Evaluation of CRISPR-Based Assays for Rapid Detection of SARS-CoV-2: A Systematic Review and Meta-Analysis

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Purpose: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the pathogen of coronavirus disease 2019. Diagnostic methods based on the clustered regularly interspaced short palindromic repeats (CRISPR) have been developed to detect SARS-CoV-2 rapidly. Therefore, a systematic review and meta-analysis were performed to assess the diagnostic accuracy of CRISPR for detecting SARS-CoV-2 infection.

Materials and Methods: Studies published before August 2021 were retrieved from four databases, using the keywords “SARS-CoV-2” and “CRISPR.” Data were collected from these publications, and the sensitivity, specificity, negative likelihood ratio (NLR), positive likelihood ratio (PLR), and diagnostic odds ratio (DOR) were calculated. The summary receiver operating characteristic curve was plotted for analysis with MetaDiSc 1.4. The Stata 15.0 software was used to draw Deeks’ funnel plots to evaluate publication bias.

Results: We performed a pooled analysis of 38 independent studies shown in 30 publications. The reference standard was reverse transcription-quantitative PCR. The results indicated that the sensitivity of CRISPR-based methods for diagnosis was 0.94 (95% CI 0.93–0.95), the specificity was 0.98 (95% CI 0.97–0.99), the PLR was 34.03 (95% CI 20.81–55.66), the NLR was 0.08 (95% CI 0.06–0.10), and the DOR was 575.74 (95% CI 382.36–866.95). The area under the curve was 0.9894.

Conclusion: Studies indicate that a diagnostic method based on CRISPR has high sensitivity and specificity. Therefore, this would be a potential diagnostic tool to improve the accuracy of SARS-CoV-2 detection.

Key Words: CRISPR-based assays, detection, SARS-CoV-2, sensitivity, specificity

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified as the pathogen of the coronavirus disease 2019 (COVID-19), and it has caused more than 1.45 million deaths worldwide by November 30, 2020.1 Patients infected with SARS-CoV-2 may exhibit symptoms such as shortness of dyspnea, high fever, and pneumonia, which are fatal for vulnerable individuals.2 Coronavirus-infected inpatients are more likely to develop acute respiratory failure, pulmonary embolism, or septic shock, resulting in death.3 Moreover, with the sharply increasing number of infected people and limited assays currently, the development of efficient, rapid, accurate, and sensitive SARS-CoV-2 sensing tools is urgent for public health in the world.4 Molecular tests and serological tests have been implemented for COVID-19 diagnosis to detect viral RNA and anti-SARS-
CoV-2 immunoglobulins, respectively. For molecular diagnostic tests, the collection of upper nasopharyngeal swabs is recommended by the US Centers for Disease Control and Prevention. So far, reverse transcription-quantitative PCR (RT-qPCR) has widely been used as the reference standard for the detection of viral RNA in SARS-CoV-2. However, it requires well-trained personnel and advanced equipment, which limits the application of RT-qPCR, especially in resource-constrained developing countries. Metagenomic next-generation sequencing is another molecular test to identify SARS-CoV-2, but the sensitivity of this method is restricted by the influence of the human host background. On the other hand, the serology tests, including immunochromatographic analysis and enzyme-linked immunosorbent assay (ELISA), are not sufficiently accurate in detecting SARS-CoV-2. In addition, asymptomatic patients are considered to play a major role in the spread of the virus. These factors increase the need for effective, cheap, and rapid alternative methods.

The clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated proteins (Cas) system shows strong collateral activity against single-stranded RNA and DNA targets through molecular immune mechanisms, providing highly accurate methods of nucleic acid detection. The mechanism of the detection system is the specific binding and cleavage activity of CRISPR-Cas. Once the primers for reverse transcription loop-mediated isothermal amplification or reverse transcription recombinase polymerase amplification recognize the specific regions of the SARS-CoV-2 genome, the targeted nucleic acid is amplified at a constant temperature. The guide RNAs then target SARS-CoV-2 E, N, or Orf1ab amplicons with the base-pairing pattern at attomolar sensitivity, ensuring the amplified nucleotide cleaved by the Cas nuclease accurately. The target nucleotide is finally identified on the detection platform with fluorescence tracking. Therefore, CRISPR is a more efficient and suitable point-of-care diagnostic method than RT-qPCR, considering its sequence-specific detection method and isothermal amplification approaches.

In this study, we conducted a systematic review and meta-analysis to assess the diagnostic accuracy of CRISPR in detecting SARS-CoV-2 infection, evaluate the quality of available evidence, and perform an in-depth analysis regarding the related research.

