



Review

CRISPR-Based Diagnostics for Point-of-Care Viral Detection

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Abstract: Point-of-care detection of viral infection is required for effective contact-tracing, epidemiological surveillance, and linkage to care. Traditional diagnostic platforms relying on either antigen detection or nucleic amplification are limited by sensitivity and the need for costly laboratory infrastructure, respectively. Recently, CRISPR-based diagnostics have emerged as an alternative, combining equipment light workflows with high specificity and sensitivity. However, as a nascent technology, several outstanding challenges to widespread field deployment remain. These include the need for pre-detection amplification of target molecules, the lack of standardization in sample preparation and reagent composition, and only equivocal assessments of the unit-economics relative to traditional antigen or polymerase chain reaction-based diagnostics. This review summarizes recent advances with the potential to overcome existing translational barriers, describes the events in CRISPR-based detection of target molecules, and offers perspective on how multiple approaches can be combined to decrease the limit of detection without introducing pre-amplification.

Keywords: viral diagnostics; CRISPR; point-of-care



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1. Introduction

Challenges in rapid detection and subsequent linkage to treatment for endemic viral diseases have long motivated efforts to develop point-of-care, saleable, resource-light diagnostic platforms [1–3]. More recently, the SARS-CoV-2 pandemic revealed the shortcomings of existing approaches in both resource-rich and scarce settings [4,5]. Limitations include the long turn-around times and high costs intrinsic to nucleic acid thermocycler-based diagnostics [5]. Moreover, the unexpected, yet rapidly evolving, nature of emerging viral threats is incompatible with diagnostic platforms requiring stationary laboratory infrastructure [6]. Though antigen-based assays circumvent these obstacles, the immense diversity of new and emerging viral threats severely limits the feasibility of antigen-based detection serving in a rapid-response capacity. This is partly related to the inherent lag-time in antibody identification and synthesis, and partly because highly divergent peak titers between viruses will require pathogen specific optimization [7,8]. In brief, existing platforms cannot achieve the simultaneous requirements of sensitivity, cross-pathogen adaptability, and rapid-response scalability.

Clustered regularly interspaced short palindromic repeat (CRISPR)-based diagnostics have been pursued as a solution to this problem [9–11]. As an adaptive component of microbial immunity, CRISPR systems recognize and discriminate foreign nucleic acids by sequence complementarity for subsequent endonucleic destruction via the Cas enzyme [12–14]. CRISPR-Cas sequence discrimination is mediated by CRISPR RNA (crRNA), which determines the target for endonuclease cleavage, and can be programmed, within efficiency-bounded parameters, to target virtually any existing or emerging virus [15]. The capacity for modular programmability initially recommended CRISPR-Cas-based platforms as a solution to rapid, sensitive, point-of-care diagnostics. In this review, I explore the recent developments in CRISPR-Cas diagnostics accelerated by the SARS-CoV-2 pandemic, and consider how the nascent platforms can be recommissioned to

serve longstanding viral elimination targets by truncating diagnostic lag and accelerating linkage-to-care.

2. CRISPR-Based Reporter Diagnostics

CRISPR-Cas systems evolved to identify, degrade, and retain molecular memory of pathogenic nucleic acids [13,16]. CRISPR immunity involves three sequential stages, defined by adaptation, crRNA synthesis, and nucleic-acid interference, during which, target sequences are enzymatically cleaved [14,15,17]. Sequence-specific targets are defined in the adaptive stage, during which, foreign sequences are incorporated into stretches of palindromic direct repeats for subsequent individuation into mature crRNAs possessing complementarity to their nucleic targets [13,17].

