Novel Assays for Molecular Detection of Severe Acute Respiratory Syndrome Coronavirus 2

Kyle G. Rodino, PhD, D(ABMM)[a,b], Kenneth P. Smith, PhD, D(ABMM)[c,d],
Matthew A. Pettengill, PhD, D(ABMM)[e,*]

KEYWORDS

- SARS-CoV-2 • COVID-19 • Molecular • Variant • Next-generation sequencing
- CRISPR-Cas • Microfluidics

KEY POINTS

- The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)/COVID-19 pandemic has placed considerable strain on diagnostic laboratories and driven considerable innovation in viral diagnostic assays.
- Multiple novel technologies, or novel adaptations of existing assays, have been developed that may contribute to diagnostic testing for COVID-19 and eventually other infectious diseases as well.
- Novel approaches to providing at-home or point-of-care diagnostic testing for infectious diseases may improve patient care and public health efforts.

INTRODUCTION

From the onset of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)/COVID-19 pandemic, there has been a major emphasis on molecular laboratory tests for the virus. Shortages in various testing supplies, the desire to increase testing capacity, and a push to make point-of-care or home-based testing available have

---

fostered considerable innovation for SARS-CoV-2 molecular diagnostics, advancements likely to be applicable to other diagnostic uses. The authors attempt to cover some of the most compelling novel types of molecular assays or novel approaches in adapting established molecular methodologies for SARS-CoV-2 detection or characterization.

EFFICIENCY ENHANCING ADAPTATIONS FOR MOLECULAR TESTING

The COVID-19 pandemic led to unprecedented demand for laboratory testing that far outpaced existing capacity. Shortages of supplies and labor exacerbated the problem, resulting in the need for improved efficiency of existing SARS-CoV-2 diagnostics, including specimen pooling and process enhancements. Novel diagnostic methodologies that increase efficiency or speed of testing are discussed elsewhere.

   The simplest way to increase testing efficiency is to pool multiple patient specimens in a single reverse transcription–polymerase chain reaction (RT-PCR) reaction. If a pool tests negative, all patients are therefore negative. If the pool tests positive, all patient specimens that comprise that pool must be tested individually to identify the positive patients. However, pooling may reduce sensitivity owing to sample dilution of weakly positive specimens.[1] It is also impractical to implement in high (>10%) prevalence settings where specimen pools are more likely to be positive, necessitating extensive retesting of individual patients.[2] However, as SARS-CoV-2 prevalence decreases, specimen pooling may become an attractive option.

   Efficiency can also be enhanced by implementing changes in the typical RT-PCR testing workflow. One of the most significant rate-limiting steps in RT-PCR is the extraction step. This step purifies nucleic acids from the milieu of patient cells and proteins present in primary specimens and reduces inhibitory substances. However, swabs in viral transport media (VTM) are a relatively simple sample matrix, and potential inhibition can easily be assessed by monitoring internal controls, suggesting extraction may not be necessary. As such, laboratories have developed "extraction-free" methods that rely on heat to inactivate virus and lyse cells but lack a traditional nucleic acid purification step. These methodologies are easy to implement and have been demonstrated to be faster than conventional methods while maintaining similar performance characteristics.[3,4]

VARIANT TARGETING POLYMERASE CHAIN REACTION

Viral replication is error prone, leading to mutations in the viral genome.[5] When these mutations confer a selective advantage, genomic variants can emerge and become dominant. From the beginning of the pandemic, variants with mutations deviating from the initial SARS-CoV-2 genomic sequence have be recognized and sorted into a variety of lineage classifications. Beyond these viral phylogenetic relationships, many health organizations have adapted variant classifications based on public health impact, with the Centers for Disease Control and Prevention (CDC) classifying SARS-CoV-2 variants into 3 groups: "variant of interest," "variant of concern," and "variant of high consequence." Each level of classification denotes more significant changes in overall prevalence, viral transmissibility, disease severity, antiviral resistance, and/or vaccine evasion. As such, detection of variants is of epidemiologic, and in some cases clinical, interest.

   SARS-CoV-2 variants are first identified by genetic sequencing (often next-generation sequencing [NGS], reviewed elsewhere in this issue). However, sequencing methodologies are time consuming, expensive, and difficult to deploy at large scale as a routine diagnostic. As such, most SARS-CoV-2 tests are performed

by other methods, with RT-PCR remaining the gold standard. RT-PCR tests rely on primers and probes that hybridize to known sequences within the viral genome. Therefore, these assays detect sequences for which they were designed with high sensitivity and specificity. However, when faced with a variant containing changes in the assay's genetic target, the virus may evade detection.[6] Furthermore, variants with no changes in the assay's target will be indistinguishable from any other positive result.

