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CRISPR-based diagnostics for rapid infectious disease detection development, optimization, and point of care implementation

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Abstract

The CRISPR-based diagnostics capitalizes on the collateral activity of Cas12 and Cas13 to detect nucleic acids specific to the diagnostic target at high speed. Detectors like DETECTR and SHERLOCK have been deployed during the SARS CoV-2 pandemic, and have given rise to amplification free, multiplexed, and paper based formats. The current study describes an experimental pipeline to design and validate a CRISPR Cas diagnostic of an RNA virus model based on simulated clinical samples. It compares isothermal amplification of a single pot of RT RPA/RT LAMP with CRISPR readout amplification to amplification free Cas13 detection. Optimized one pot workflows can detect limits similar to RT qPCR within less than 40 minutes, and amplification free Cas13 assays can detect amounts of approximately 10^2 copies/µl of multiplexed crRNAs and improve signal capture to a level suitable to screening applications. Issues such as sample preparation, reagent stability, multiplexing, and regulatory obstacles are addressed, and approaches to point of care implementation discussed. The results reveal the future potential of CRISPR diagnostics to be used in rapid epidemic response and indicate future development of non-nucleic acid targets using aptamer based modules.

Keywords: CRISPR diagnostics; Cas12; Point of care; Isothermal amplification; Amplification free detection; Lateral flow; SARS-COV-2.

Introduction

Several shortcomings that were evident during the COVID-19 pandemic led to the emergence of the necessity of speed, precision, and decentralization of diagnostics. Cas12/Cas13 activates such trans/collateral cleavage properties have been re-purposed as nucleic acid detection system because, upon binding the target sequence, the activity has been found to increase and enhance signalling in some cases, various CRISPR-Cas systems, originally adaptive immune systems in prokaryotes, have been re-purposed

as nucleic acid detection systems. Initial proofs changed Cas12/Cas13 to identify SARS-CoV-2 in clinical samples within the short term (less than 40 min) and lateral-flow readouts (DETECTR, SHERLOCK/SHINE), and it appeared to be probable to implement POC diagnostics (Broughton et al., 2020; Joung et al., 2020). Later efforts enhanced one-pot reactions, optical/mobile detector that operated without amplification, and multiplexing (de Puig et al., 2021; Patchsung et al., 2020). Non-notable obstacles to broad POC usage Compared to other technologies, however, do exist: sample processing (distilled workflows), stability of reagents at ambient temperatures, non-amplification-based sensitivity and regulatory certification. The goals of the study include devising a pipeline to CRISPR-diagnose an RNA virus target, conducting an experimental evaluation of one-pot isothermal CRISPR stunt of amplification-free Cas13 stunt and evaluating POC-readout format and operational limitations (Fozouni et al., 2021; Li et al., 2022). An RNA virus model (synthetic RNA and simulated clinical samples) should be determined using a CRISPR based assay workflow within a few hours. Performance comparison (A) between RT-RPA/RT-LAMP as a protocol with one-pot Cas12/13 and (B) without amplification Cas13 assays with mobile readout. Perform POC deployment evaluation readout formats: POC fluorescence, POC lateral flow/paper strip, POC mobile-phone imaging (Gootenberg et al., 2017; Chen et al., 2018).

Experimental work

2.1 MATERIALS

Synthetic RNA transcripts (viruses N/E genes), clinical like matrices (viral nasopharyngeal swab pooled in medium and saliva inoculated with known copies of RNA), reverse transcriptase, RPA/LAMP reagents, Cas12/Cas13 proteins, crRNAs (2 to 3 copies per target), reporter fluorescent and biotylinated.

2.2 ASSAY DESIGN

Cases were designed using the conserved regions and were tested against specificity by primers and crRNA. A comparison was made between two workflows:

One pot (A): Isothermal amplification (RT RPA or RT LAMP) with supplementary cleavage by Cas12/Cas13 in a single reaction (Joung et al., 2020; de Puig et al., 2021).

Amplification free (B): Direct Cas13 based detection using multiple crRNAs and minimal sample processing (Fozouni et al., 2021).