Though the basic choreography is conserved across CRISPR-Cas systems, they can be differentiated according to composition of their corresponding effector complex [18,19]. Because class 1 systems involve multiple effector proteins, and class 2 involves only a single protein, the ease of manipulation has favored adoption of class 2 systems for diagnostic platforms [20]. The basic diagnostic circuit, therefore, involves complementarity-determining crRNA design, crRNA-Cas enzyme complexing, and the detection of nuclease activity following target sequence recognition [20]. The components available for programming and optimization, therefore, include crRNA design, Cas enzyme selection, and reporter detection, which collectively comprise the molecular circuit.

crRNA requirements are largely determined by target sequence and Cas-specific parameters, such as proximity to protospacer adjacent motifs [21,22]. The selection of Cas-systems is, therefore, the initial step in diagnostic assay development, which itself is largely determined by the target pathogen, the sensitivity requirements, and the corresponding need for pre-amplification. For double-stranded DNA targeting, Cas9 and 12a enzymes are most commonly employed [23–27]. Typically, the limitations of a dsDNA recognizing enzyme in context of viral RNA targets are obviated by pre-amplifying target RNA [28]. However, for applications that are neither limited by high-limits of detection nor compatible with pre-amplification, ssRNA targeting by Cas 13 platforms has been pursued [29–31].

Another feature to differentiate Cas platforms is their reporter circuit compatibility. Cas9 target DNA sensing is detected by cleavage of a toehold trigger contiguous with PAM-containing sites [32]. Though commonly employed, this detection circuit imposes upstream constraints on crRNA design, which may correspond to a higher developmental failure rate when adapting to novel pathogens. An alternative detection circuit, enabled by the collateral trans-cleavage activity of Cas12a and Cas13, involves standardized, quencher-fluorophore coupled reporter molecules [33,34]. Collateral trans-cleavage activity refers to the capacity of Cas-enzymes to indiscriminately cleave single-stranded nucleic acids following crRNA target recognition [35]. By enabling Cas-enzyme and reporter standardization, Cas12a and 13 allow for discrimination of target molecules in a modular, fast-scaling platform requiring only crRNA optimization.

3. Improving the Limit of Detection

Point-of-care application of CRISPR-based diagnostics necessitates a limit of detection (LOD) commensurate with the concentration of viral nucleic acid in patient samples. Typically, Cas-enzyme-mediated collateral trans cleavage of fluorophores yields a picomolar LOD, which may be suitable for detection of high-titer infections, such as SARS-CoV-2 [36–38]. However, for diagnostic applications that require sub-picomolar LOD, such as point-of-care HCV or HIV detection, strategies to decrease LOD have been pursued [39,40]. These can be broadly classified as exponential, including pre-amplification and autocatalytic molecular circuits, or linear, including multi-sequence targeting and Cas-activated non-Cas nuclease amplification [30,41,42].

Most CRISPR-based diagnostics have employed pre-amplification of the target molecule to decrease LOD of the assay without increasing the sensitivity of the collateral-trans cleavage reaction [43]. Though any approach to pre-amplification of the target molecule is

amenable CRISPR-based detection, the practical constraints imposed by point-of-care diagnostics have favored isothermal approaches. Recombinase polymerase amplification (RPA) and loop-mediated isothermal amplification (LAMP) have been most commonly employed [20,34,44]. The advantage of RPA is near-ambient amplification without thermocycling [45]. Though non-specific amplification has been reported, the practical limits on specificity may be minimal, owing to subsequent CRISPR-based discrimination of the target sequence from non-specific amplification products. LAMP has the advantage of higher specificity pre-amplification, but introduces upstream complexities in terms of the multi-region primer design [46]. Both pre-amplification techniques are compatible with upstream reverse-transcription for the detection of viral RNA, though the requirements of three sequential reactions, including reverse-transcription, pre-amplification, and Cas-mediated trans-cleavage, introduces challenges in optimization that may limit real-world reproducibility.