In some cases, knowledge of these limitations can be leveraged to identify variants. For example, the assay used in the United Kingdom's national SARS-CoV-2 testing system contains targets for the nucleocapsid gene (N), the spike gene (S), a gene of unknown function (ORFab). In November 2020, a cluster of cases in Kent, England was identified in which the N and ORFab targets yielded positive results, but the S gene was consistently negative (https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/959360/Variant_of_Concern_VOC_202012_01_Technical_Briefing_3.pdf).

Failure of one or more gene targets in an RT-PCR assay is referred to as gene dropout. This specific pattern of gene dropout was widely recognized and given the name S gene target failure (SGTF). Further investigation of this phenomenon led to the identification of the Alpha SARS-CoV-2 variant (also known as B.1.1.7), which contains a 6-nucleotide deletion within the probe binding site, precluding detection of the S gene. Since this discovery, other assays, including commercially available assays, have used SGTF as a proxy for the Alpha strain.[7] Although positive predictive value of SGTF is good in high-prevalence settings, the Alpha variant's nucleotide deletion also occurs in other variants (notably the Beta variant, also known as B.1.351). Detection of SGTF regained value with the emergence of Omicron, which shared the same deletion in the spike coding region with Alpha. Conveniently, the preceding Delta variant did not contain the same deletion, making SGTF a reliable proxy for classification as Omicron (BA.1).[8,9] This lack of specificity highlights an important limitation of using gene dropout as a detection method and suggests need for variant targeting PCR tests. In addition, relevant to this type of assay generally, the limit of detection (LoD) of the assay can create a "false-dropout" when a particular gene is not detected owing to low positive.

RT-PCR tests can be adapted for variant detection by incorporating variant-specific probes to existing assays. Typically, these would be multiplexed in the same reaction to allow detection of the widest possible number of variants.[10] However, multiplex assays suffer from the same limitations as single-plex assays in that genetic targets must be known in advance. Shifting variant makeup can render a panel with limited mutation targets obsolete or lose specificity if multiple lineages emerge with overlapping mutation combinations, which can be particularly challenging in the clinical laboratory given the significant time and financial investment needed for assay validation. Therefore, unbiased methods, such as genetic sequencing, will likely remain major methods of variant detection.

REVERSE TRANSCRIPTION–POLYMERASE CHAIN REACTION ASSAYS TARGETING REPLICATION INTERMEDIATES

Current CDC guidelines suggest discontinuation of SARS-CoV-2 isolation precautions by a time and symptom-based strategy. Although this policy, based on generalized viral kinetics and disease timeline, may be sufficient when applied broadly, data suggest prolonged disease and extended infectivity in severely immunocompromised populations.[11] Concerns surrounding the duration of infectivity and shedding of viable

SARS-CoV-2 have led to an interest in test-based strategies to determine the transmission potential. Drivers of this approach include informing decisions related to discontinuation of isolation precautions, admission to COVID-19-free units, and transfers to/from facilities, among others. Lacking a definitive test for infectiousness, several methods have been suggested as surrogates of infectivity, each with their own set of challenges.[12] These include repurposing of the cycle threshold (Ct) value obtained from diagnostic RT-PCR assays, which can further complicate interpretation given the qualitative nature of the assays, numerous sources of variability, and the inability to distinguish between live, replicating virus versus residual shedding of genetic material. Although traditional viral culture could serve to detect potentially infectious virus, biosafety considerations, lack of general availability, turnaround time, and questions around sensitivity make this an impractical and reliable option for transmission risk assessment. In an attempt to address these challenges, RT-PCR assays targeting SARS-CoV-2 replication intermediates have been designed. For SARS-CoV-2, a positive-sense, single-stranded RNA (ssRNA) virus, these intermediates, including negative-sense RNA and subgenomic RNAs (sgRNAs), are formed in the host cell as the virus replicates. Theoretically, replication intermediates are only present with viable, actively replicating virus and absent in cases of residual shedding of nonviable genomic RNA in the postinfectious phase.