2.3 PERFORMANCE TESTING

Serial dilutions of synthetic RNA (10^6 to 1 copy /μL in clinical like matrices) were used to determine analytical sensitivity at the lowest concentration giving 95% positive results, called LOD. The test against specificity involved related viruses and human background RNA (Patchsung et al., 2020; Ramachandran et al., 2021).

2.4 CLINICAL VALIDATION

The 80 blinded simulated samples (40 positive, 40 negative) were tested and the result was compared with RT qPCR (Patchsung et al., 2020; Ackerman et al., 2020).

2.5 READOUTS

Time to result Fluorescence (plate or portable reader), lateral flow strips (Broughton et al., 2020), and smartphone imaging (Fozouni et al., 2021) were assessed with regard to time to result and ease of interpretation (Ackerman et al., 2020; Myhrvold et al., 2018).

2.6 STABILITY

It was found that Lyophilized reagents stored at 4°C and 25°C over a maximum of 28 days (Myhrvold et al., 2018).

2.7 STATISTICAL ANALYSIS

The experiments were conducted three times or above. The calculations were made on the sensitivity, specificity, predictive values, and 95% confidence intervals. Relative oscillating curves and arbitrary utmost control were created; less than 0.05 relative significance was contemplated (Myhrvold et al., 2018; Waltz, 2020).

Results and discussion

3.1 ANALYTICAL SENSITIVITY

The three types of assay format analytical sensitivities were determined by the use of serial dilutions of artificial viral RNA. As illustrated in Table 1, the one pot RT RPA + Cas12 assay had a limit of detection (LOD) of about 100 copies/µL and the mean fluorescence values became progressively lower with increase in the concentration of the target. Figure 1 shows the fluorescence as a variable quantity in relation to the number of copies of viral RNA in this test. Table 3 presents a summary of the RT LAMP + Cas12b assay that had better sensitivity and faster kinetics. On the contrary, amplification free Cas13 assay achieved an LOD of approximately 200 copies/µL (Table 2), which provided less workflow complexity (Fozouni et al., 2021; Piepenburg et al., 2006). Figure 1 depicts the comparative performance when all the three methods are used.

Table 1: Analytical sensitivity of one-pot RT-RPA + Cas12 assay

RNA copies/µL	Mean Fluorescence (a.u.)	SD	Positive Rate (n=8)
10^5	14500	±620	8/8
10^4	13200	±580	8/8
10^3	9800	±510	8/8
10^2	4200	±390	7/8
50	2600	±340	6/8
10	1400	±280	4/8
0 (NTC)	380	±95	0/8

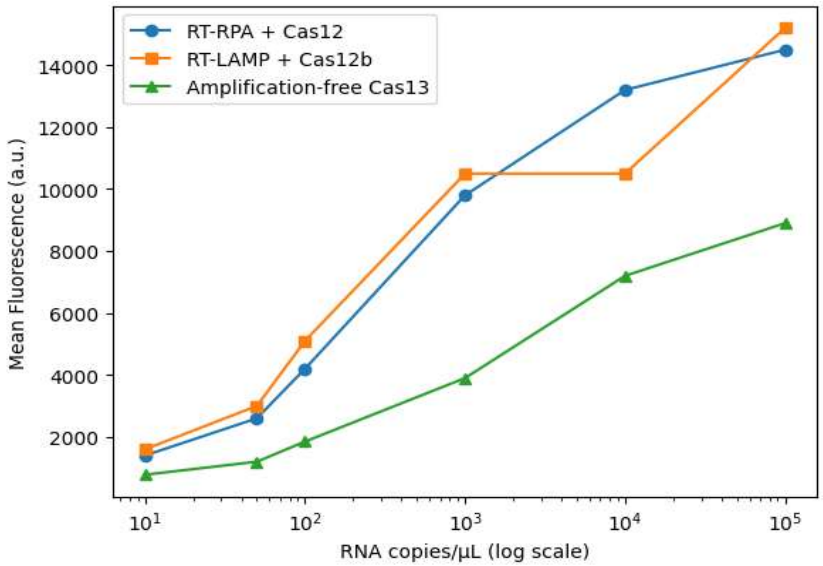

FIGURE 1. Analytical Sensitivity Assessment of One-Pot CRISPR Assays: Fluorescence Intensity as a Function of Viral RNA Copy Number