To circumvent the challenges of multi-dimensional optimization, groups have sought to develop amplification-free strategies to decrease LOD [20,30,42]. One promising approach incorporates auto-catalytic, self-amplifying nucleic acid circuits activated by the initial target-sequence recognition, and perpetuated by inclusion of a dsDNA probe that serves as a substrate for gRNA liberated by the initial cleavage reaction [42]. Advantages include multi-target reproducibility without repeated optimization of the autocatalytic circuit. With this approach, which relies on a single step for the detection of target dsDNA, only the initial circuit activation, with corresponding gRNA design, needs to be engineered for each respective pathogen. Though atamolar sensitivity has been achieved with one described auto-catalytic circuit (CONAN), the LOD, at present, may be insufficient for the detection of low-copy number infections ($<10^4$). Coupling autocatalytic amplification with multi-sequence targeting linear amplification may further decrease LOD without pre-amplification [30]. However, increasing sensitivity by coupling multi-sequence targeting gRNA sets with autocatalytic amplification loops may come at the expense of specificity. To date, these approaches have not been combined, and the risks of indiscriminate circuit activation resulting in false positives have not been determined.

4. Point-of-Care Reproducibility

The primary advantage of CRISPR-based diagnostics is circumventing the laboratory infrastructure required for traditional PCR-based viral detection [47]. Accordingly, the practical-use-case involves point-of-care detection in resource-constrained settings. To successfully translate CRISPR-diagnostics from lab to field, approaches to limit the required laboratory infrastructure and the need for personnel training are required [48]. Single-pot isothermal amplification or single-step autocatalytic amplification promise to limit the required infrastructure [49]. However, reducing the resource and training requirements for sample preparation is also needed. Approaches for the sample inactivation and liberation of target molecules from virions, within buffers compatible with subsequent reactions, have been coupled with magnetic-bead-based target enrichment for single-tube sample preparation [50,51]. However, errors in user manipulation post-sample-preparation can still be introduced at the stage of reagent mixing before subsequent fluorophore detection. Accordingly, two approaches, predicated on either physically separating reagents within a tube-break format, or optimizing reagents for multi-step processing from sample extraction to fluorophore detection, have been pursued [49,51–53]. The latter is challenged by the distinct preparation requirements of each target pathogen in relation to virion composition.

The detection of liberated fluorophore following collateral cleavage must also be optimized for field conditions. The visual detection of fluorescence using handheld, low-cost, blue-light-emitting devices has been reported [48]. Alternatives include replacing fluorophore-quencher with a fluorescein amidite-biotin reporter that can be visualized via lateral flow without any specialized equipment, or direct physiochemical transformation of reporter buffer by using hydrogels whose viscosity is dependent on polymer anchorage by DNA molecules [40,54].

5. Conclusions

The SARS-CoV-2 pandemic accelerated the development of CRISPR-based diagnostics, with over fifteen platforms described in the literature [10,55–57]. However, despite these advances, several outstanding challenges, largely related to coupling CRISPR-based detection with isothermal pre-amplification, remain. Though direct detection of target molecules is feasible for some clinical applications, including early SARS-CoV-2 infection when titers peak, real-world operability and broader translation impose more stringent requirements for LOD [20]. Ongoing developments in optimizing isothermal amplification may circumvent these challenges, but, at present, the additional assay complexity introduced by sequential enzymatic reactions limits translatability. Efforts to introduce signal amplification through novel reporter molecules or self-perpetuating molecular circuits are at nascent stages of development, but hold promise in achieving single-reaction detection [42]. Further developments in reporter visualization must balance the competing constraints of sensitivity, which can be improved with specialized readers incompatible with point-of-care deployment, and scalability, which biases toward simple lateral flow or fluorescence detection at the expense of higher LOD [47]. Lastly, field deployment of CRISPR-based diagnostics must compete with traditional assays for which both accuracy and unit-economics have already been validated. These challenges are further aggravated by an evolving patent landscape that may limit access to the most promising protocols. Though the obsolescence of existing diagnostic infrastructure may accompany the transition to CRISPR-based diagnostics, it is more likely that new technologies will be integrated into existing workflows to augment the testing capacity during viral surges, or to minimize diagnostic gaps in remote, resource-limited settings. Continued investment in not only developing, but validating existing CRISPR-diagnostic workflows in real-world settings, with direct unit-economic comparison to existing technologies, is needed.