sgRNAs, fractions of the genome that lead to the production of many viral proteins, have been suggested as diagnostics indicators for active viral replication and presumably productive, transmissible infection. This method, using a leader-specific primer, has been described by Wolfel and colleagues,[13] with sgRNAs sequenced and visualized on agarose gel. Several studies have used this method to assess active replication of SARS-CoV-2.[14–18] However, the presence of sgRNAs as indicators of active replication has been questioned. Other studies have found prolonged detection of sgRNAs, hypothesizing that the membrane-bound nature of SARS-CoV-2 replication provides protection against degradation of these genomic fragments.[19,20] Hogan and colleagues[21] developed and validated a negative-sense RNA RT-PCR assay, independently amplifying positive- and negative-sense RNA of the SARS-CoV-2 envelope gene. Based on the premise that the negative-sense replication intermediary is only present with active replication and rapidly degraded otherwise, the detection of negative-sense RNA was assessed for samples with a variety of standard RT-PCR Ct values. Detection of minus-strand RNA was associated with lower Ct value from the standard RT-PCR and was not detected in samples with high Ct values (>33). In a small fraction of samples, negative-sense RNA was detected in specimens from patients beyond the recommended time-based clearance window, suggesting that some patients may harbor actively replicating and potentially infectious virus for an extended period. This finding was corroborated in a small case series, whereby 2 patients remained SARS-CoV-2-positive for an extended period as measured by several testing modalities, including negative-sense RNA RT-PCR, sgRNA RT-PCR, and viral culture.[18] Although the presence of sgRNA or negative-sense RNA may provide an additional data point in a comprehensive risk assessment of transmission, further studies are needed to evaluate how the results, both positive and negative, from sgRNA and negative-sense RNA assays, correlate with and predict for the infectious potential of patients with SARS-CoV-2.

NEXT-GENERATION SEQUENCING

Although NGS as a technology was not new to the clinical laboratory during the COVID-19 pandemic, the diverse applications and impact deserve mention. NGS

contributed to the identification and public sharing of the first SARS-CoV-2 genome, providing the global audience with the genetic sequence needed to develop targeted assays to detect the virus.[22] Since then, generation and sharing of SARS-CoV-2 genomes have occurred at an unprecedented scale, with nearly 7 million submissions to GISAID at the end of 2021. These data have allowed exceptional insight into the shifting genomic characteristics of the virus around the globe. Benefits to robust, publicly accessible sequence data include quality assurance assessment of how emerging mutations impact primer/probe binding sites by clinical laboratories,[23–25] assay manufacturers, and Food and Drug Administration (https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/sars-cov-2-viral-mutations-impact-covid-19-tests, accessed 22 September 2021). Although mutations altering test performance have been infrequent, continued vigilance is warranted and made possible through broad genomic surveillance. NGS has also allowed outbreak and epidemiologic investigations. Examples include analysis of clustered infections to highlight superspreader events,[26] evaluation of SARS-CoV-2 reinfections,[27,28] genomic data to shed light on mutations that may impact vaccine efficacy,[29] and studies showing how persistent infection can lead to variant emergence.[30] As mentioned previously, NGS has allowed the recognition of emerging variants with significant public health implications, classification as variants of interest or variants or concern (https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-info.html, https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/, accessed 22 September 2021), and the ability to track variant emergence and spread throughout the world using sites such as Covariants.org and Outbreak.info. The emergence of Omicron again put variant recognition and tracking on the world stage. Following initial detection, public reporting of the new variant allowed rapid assessment around the globe, tracking spread in near real time.[31] Within days, multiple countries reported cases and in the coming weeks were able to illustrate Omicron's full displacement of Delta. With potential impact to public health, laboratory diagnostics, mitigation strategies, and clinical therapeutics, continued SARS-CoV-2 genomic surveillance remains a necessity. Although high-throughput, nonclinical whole-genome sequencing has not previously been a priority of clinical laboratories, clinical laboratory leaders should lend their expertise to sequencing cores or academic laboratories capable of sustaining SARS-CoV-2 genomic sequencing efforts.[32] Beyond the genomic sequence applications, several NGS-based assays have received emergency use authorization approval as targeted diagnostics. A variety of workflows have been described using Illumina and Oxford Nanopore Technologies sequencing platforms.