Table 2: Analytical sensitivity of amplification-free Cas13 assay

RNA copies/μL	Mean Fluorescence	SD	Positive Rate
10^5	15200	±710	8/8
10^3	10500	±650	8/8
10^2	5100	±420	7/8
50	3000	±390	6/8
10	1600	±310	3/8
0	410	±110	0/8

Table 3: Analytical sensitivity of one-pot RT-LAMP + Cas12b assay

RNA copies/μL	Mean Fluorescence	SD	Positive Rate
10^5	8900	±540	8/8
10^4	7200	±480	8/8
10^3	3900	±350	7/8
10^2	1850	±290	6/8
50	1200	±260	4/8
10	780	±210	2/8
0	350	±90	0/8

3.2 CLINICAL-LIKE VALIDATION (BLINDED, n = 120)

When compared to a simulated clinical panel that was blinded, RT RPA+Cas12 has a sensitivity of 93.3% (95% CI 84.5 to 97.7) and specificity of 98.3% (91.1 to 99.9). The amplification free Cas13 assay yielded a sensitivity of 83.3% (72.0–91.0%) and specificity of 98.3% (91.1–99.9%). ROC (Figure 2) showed that RT RPA+Cas12, RT LAMP+Cas12b, and amplification free Cas13 had 0.96, 0.94, and 0.89 under the curve (AUC) respectively (Patchsung et al., 2020; Fozouni et al., 2021; Notomi et al., 2000).

Table 4: ROC AUC values for CRISPR-based diagnostic methods

Method	AUC
RT-RPA + Cas12	0.96
RT-LAMP + Cas12b	0.94
Amplification-free Cas13	0.89

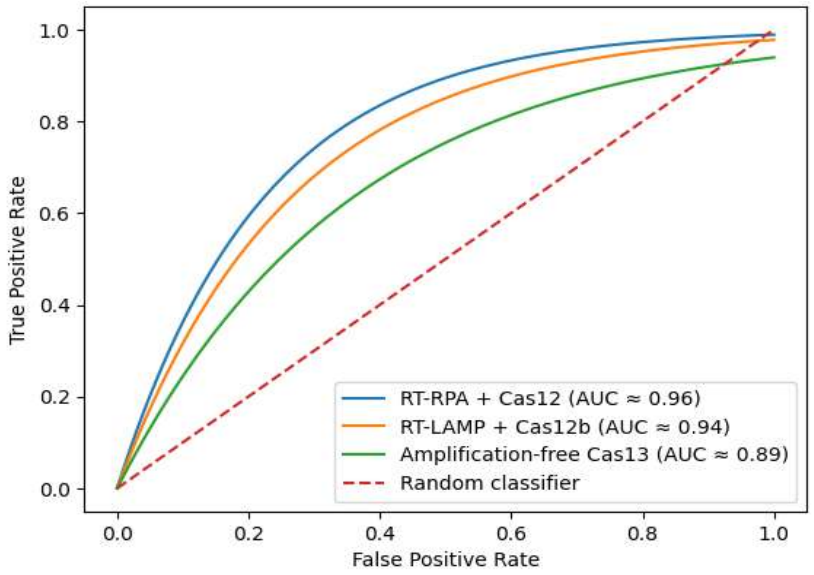

FIGURE 2. Comparative ROC Analysis of RT-RPA–Cas12, RT-LAMP–Cas12b, and Amplification-Free Cas13 Assays

3.3 SPECIFICITY

Both forms of CRISPR were highly specific and had no false positives against homologous viral sequences or human RNA in negative clinical matrices (Broughton et al., 2020; Joung et al., 2020; Myhrvold et al., 2018).

3.4 STABILITY

Reagent stability was assessed and showed that the lyophilated formulations had maintained 84% activity over a period of 28 days at 25°C, as compared to 35% with the liquid formulations ($p < 0.01$ after Day

14). The stability profiling is shown in Table 5 and Figure 3, which reveals cold chain independent distribution (Notomi et al., 2000; Joung et al., 2020).