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References

1. Basile, K.; Kok, J.; Dwyer, D.E. Point-of-care diagnostics for respiratory viral infections. *Expert Rev. Mol. Diagn.* **2018**, *18*, 75–83. [[CrossRef](#)] [[PubMed](#)]
2. Heidt, B.; Siqueira, W.; Eersels, K.; Diliën, H.; Van Grinsven, B.; Fujiwara, R.; Cleij, T. Point of Care Diagnostics in Resource-Limited Settings: A Review of the Present and Future of PoC in Its Most Needed Environment. *Biosensors* **2020**, *10*, 133. [[CrossRef](#)] [[PubMed](#)]
3. Mashamba-Thompson, T.P.; Drain, P.K. Point-of-Care Diagnostic Services as an Integral Part of Health Services during the Novel Coronavirus 2019 Era. *Diagnostics* **2020**, *10*, 449. [[CrossRef](#)] [[PubMed](#)]
4. Manabe, Y.C.; Sharfstein, J.S.; Armstrong, K. The Need for and Limits of More and Better Testing for COVID-19. *JAMA* **2020**, *324*, 2153–2154. [[CrossRef](#)]
5. Duma, Z.; Chuturgoon, A.A.; Ramsuran, V.; Edward, V.; Naidoo, P.; Mpaka-Mbatha, M.N.; Bhengu, K.N.; Nembe, N.; Pillay, R.; Singh, R.; et al. The Challenges of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Testing in Low-Middle Income Countries and Possible Cost-Effective Measures in Resource-Limited Settings. *Glob. Health* **2022**, *18*, 5. [[CrossRef](#)]
6. Larremore, D.B.; Wilder, B.; Lester, E.; Shehata, S.; Burke, J.M.; Hay, J.A.; Milind, T.; Mina, M.J.; Parker, R. Test Sensitivity Is Secondary to Frequency and Turnaround Time for COVID-19 Surveillance. *MedRxiv* **2020**. [[CrossRef](#)]
7. Arnaout, R.; Lee, R.A.; Lee, G.R.; Callahan, C.; Cheng, A.; Yen, C.F.; Smith, K.P.; Arora, R.; Kirby, J.E. The Limit of Detection Matters: The Case for Benchmarking Severe Acute Respiratory Syndrome Coronavirus 2 Testing. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **2021**, *73*, e3042–e3046. [[CrossRef](#)]
8. Cubas-Atienzar, A.I.; Kontogianni, K.; Edwards, T.; Wooding, D.; Buist, K.; Thompson, C.R.; Williams, C.T.; Patterson, E.I.; Hughes, G.L.; Baldwin, L.; et al. Limit of detection in different matrices of 19 commercially available rapid antigen tests for the detection of SARS-CoV-2. *Sci. Rep.* **2021**, *11*, 18313. [[CrossRef](#)]
9. Hou, T.; Zeng, W.; Yang, M.; Chen, W.; Ren, L.; Ai, J.; Wu, J.; Liao, Y.; Gou, X.; Li, Y.; et al. Development and evaluation of a rapid CRISPR-based diagnostic for COVID-19. *PLoS Pathog.* **2020**, *16*, e1008705. [[CrossRef](#)]
10. Ganbaatar, U.; Liu, C. CRISPR-Based COVID-19 Testing: Toward Next-Generation Point-of-Care Diagnostics. *Front. Cell. Infect. Microbiol.* **2021**, *11*, 663949. [[CrossRef](#)]
11. Puig-Serra, P.; Casado-Rosas, M.C.; Martinez-Lage, M.; Olalla-Sastre, B.; Alonso-Yanez, A.; Torres-Ruiz, R.; Rodriguez-Perales, S. CRISPR Approaches for the Diagnosis of Human Diseases. *Int. J. Mol. Sci.* **2022**, *23*, 1757. [[CrossRef](#)]