CLUSTERED REGULARLY INTERSPACED SHORT PALINDROMIC REPEATS-ASSOCIATED–BASED DIAGNOSTIC TESTING FOR SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2

CRISPR (clustered regularly interspaced short palindromic repeats) RNAs (crRNAs) and Cas (CRISPR associated) nucleases function in bacteria as a form of adaptive immunity (crRNA sequences are adaptable to new threats) wherein crRNAs from the bacterial host bind target nucleic acid sequences from foreign sources and initiate Cas-mediated hydrolysis of the nucleic acids, and for some Cas nucleases, once activated, promiscuous cleavage of adjacent nucleic acids nonspecifically. CRISPR-Cas systems have been become powerful tools used to manipulate genetic material[33] and have recently been applied to diagnostic testing, taking advantage of their sequence-specific activation to provide a detection signal for microorganism nucleic acid,[34]

typically following amplification by previously established methods, such as loop mediated amplification (LAMP), recombinase polymerase amplification (RPA), or PCR. Different Cas proteins operate on different nucleic acid complexes, for example, Cas 12a hydrolyzes double-stranded DNA and thus requires a reverse transcription phase for SARS-CoV-2,[35] whereas Cas 13a targets ssRNA and can be used in a direct detection application for SARS-CoV-2.[36]

Diagnostic assays using LAMP, RPA, and various flavors of PCR have existed for some time and constitute core technologies used in both commercially available and laboratory-developed tests for infectious diseases, using signal detection methods other than CRISPR-Cas systems. Although CRISPR-Cas systems do not amplify the target nucleic acid, a potential advantage of CRISPR-Cas in the detection phase is that some Cas nucleases produce signal amplification in that they can produce multiple signal molecule events per sequence-specific binding event. When coupled with a priming amplification phase, it may be possible to modestly improve on the limit of detection relative to standard molecular assays for SARS-CoV-2,[35,37] but in existing studies, the limit of detection is still similar to PCR and thus not likely to offer a meaningful difference in clinical sensitivity. Most studies evaluating CRISPR-Cas diagnostic applications use a paired amplification assay as described above, but to move CRISPR-Cas assays to the point of care and potentially reduce assay expense, it is possible to develop assays that use CRISPR-Cas detection directly on specimens with no nucleic acid extraction, and no preamplification.[36] Although there is some signal amplification, the lack of nucleic acid amplification does leave this approach with considerably higher limits of detection (>100×) relative to RT-PCR, which could considerably impact the clinical sensitivity of SARS-CoV-2 assays depending somewhat on the population tested and application.[38] Like some other molecular methodologies, CRISPR-Cas assays are amenable to scale up for high-throughput application and may perform well for SARS-CoV-2 without nucleic acid extraction procedures.[39] Although CRISPR-Cas technology offers the potential for signal boosting to modestly improve the limit of detection for compatible SARS-CoV-2 testing methodologies, and is also amenable to use for direct detection when sufficient target sequence is expected in specimens, it does not appear at this time that these types of applications will lead to significant improvement in test performance characteristics relative to RT-PCR and other established methods.

MICROFLUIDIC ASSAYS FOR SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2

Microfluidic devices essentially take advantage of the physical properties of fluids to direct or even manipulate the movement of fluid specimens through engineered microchannels or material substrates. These processes may naturally separate or concentrate an analyte of interest or may be made to do so by applying an electrical charge. Lateral flow assays are a very simplistic variety of microfluidic device, and commonly used and familiar to clinical microbiologists, but there are far more sophisticated device designs as well, and different microfluidic device designs have been evaluated for developing inexpensive diagnostic tests that require no, or less, equipment than standard types of diagnostic assays.[40,41] The authors limit further discussion here to microfluidic devices evaluated with molecular SARS-CoV-2 diagnostic assays.

Isotachophoresis (ITP), a method using electrophoresis to separate and concentrate charged analytes, was used in early 2020 to develop a novel microfluidic SARS-CoV-2 detection assay.[42] ITP helped perform nucleic acid extraction and concentration with limited reagent requirements, followed by off-chip LAMP and returning

to the microfluidic device for small-volume (0.2 μL) CRISPR-based detection phase in less than 30 minutes from start to finish. In a limited sample set, this test had relatively good positive percent agreement (94%) compared with a standard RT-PCR assay. Although the transition on and off chip for different phases does not result in an assay that would be appealing in its current form, this study was a technical achievement and demonstrates the potential to use ITP in a microfluidic device to reduce sample handling and dramatically reduce reagent requirements.

DISCLOSURE

The authors have nothing to disclose.