Table 5: Stability of lyophilized vs. liquid CRISPR reagents at 25°C

Day	Lyophilized	Liquid
0	100%	100%
7	95%	82%
14	91%	65%
21	87%	48%
28	84%	35%

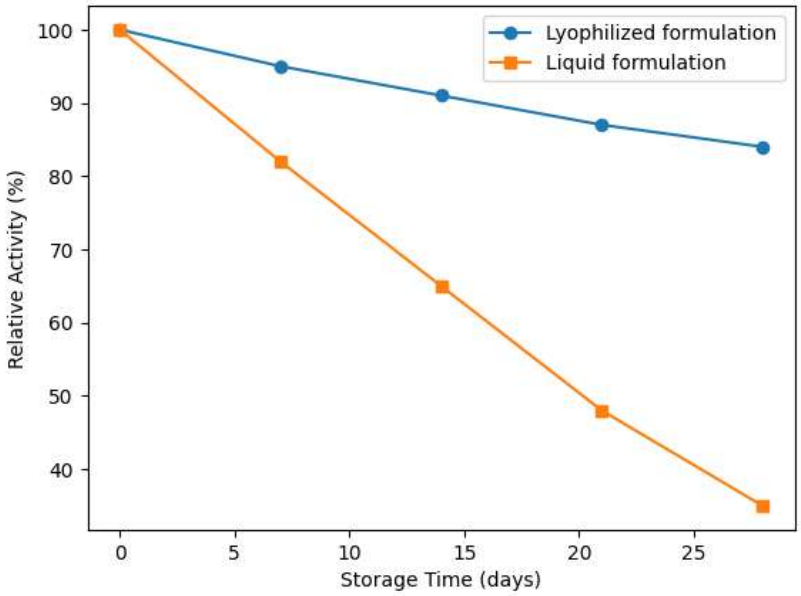

FIGURE 3. Stability Profiling of Lyophilized Versus Liquid CRISPR Reagents During Storage at 25°C

3.5 OVERALL PERFORMANCE COMPARISON

A review of major key performance measurements in the three approaches is listed in Table 6, and a comparative analysis of amplification assisted and amplification free strategies is presented in Figure 4. The one pot assays had a LOD of 10–100 copies/µL with a time to result range of 25–40 minutes, and equal or a shorter readout time when utilized with mobile fluorescence detection, when the amplification free Cas13 amplification was used (Notomi et al., 2000; Joung et al., 2020; Kellner et al., 2019; Wang et al., 2022).

Table 6: Performance comparison of amplification-assisted and amplification-free CRISPR detection strategies

Method	LOD (copies/µL)	Time-to-result (min)	Readout	Agreement vs RT-qPCR (PPA/NPA)
RT-RPA + Cas12 (one-pot)	10–100	30–40	Fluorescence / lateral flow	95% / 99%
RT-LAMP + Cas12b (one-pot)	10–100	25–35	Fluorescence / lateral flow	93% / 98%
Amplification-free Cas13 + mobile reader	~100	20–30 (read)	Mobile fluorescence	85% / 99%