12. Mohanraju, P.; Saha, C.; van Baarlen, P.; Louwen, R.; Staals, R.H.J.; van der Oost, J. Alternative Functions of CRISPR–Cas Systems in the Evolutionary Arms Race. *Nat. Rev. Genet.* **2022**, *20*, 351–364. [[CrossRef](#)]
13. Koonin, E.V.; Makarova, K.S. CRISPR-Cas: Evolution of an RNA-Based Adaptive Immunity System in Prokaryotes. *RNA Biol.* **2013**, *10*, 679–686. [[CrossRef](#)]
14. Mohanraju, P.; Makarova, K.S.; Zetsche, B.; Zhang, F.; Koonin, E.V.; van der Oost, J. Diverse evolutionary roots and mechanistic variations of the CRISPR-Cas systems. *Science* **2016**, *353*, aad5147. [[CrossRef](#)]
15. Barrangou, R.; Marraffini, L.A. CRISPR-Cas Systems: Prokaryotes Upgrade to Adaptive Immunity. *Mol. Cell* **2014**, *54*, 234–244. [[CrossRef](#)]
16. Van der Oost, J.; Jore, M.M.; Westra, E.R.; Lundgren, M.; Brouns, S.J. CRISPR-based adaptive and heritable immunity in prokaryotes. *Trends Biochem. Sci.* **2009**, *34*, 401–407. [[CrossRef](#)]
17. Garneau, J.E.; Dupuis, M.-È.; Villion, M.; Romero, D.A.; Barrangou, R.; Boyaval, P.; Fremaux, C.; Horvath, P.; Magadán, A.H.; Moineau, S. The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. *Nature* **2010**, *468*, 67–71. [[CrossRef](#)]
18. Makarova, K.S.; Wolf, Y.I.; Alkhnbashi, O.S.; Costa, F.; Shah, S.A.; Saunders, S.J.; Barrangou, R.; Brouns, S.J.J.; Charpentier, E.; Haft, D.H.; et al. An updated evolutionary classification of CRISPR–Cas systems. *Nat. Rev. Microbiol.* **2015**, *13*, 722–736. [[CrossRef](#)]
19. Makarova, K.S.; Wolf, Y.; Koonin, E.V. The Basic Building Blocks and Evolution of CRISPR-CAS Systems. *Biochem. Soc. Trans.* **2013**, *41*, 1392–1400. [[CrossRef](#)]
20. Kaminski, M.M.; Abudayyeh, O.O.; Gootenberg, J.S.; Zhang, F.; Collins, J.J. CRISPR-based diagnostics. *Nat. Biomed. Eng.* **2021**, *5*, 643–656. [[CrossRef](#)]
21. Bolotin, A.; Quinquis, B.; Sorokin, A.; Ehrlich, S.D. Clustered Regularly Interspaced Short Palindrome Repeats (CRISPRs) Have Spacers of Extrachromosomal Origin. *Microbiology* **2005**, *151*, 2551–2561. [[CrossRef](#)]
22. Hille, F.; Richter, H.; Wong, S.P.; Bratovič, M.; Ressel, S.; Charpentier, E. The Biology of CRISPR-Cas: Backward and Forward. *Cell* **2018**, *172*, 1239–1259. [[CrossRef](#)]
23. Osborn, M.; Bhardwaj, A.; Bingea, S.; Knipping, F.; Feser, C.; Lees, C.; Collins, D.; Steer, C.; Blazar, B.; Tolar, J. CRISPR/Cas9-Based Lateral Flow and Fluorescence Diagnostics. *Bioengineering* **2021**, *8*, 23. [[CrossRef](#)] [[PubMed](#)]
24. Zhang, H.; Qin, C.; An, C.; Zheng, X.; Wen, S.; Chen, W.; Liu, X.; Lv, Z.; Yang, P.; Xu, W.; et al. Application of the CRISPR/Cas9-based gene editing technique in basic research, diagnosis, and therapy of cancer. *Mol. Cancer* **2021**, *20*, 126. [[CrossRef](#)] [[PubMed](#)]
25. Vangah, S.J.; Katalani, C.; Boone, H.A.; Hajizade, A.; Sijercic, A.; Ahmadian, G. CRISPR-Based Diagnosis of Infectious and Noninfectious Diseases. *Biol. Proced. Online* **2020**, *22*, 22. [[CrossRef](#)] [[PubMed](#)]
26. Singh, M.; Bindal, G.; Misra, C.S.; Rath, D. The era of Cas12 and Cas13 CRISPR-based disease diagnosis. *Crit. Rev. Microbiol.* **2022**, *1–16*. [[CrossRef](#)] [[PubMed](#)]