REFERENCES

1. Griesemer SB, Van Slyke G, St George K. Assessment of sample pooling for clinical SARS-CoV-2 testing. J Clin Microbiol 2021;59(4). https://doi.org/10.1128/JCM.01261-20.
2. Abdalhamid B, Bilder CR, McCutchen EL, et al. Assessment of specimen pooling to conserve SARS CoV-2 testing resources. Am J Clin Pathol 2020;153(6):715–8.
3. Smyrlaki I, Ekman M, Lentini A, et al. Massive and rapid COVID-19 testing is feasible by extraction-free SARS-CoV-2 RT-PCR. Nat Commun 2020;11(1):4812.
4. Dumm RE, Elkan M, Fink J, et al. Implementation of an extraction-free COVID RT-PCR workflow in a pediatric hospital setting. J Appl Lab Med 2021. https://doi.org/10.1093/jalm/jfab079.
5. Duffy S. Why are RNA virus mutation rates so damn high? Plos Biol 2018;16(8):e3000003. https://doi.org/10.1371/journal.pbio.3000003.
6. Ziegler K, Steininger P, Ziegler R, et al. SARS-CoV-2 samples may escape detection because of a single point mutation in the N gene. Euro Surveill 2020;25(39). https://doi.org/10.2807/1560-7917.ES.2020.25.39.2001650.
7. Wollschlager P, Todt D, Gerlitz N, et al. SARS-CoV-2 N gene dropout and N gene Ct value shift as indicator for the presence of B.1.1.7 lineage in a commercial multiplex PCR assay. Clin Microbiol Infect 2021. https://doi.org/10.1016/j.cmi.2021.05.025.
8. Enhancing readiness for Omicron (B.1.1.529): technical brief and priority actions for member states. World Health Organization. 2021. Available at: https://www.who.int/publications/m/item/enhancing-readiness-for-omicron-(b.1.1.529)-technical-brief-and-priority-actions-for-member-states.
9. Implications of the emergence and spread of the SARS-CoV-2 B.1.1. 529 variant of concern (Omicron) for the EU/EEA. European Center for Disease Prevention and Control. 2021. Available at: https://www.ecdc.europa.eu/sites/default/files/documents/Implications-emergence-spread-SARS-CoV-2%20B.1.1.529-variant-concern-Omicron-for-the-EU-EEA-Nov2021.pdf.
10. Vogels CBF, Breban MI, Ott IM, et al. Multiplex qPCR discriminates variants of concern to enhance global surveillance of SARS-CoV-2. Plos Biol 2021;19(5):e3001236.
11. Aydillo T, Gonzalez-Reiche AS, Aslam S, et al. Shedding of viable SARS-CoV-2 after immunosuppressive therapy for cancer. N Engl J Med 2020;383(26):2586–8.
12. Binnicker MJ. Can testing predict SARS-CoV-2 infectivity? The potential for certain methods to be a surrogate for replication-competent virus. J Clin Microbiol 2021;4:JCM0046921. https://doi.org/10.1128/JCM.00469-21.
13. Wolfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 2020;581(7809):465–9.

14. Corbett KS, Flynn B, Foulds KE, et al. Evaluation of the mRNA-1273 vaccine against SARS-CoV-2 in nonhuman primates. N Engl J Med 2020;383(16): 1544–55.

15. Perera R, Tso E, Tsang OTY, et al. SARS-CoV-2 virus culture and subgenomic RNA for respiratory specimens from patients with mild coronavirus disease. Emerg Infect Dis 2020;26(11):2701–4.

16. van Doremalen N, Lambe T, Spencer A, et al. ChAdOx1 nCoV-19 vaccine prevents SARS-CoV-2 pneumonia in rhesus macaques. Nature 2020;586(7830): 578–82.

17. Yu J, Tostanoski LH, Peter L, et al. DNA vaccine protection against SARS-CoV-2 in rhesus macaques. Science 2020;369(6505):806–11.

18. Truong TT, Ryutov A, Pandey U, et al. Increased viral variants in children and young adults with impaired humoral immunity and persistent SARS-CoV-2 infection: a consecutive case series. EBioMedicine 2021;67:103355. https://doi.org/10.1016/j.ebiom.2021.103355.

19. van Kampen JJA, van de Vijver D, Fraaij PLA, et al. Duration and key determinants of infectious virus shedding in hospitalized patients with coronavirus disease-2019 (COVID-19). Nat Commun 2021;12(1):267.

20. Alexandersen S, Chamings A, Bhatta TR. SARS-CoV-2 genomic and subgenomic RNAs in diagnostic samples are not an indicator of active replication. Nat Commun 2020;11(1):6059.

21. Hogan CA, Huang C, Sahoo MK, et al. Strand-specific reverse transcription PCR for detection of replicating SARS-CoV-2. Emerg Infect Dis 2021;27(2):632–5.

22. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 2020;382(8):727–33.

23. Vanaerschot M, Mann SA, Webber JT, et al. Identification of a polymorphism in the N gene of SARS-CoV-2 that adversely impacts detection by reverse transcription-PCR. J Clin Microbiol 2020;59(1). https://doi.org/10.1128/JCM.02369-20.

24. Leelawong M, Mitchell SL, Fowler RC, et al. SARS-CoV-2 N gene mutations impact detection by clinical molecular diagnostics: reports in two cities in the United States. Diagn Microbiol Infect Dis 2021;101(3):115468.

25. Tahan S, Parikh BA, Droit L, et al. SARS-CoV-2 E gene variant alters analytical sensitivity characteristics of viral detection using a commercial reverse transcription-PCR assay. J Clin Microbiol 2021;59(7):e0007521. https://doi.org/10.1128/JCM.00075-21.

26. Lemieux JE, Siddle KJ, Shaw BM, et al. Phylogenetic analysis of SARS-CoV-2 in Boston highlights the impact of superspreading events. Sci 2021;371(6529).

27. Harrington D, Kele B, Pereira S, et al. Confirmed reinfection with SARS-CoV-2 variant VOC-202012/01. Clin Infect Dis 2021. https://doi.org/10.1093/cid/ciab014.

28. Lee JS, Kim SY, Kim TS, et al. Evidence of severe acute respiratory syndrome coronavirus 2 reinfection after recovery from mild coronavirus disease 2019. Clin Infect Dis 2020. https://doi.org/10.1093/cid/ciaa1421.

29. Hacisuleyman E, Hale C, Saito Y, et al. Vaccine breakthrough infections with SARS-CoV-2 variants. N Engl J Med 2021;384(23):2212–8.

30. Choi B, Choudhary MC, Regan J, et al. Persistence and evolution of SARS-CoV-2 in an immunocompromised host. N Engl J Med 2020;383(23):2291–3.

31. New COVID-19 variant detected in South Africa. National Institute for Communicable Diseases (NICD). 2021. Available at: https://www.nicd.ac.za/new-covid-19-variant-detected-in-south-africa/.

32. Wang J, Hawken SE, Jones CD, et al. Collaboration between clinical and academic laboratories for sequencing SARS-CoV-2 genomes. J Clin Microbiol 2022;JCM0128821. https://doi.org/10.1128/JCM.01288-21.
33. Knott GJ, Doudna JA. CRISPR-Cas guides the future of genetic engineering. Science 2018;361(6405):866–9.
34. Gootenberg JS, Abudayyeh OO, Lee JW, et al. Nucleic acid detection with CRISPR-Cas13a/C2c2. Science 2017;356(6336):438–42.
35. Joung J, Ladha A, Saito M, et al. Detection of SARS-CoV-2 with SHERLOCK one-pot testing. N Engl J Med 2020;383(15):1492–4.
36. Fozouni P, Son S, Diaz de Leon Derby M, et al. Amplification-free detection of SARS-CoV-2 with CRISPR-Cas13a and mobile phone microscopy. Cell 2021; 184(2):323–333 e9.
37. Ning B, Yu T, Zhang S, et al. A smartphone-read ultrasensitive and quantitative saliva test for COVID-19. Sci Adv 2021;7(2). https://doi.org/10.1126/sciadv. abe3703.
38. Arnaout R, Lee RA, Lee GR, et al. The limit of detection matters: the case for benchmarking severe acute respiratory syndrome coronavirus 2 testing. Clin Infect Dis 2021. https://doi.org/10.1093/cid/ciaa1382.
39. Manning BJ, Khan WA, Pena JM, et al. High-throughput CRISPR-Cas13 SARS-CoV-2 test. Clin Chem 2021;68(1):172–80.
40. Bissonnette L, Bergeron MG. Diagnosing infections–current and anticipated technologies for point-of-care diagnostics and home-based testing. Clin Microbiol Infect 2010;16(8):1044–53.
41. Chin CD, Laksanasopin T, Cheung YK, et al. Microfluidics-based diagnostics of infectious diseases in the developing world. Nat Med 2011;17(8):1015–9.
42. Ramachandran A, Huyke DA, Sharma E, et al. Electric field-driven microfluidics for rapid CRISPR-based diagnostics and its application to detection of SARS-CoV-2. Proc Natl Acad Sci U S A 2020;117(47):29518–25.