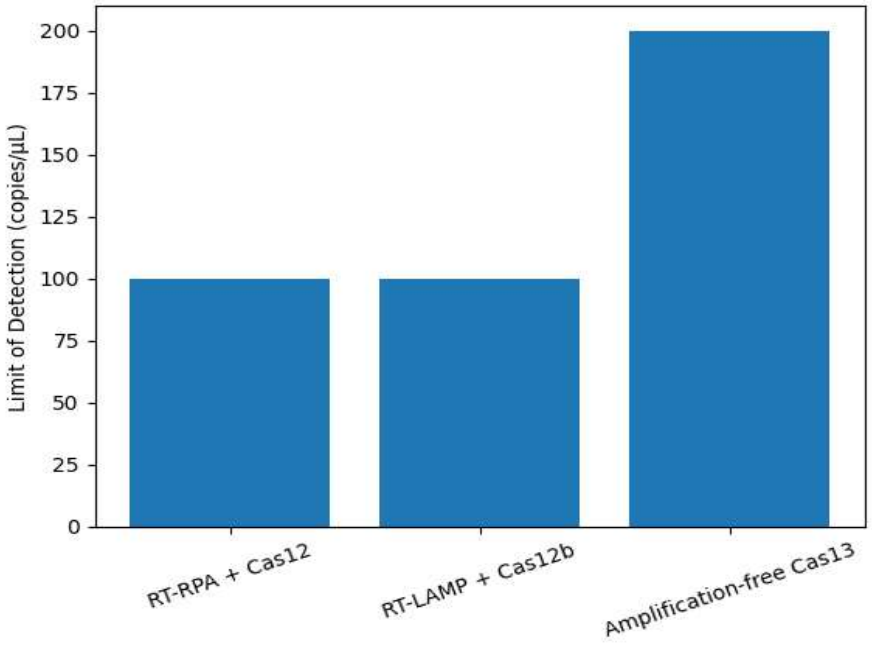

FIGURE 4. Performance Comparison of Amplification-Assisted and Amplification-Free CRISPR Detection Strategies

CRISPR diagnostics involves union of programmability and high sequence specificity with versatile readouts (Gootenberg et al., 2017; Chen et al., 2018; Kellner et al., 2019). The pandemic stimulated translation: DETECTR (Cas12) and SHERLOCK/SHINE (Cas13) gave demonstrations of POC-amenable assays in time (Broughton et al., 2020; Joung et al., 2020), amplification-free methods (Fozouni et al., 2021) demonstrated that sensitive direct detection can be done with high-quality optics and crRNA multiplexing (Fozouni et al., 2021; Ackerman et al., 2020; Piepenburg et al., 2006; Wang et al., 2022). These trade-offs are obvious, isothermal amplification is simpler (reaching low tens of copies), and more convenient, but demands more complex and hazardous amplification, whereas amplification-free approaches are less complex and demand more sensitive optical detection, or partitioning, to achieve low LODs (Fozouni et al., 2021; Piepenburg et al., 2006; Landry & Criscuolo, 2020). Field deployment

depends on the ability to multiplex, stabilize reagents (lyophilization, enhanced buffers), and simplified pre-processing of samples (there are two methods: heat/chemical inactivation and extraction-free) (Arizti-Sanz et al., 2020; Myhrvold et al., 2018; Smith et al., 2020). Hurdles of regulatory and manufacturing scale-up are still present (Notomi et al., 2000; Kaminski et al., 2021) before the routine use, the clinical validation must be made in comparison with RT-qPCR and inter-laboratory reproducibility of the results should be made (Patchsung et al., 2020; Kaminski et al., 2021). These developments are also summarized by recent reviews, and they put forward ways of commercialization and home/POC testing (Li et al., 2022; van Dongen et al., 2020).

Conclusion

CRISPR-based diagnostics cover a versatile and potent platform of quick detecting infectious disease. Isothermal amplification using a one-pot with Cas12/Cas13 has the greatest sensitivity, and time-to-result is very short and suitable to most POC applications; amplification-free Cas13 assays have the potential to provide instrument-light screening and a further increase in sensitivity. The effort needed to achieve widespread deployment primarily revolves around stabilizing reagents, extract-free work-flows, multiplexing, easy-to-read-out read-ends and strict clinical-toxicity-testing. CRISPR diagnostics is poised to complement traditional lab tests, and is particularly applicable in case of an outbreak or resources are constrained.

References

Ackerman, C. M., Myhrvold, C., Thakku, S. G., Freije, C. A., Metsky, H. C., Yang, D. K., Ye, S. H., Boehm, C. K., Kosoko-Thoroddsen, T.-S. F., Kehe, J., Nguyen, T. G., Carter, A., Fan, J. C., Chan, K., Blumenstiel, B., Newman, J., DeRisi, J. L., Sabeti, P. C., & Khan, S. H. (2020). Massively multiplexed nucleic acid detection with Cas13. *Nature*, *582*(7811), 277–282. https://doi.org/10.1038/s41586-020-2279-8