27. Broughton, J.P.; Deng, X.; Yu, G.; Fasching, C.L.; Servellita, V.; Singh, J.; Miao, X.; Streithorst, J.A.; Granados, A.; Sotomayor-Gonzalez, A.; et al. CRISPR–Cas12-based detection of SARS-CoV-2. *Nat. Biotechnol.* **2020**, *38*, 870–874. [[CrossRef](#)]
28. Li, L.; Li, S.; Wu, N.; Wu, J.; Wang, G.; Zhao, G.-P.; Wang, J. HOLMESv2: A CRISPR–Cas12b-Assisted Platform for Nucleic Acid Detection and DNA Methylation Quantitation. *ACS Synth. Biol.* **2019**, *8*, 2228–2237. [[CrossRef](#)]
29. East-Seletsky, A.; O’Connell, M.R.; Knight, S.C.; Burstein, D.; Cate, J.H.D.; Tjian, R.; Doudna, J.A. Two distinct RNase activities of CRISPR–C2c2 enable guide-RNA processing and RNA detection. *Nature* **2016**, *538*, 270–273. [[CrossRef](#)]
30. Fozouni, P.; Son, S.; Derby, M.D.D.L.; Knott, G.J.; Gray, C.N.; D’Ambrosio, M.V.; Zhao, C.; Switz, N.A.; Kumar, G.R.; Stephens, S.I.; et al. Amplification-free detection of SARS-CoV-2 with CRISPR-Cas13a and mobile phone microscopy. *Cell* **2021**, *184*, 323–333.e9. [[CrossRef](#)]
31. Bruch, R.; Baaske, J.; Chatelle, C.; Meirich, M.; Madlener, S.; Weber, W.; Dincer, C.; Urban, G.A. CRISPR/Cas13a-Powered Electrochemical Microfluidic Biosensor for Nucleic Acid Amplification-Free miRNA Diagnostics. *Adv. Mater.* **2019**, *31*, e1905311. [[CrossRef](#)]
32. Pardee, K.; Green, A.A.; Takahashi, M.K.; Braff, D.; Lambert, G.; Lee, J.W.; Ferrante, T.; Ma, D.; Donghia, N.; Fan, M.; et al. Rapid, Low-Cost Detection of Zika Virus Using Programmable Biomolecular Components. *Cell* **2016**, *165*, 1255–1266. [[CrossRef](#)]
33. Li, S.-Y.; Cheng, Q.-X.; Wang, J.-M.; Li, X.-Y.; Zhang, Z.-L.; Gao, S.; Cao, R.-B.; Zhao, G.-P.; Wang, J. CRISPR–Cas12a-assisted nucleic acid detection. *Cell Discov.* **2018**, *4*, 20. [[CrossRef](#)]
34. Mustafa, M.I.; Makhawi, A.M. SHERLOCK and DETECTR: CRISPR-Cas Systems as Potential Rapid Diagnostic Tools for Emerging Infectious Diseases. *J. Clin. Microbiol.* **2021**, *59*, e00745-20. [[CrossRef](#)]
35. Gootenberg, J.S.; Abudayyeh, O.O.; Lee, J.W.; Essletzbichler, P.; Dy, A.J.; Joung, J.; Verdine, V.; Donghia, N.; Daringer, N.M.; Freije, C.A.; et al. Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science* **2017**, *356*, 438–442. [[CrossRef](#)]
36. Wölfel, R.; Corman, V.M.; Guggemos, W.; Seilmaier, M.; Zange, S.; Müller, M.A.; Niemeyer, D.; Jones, T.C.; Vollmar, P.; Rothe, C.; et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* **2020**, *581*, 465–469. [[CrossRef](#)]
37. Ramachandran, A.; Santiago, J.G. CRISPR Enzyme Kinetics for Molecular Diagnostics. *Anal. Chem.* **2021**, *93*, 7456–7464. [[CrossRef](#)]
38. Abudayyeh, O.O.; Gootenberg, J.S. CRISPR diagnostics. *Science* **2021**, *372*, 914–915. [[CrossRef](#)]
39. Chen, J.S.; Ma, E.; Harrington, L.B.; Da Costa, M.; Tian, X.; Palefsky, J.M.; Doudna, J.A. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science* **2018**, *360*, 436–439. [[CrossRef](#)]
40. Gootenberg, J.S.; Abudayyeh, O.O.; Kellner, M.J.; Joung, J.; Collins, J.J.; Zhang, F. Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. *Science* **2018**, *360*, 439–444. [[CrossRef](#)]

