez OP, Munoz-Valle JF. 2021. RT-qPCR assays for rapid detection of the N501Y, 69-70del, K417N, and E484K SARS-CoV-2 mutations: a screening strategy to identify variants with clinical impact. Front Cell Infect Microbiol 11:672562. https://doi.org/10.3389/fcimb.2021.672562.
26. Wang H, Jean S, Eltringham R, Madison J, Snyder P, Tu H, Jones DM, Leber AL. 2021. Mutation-specific SARS-CoV-2 PCR screen: rapid and accurate detection of variants of concern and the identification of a newly emerging variant with spike L452R mutation. J Clin Microbiol 59:e00926-21. https://doi.org/10.1128/JCM.00926-21.
27. Matic N, Lowe CF, Ritchie G, Stefanovic A, Lawson T, Jang W, Young M, Dong W, Brumme ZL, Brumme CJ, Leung V, Romney MG. 2021. Rapid detection of SARS-CoV-2 variants of concern, including B.1.1.28/P.1, British Columbia, Canada. Emerg Infect Dis 27:1673–1676. https://doi.org/10.3201/eid2706.210532.
28. Wang PM, Liu L, Iketani S, Luo Y. 2021. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. bioRxiv https://doi.org/10.1101/2021.01.25.428137.