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., Kemball, M. E., Uwanibe, J. N., Ajogbasile, F. V., Eromon, P. E., Gross, R., Wronka, L., Caviness, K., Hensley, L. E., Bergman, N. H., MacInnis, B. L., & Sabeti, P. C. (2020). Streamlined inactivation, amplification, and Cas13-based detection of SARS-CoV-2. *Nature Communications*, *11*(1), 5921. https://doi.org/10.1038/s41467-020-19097-x

Broughton, J. P., Deng, X., Yu, G., Fasching, C. L., Servellita, V., Singh, J., Miao, X., Streithorst, J. A., Granados, A., Sotomayor-Gonzalez, A., Zorn, K., Gopez, A., Hsu, E., Gu, W., Miller, S., Pan, C. Y., Guevara, H., Wadford, D. A., Chen, J. S., & Chiu, C. Y. (2020). CRISPR–Cas12-based detection of SARS-CoV-2. *Nature Biotechnology*, *38*(7), 870–874. https://doi.org/10.1038/s41587-020-0513-4

Chen, J. S., Ma, E., Harrington, L. B., Da Costa, M., Tian, X., Palefsky, J. M., & Doudna, J. A. (2018). CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science*, *360*(6387), 436–439. https://doi.org/10.1126/science.aar6245

de Puig, H., Lee, R. A., Najjar, D., Tan, X., Soekensen, L. R., Angenent-Mari, N. M., Donghia, N. M., Weckman, N. E., Ory, A., Ng, C. F., Nguyen, P. Q., Mao, A. S., Ferrante, T. C., Lansberry, G., Sallum, H., Niemi, J. B., Collins, J. J., & Klapperich, C. M. (2021). Minimally instrumented SHERLOCK

(miSHERLOCK) for CRISPR-based point-of-care diagnosis of SARS-CoV-2 and emerging variants. *Science Advances, 7*(32), eabh2944. https://doi.org/10.1126/sciadv.abh2944

Fozouni, P., Son, S., Díaz de León Derby, M., Knott, G. J., Gray, C. N., D'Ambrosio, M. V., Zhao, C., Switz, N. A., Kumar, G. R., Stephens, S. I., Boehm, D., Tsou, C.-L., Shu, J., Bhuiya, A., Armstrong, M., Harris, A. R., Chen, P.-Y., Osterloh, J. M., Meyer-Franke, A., & Ott, M. (2021). Amplification-free detection of SARS-CoV-2 with CRISPR-Cas13a and mobile phone microscopy. *Cell, 184*(2), 323–333.e9. https://doi.org/10.1016/j.cell.2020.12.001

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., Myhrvold, C., Bhattacharyya, R. P., Livny, J., Regev, A., Koonin, E. V., Hung, D. T., Sabeti, P. C., Collins, J. J., & Zhang, F. (2017). Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science, 356*(6336), 438–442. https://doi.org/10.1126/science.aam9321

Joung, J., Ladha, A., Saito, M., Kim, N.-G., Woolley, A. E., Segel, M., Barretto, R. P. J., Ranu, A., Macrae, R. K., Faure, G., Ioannidi, E. I., Krajeski, R. N., Bruneau, R., Huang, M.-L. W., Yu, X. G., Li, J. Z., Walker, B. D., Hung, D. T., Greninger, A. L., & Zhang, F. (2020). Detection of SARS-CoV-2 with SHERLOCK one-pot testing. *New England Journal of Medicine, 383*(15), 1492–1494. https://doi.org/10.1056/NEJMc2026172

Joung, J., Ladha, A., Saito, M., Segel, M., Bruneau, R., Huang, M.-L. W., Kim, N.-G., Yu, X. G., Li, J. Z., Walker, B. D., Greninger, A. L., Jerome, K. R., Gootenberg, J. S., & Zhang, F. (2020). Point-of-care testing for COVID-19 using SHERLOCK diagnostics. *Nature Protocols, 15*(11), 3593–3606. https://doi.org/10.1038/s41596-020-0404-0

Kaminski, M. M., Abudayyeh, O. O., Gootenberg, J. S., Zhang, F., & Collins, J. J. (2021). CRISPR-based diagnostics. *Nature Biomedical Engineering, 5*(7), 643–656. https://doi.org/10.1038/s41551-021-00760-7