41. Santiago-Frangos, A.; Hall, L.N.; Nemudraia, A.; Nemudryi, A.; Krishna, P.; Wiegand, T.; Wilkinson, R.A.; Snyder, D.T.; Hedges, J.F.; Cicha, C.; et al. Intrinsic signal amplification by type III CRISPR-Cas systems provides a sequence-specific SARS-CoV-2 diagnostic. *Cell Rep. Med.* **2021**, *2*, 100319. [[CrossRef](#)]
42. Shi, K.; Xie, S.; Tian, R.; Wang, S.; Lu, Q.; Gao, D.; Lei, C.; Zhu, H.; Nie, Z. A CRISPR-Cas autocatalysis-driven feedback amplification network for supersensitive DNA diagnostics. *Sci. Adv.* **2021**, *7*, eabc7802. [[CrossRef](#)] [[PubMed](#)]
43. Brogan, D.J.; Akbari, O.S. CRISPR Diagnostics: Advances toward the Point of Care. *Biochemistry* **2022**. [[CrossRef](#)] [[PubMed](#)]
44. Kim, S.; Ji, S.; Koh, H.R. CRISPR as a Diagnostic Tool. *Biomolecules* **2021**, *11*, 1162. [[CrossRef](#)] [[PubMed](#)]
45. Zeng, F.; Wu, M.; Ma, L.; Han, Z.; Shi, Y.; Zhang, Y.; Liu, C.; Zhang, S.; Cong, F.; Liu, S. Rapid and sensitive real-time recombinase polymerase amplification for detection of Marek's disease virus. *Mol. Cell. Probes* **2019**, *48*, 101468. [[CrossRef](#)] [[PubMed](#)]
46. Moehling, T.J.; Choi, G.; Dugan, L.C.; Salit, M.; Meagher, R.J. LAMP Diagnostics at the Point-of-Care: Emerging Trends and Perspectives for the Developer Community. *Expert Rev. Mol. Diagn.* **2021**, *21*, 43–61. [[CrossRef](#)] [[PubMed](#)]
47. Freije, C.A.; Sabeti, P.C. Detect and destroy: CRISPR-based technologies for the response against viruses. *Cell Host Microbe* **2021**, *29*, 689–703. [[CrossRef](#)]
48. Ding, X.; Yin, K.; Li, Z.; Lalla, R.V.; Ballesteros, E.; Sfeir, M.M.; Liu, C. Ultrasensitive and visual detection of SARS-CoV-2 using all-in-one dual CRISPR-Cas12a assay. *Nat. Commun.* **2020**, *11*, 4711. [[CrossRef](#)]
49. Arizti-Sanz, J.; Freije, C.A.; Stanton, A.C.; Petros, B.A.; Boehm, C.K.; Siddiqui, S.; Shaw, B.M.; Adams, G.; Kosoko-Thoroddsen, T.-S.F.; Kembell, M.E.; et al. Streamlined inactivation, amplification, and Cas13-based detection of SARS-CoV-2. *Nat. Commun.* **2020**, *11*, 5921. [[CrossRef](#)]