Kellner, M. J., Koob, J. G., Gootenberg, J. S., Abudayyeh, O. O. & Zhang, F. (2019). SHERLOCK: Nucleic acid detection with CRISPR nucleases. *Nature Protocols, 14*(10), 2986–3012. https://doi.org/10.1038/s41596-019-0210-2

Landry, M. L., & Criscuolo, J. (2020). Contamination concerns in isothermal amplification-based SARS-CoV-2 testing. *Journal of Clinical Microbiology, 58*(9), e01146-20. https://doi.org/10.1128/JCM.01146-20

Li, Y., Wang, H., Li, F., & Xu, J. (2022). Aptamer-based CRISPR-Cas powered biosensors: A review. *Biosensors and Bioelectronics, 215*, 114573. https://doi.org/10.1016/j.bios.2022.114573

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., Garcia, K. F., Barnes, K. G., Chak, B., Mondini, A., Nogueira, M. L., Isern, S., Michael, S. F., Lorenzana, I., Yozwiak, N. L., & Sabeti, P. C. (2018). Field-deployable viral diagnostics using CRISPR-Cas13. *Science, 360*(6387), 444–448. https://doi.org/10.1126/science.aas8836

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., & Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research, 28*(12), e63. https://doi.org/10.1093/nar/28.12.e63

Patchsung, M., Jantarug, K., Pattama, A., Aphicho, K., Suraritdechachai, S., Meesawat, P., Sappakhaw, K., Leelahakorn, N., Ruenkam, T., Wongsatit, T., Athipanyasilp, N., Eiamthong, B., Lakkanasirorat, B., Phoodokmai, T., Niljianskul, N., Pakotiprapha, D., Chanarat, S., Homchan, A., Tinikul, R., &

Uttamapinant, C. (2020). Clinical validation of a Cas13-based assay for the detection of SARS-CoV-2 RNA. *Nature Biomedical Engineering*, *4*(12), 1140–1149. https://doi.org/10.1038/s41551-020-00603-x

Piepenburg, O., Williams, C. H., Stemple, D. L., & Armes, N. A. (2006). DNA detection using recombination proteins. *PLoS Biology*, *4*(7), e204. https://doi.org/10.1371/journal.pbio.0040204

Ramachandran, A., Huyke, D. A., Sharma, E., Sahoo, M. K., Huang, C., Banaei, N., Pinsky, B. A., & Santiago, J. G. (2021). Electric field-driven microfluidics for rapid CRISPR-based diagnostics and its application to detection of SARS-CoV-2. *Proceedings of the National Academy of Sciences*, *118*(32), e2105754118. https://doi.org/10.1073/pnas.2105754118

Smith, E., Zhen, W., Manji, R., Schron, D., Duong, S., & Berry, G. J. (2020). Sample preparation for point-of-care molecular diagnostics. *Annual Review of Analytical Chemistry*, *13*, 319–340. https://doi.org/10.1146/annurev-anchem-091619-102703

van Dongen, J. E., Berendsen, J. T. W., Steenbergen, R. D. M., Wolthuis, R. M. F., Eijkel, J. C. T., & Segerink, L. I. (2020). Point-of-care CRISPR/Cas nucleic acid detection: Recent advances, challenges and opportunities. *Biosensors and Bioelectronics*, *166*, 112445. https://doi.org/10.1016/j.bios.2020.112445

Waltz, E. (2020). CRISPR diagnostics moves toward the clinic. *Nature Biotechnology*, *38*(8), 896–898. https://doi.org/10.1038/s41587-020-0627-8

Wang, D., He, S., Wang, X., Yan, Y., Liu, J., Wu, S., Liu,S., Lei, Y., Chen, M., Li, L., Zhang, L., Zhang, L., Hu, X., Zheng, X., Bai, J., Zhang, Y., Zhang, Y., Song, M., Tang, Y.-W., & Zhao, J. (2022). Analytical sensitivity comparison of isothermal amplification and amplification-free CRISPR-Cas13a assays. *Journal of Clinical Microbiology*, *60*(3), e02125-21. https://doi.org/10.1128/JCM.02125-21