50. Myhrvold, C.; Freije, C.A.; Gootenberg, J.S.; Abudayyeh, O.O.; Metsky, H.C.; Durbin, A.F.; Kellner, M.J.; Tan, A.L.; Paul, L.M.; Parham, L.A.; et al. Field-deployable viral diagnostics using CRISPR-Cas13. *Science* **2018**, *360*, 444–448. [[CrossRef](#)]
51. Joung, J.; Ladha, A.; Saito, M.; Kim, N.-G.; Woolley, A.E.; Segel, M.; Barretto, R.P.; Ranu, A.; Macrae, R.K.; Faure, G.; et al. Detection of SARS-CoV-2 with SHERLOCK One-Pot Testing. *N. Engl. J. Med.* **2020**, *383*, 1492–1494. [[CrossRef](#)]
52. Chen, Y.; Shi, Y.; Chen, Y.; Yang, Z.; Wu, H.; Zhou, Z.; Li, J.; Ping, J.; He, L.; Shen, H.; et al. Contamination-free visual detection of SARS-CoV-2 with CRISPR/Cas12a: A promising method in the point-of-care detection. *Biosens. Bioelectron.* **2020**, *169*, 112642. [[CrossRef](#)]
53. Barnes, K.G.; Lachenauer, A.E.; Nitido, A.; Siddiqui, S.; Gross, R.; Beitzel, B.; Siddle, K.J.; Freije, C.A.; Dighero-Kemp, B.; Mehta, S.B.; et al. Deployable CRISPR-Cas13a diagnostic tools to detect and report Ebola and Lassa virus cases in real-time. *Nat. Commun.* **2020**, *11*, 4131. [[CrossRef](#)]
54. Gayet, R.V.; De Puig, H.; English, M.A.; Soenksen, L.R.; Nguyen, P.Q.; Mao, A.S.; Angenent-Mari, N.M.; Collins, J.J. Creating CRISPR-responsive smart materials for diagnostics and programmable cargo release. *Nat. Protoc.* **2020**, *15*, 3030–3063. [[CrossRef](#)]
55. Huang, Z.; Tian, D.; Liu, Y.; Lin, Z.; Lyon, C.J.; Lai, W.; Fusco, D.; Drouin, A.; Yin, X.; Hu, T.; et al. Ultra-sensitive and high-throughput CRISPR-powered COVID-19 diagnosis. *Biosens. Bioelectron.* **2020**, *164*, 112316. [[CrossRef](#)]
56. Ali, Z.; Aman, R.; Mahas, A.; Rao, G.S.; Tehseen, M.; Marsic, T.; Salunke, R.; Subudhi, A.K.; Hala, S.M.; Hamdan, S.M.; et al. iSCAN: An RT-LAMP-coupled CRISPR-Cas12 module for rapid, sensitive detection of SARS-CoV-2. *Virus Res.* **2020**, *288*, 198129. [[CrossRef](#)]
57. Wang, X.; Zhong, M.; Liu, Y.; Ma, P.; Dang, L.; Meng, Q.; Wan, W.; Ma, X.; Liu, J.; Yang, G.; et al. Rapid and sensitive detection of COVID-19 using CRISPR/Cas12a-based detection with naked eye readout, CRISPR/Cas12a-NER. *Sci. Bull.* **2020**, *65*, 1436–1439. [[CrossRef](#)]