MATERIALS AND METHODS

Search strategy and source
This study was conducted according to the PRISMA guidelines. We selected four databases, including PubMed, Embase, Cochrane Library, and Web of Science, and searched for data using “SARS-CoV-2” and “CRISPR” as keywords. All of the scientific papers were published before August 2021, without language restriction. All synonyms of the above-mentioned keywords were also included in the search formula for more comprehensive literature.

Study screening and selection
The retrieved publications were independently selected by four researchers. Based on the predetermined inclusion and exclusion criteria, data were extracted by analyzing the titles, abstracts, and full texts of the studies. All disagreements were resolved through discussion and consultation.

Inclusion and exclusion criteria
The publications that met all of the following criteria were included based on preset conditions: 1) the investigators’ experimental objectives included the role of CRISPR in the diagnosis of COVID-19 infection; 2) the study type was a diagnostic accuracy test, and the diagnostic accuracy was evaluated by comparing the index to be tested with the standard reference method; and 3) the data provided by the study could identify true positive (TP), false positive (FP), true negative (TN), false negative (FN), or sensitivity and specificity.

Exclusion criteria were as follows: 1) studies that were animal experiments; 2) studies where the reference method was not mentioned; 3) letters, conference abstracts, reviews, editorials, or erratum; and 4) duplicated publications or those with no description of the available data.

Data extraction
The EndNoteX9 software was used for file management and data extraction from articles. Excel standardized electronic data entry form was used to pool the required information, including the author’s name, publishing year, study type, sample size, reference standard, and indicators. In addition, the diagnostic features of SARS-CoV-2 were extracted along with TP, FP, TN, and FN. We reviewed the extracted information, resolved all disagreements through negotiated discussion, and excluded duplicate data.

Quality assessment standard
Four investigators evaluated the quality of the included studies independently in accordance with the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) guidelines regarding four main steps: case selection, trial to be assessed, reference standard, and case process and progress. The assessment of all four components was applied to analyze the risk of bias, while the assessment of the first three components was applied for the evaluation of clinical applicability. Issues with other iconic study designs were included in the risk of biased judgments, which were related to the potential for judicial bias. Responses of “Yes,” “No,” or “Indeterminate” corresponded to a risk of bias rating of “Low,” “High,” or “Indeterminate,” based on the questions included in each section.
Data analysis
We used the MetaDiSc 1.4 software (Ramony Cajal Hospital, Madrid, Spain) for statistical analysis following standard methods, and used the Stata 15.0 software (StataCorp LLC, College Station, TX, USA) to draw Deeks’ funnel plot and test funnel plot symmetry as well as publication bias. Spearman correlation coefficient and Cochran-Q were performed to analyze the heterogeneity of the included data, and a fixed-effects model or random-effects model was selected based on the result value. The sensitivity, specificity, positive likelihood ratio (PLR), and negative likelihood ratio (NLR) were calculated and analyzed by drawing a forest plot using MetaDiSc 1.4. The effect value and its 95% confidence interval (CI) were shown in the forest plot. In addition, the area under the curve (AUC) was calculated using the summary receiver operating characteristic (SROC) curve to obtain the specificity and sensitivity. Then, the total efficiency of CRISPR was assessed using diagnostic odds ratio (DOR) and AUC. The Review Manager 5.3 software (The Nordic Cochrane Centre, Copenhagen, Denmark) was used to evaluate the quality of the included studies.

RESULTS

Summary of the included studies
After searching through all four literature databases, we obtained 547 related documents, from which 374 were selected after the removal of duplicated publications. A total of 156 studies were removed for their uncorrelated titles or “CRISPR detection” not mentioned in their abstracts. We read through the text afterwards, and 188 studies were excluded for various reasons. Finally, 30 articles were selected with a total of 38 groups of data (Fig. 1). The effect-indicator proposed in each literature was involved in the composition of the data extracted. Table 1 shows the characteristics of these studies in detail.

Methodological quality evaluation
The quality of the included studies was evaluated by analyzing the data in terms of case selection, index detection, reference standard, and case process and progress using Review Manager 5.3. Fig. 2A summarizes the results of the QUADAS-2 assessment, and Fig. 2B shows the independent quality assessment of each study. The results indicated that for case selection, seven studies had a risk of bias due to the unclear case-control study design and the unknown inclusion of consecutive or randomized case conditions. In the index test field, three studies were at higher risk since the interpretation of the index test results was made when the reference standard results were known. Both the reference standard field and the flow rate and time were considered to have a low risk of bias.

Merged analysis results
Overall, the sensitivity of CRISPR in the diagnosis of COVID-19

Fig. 1. Flow diagram of study identification and inclusion.
was 0.94 (95% CI 0.93–0.95, I²=52.8%) (Fig. 3A), and the specificity was 0.98 (95% CI 0.97–0.99, I²=65.0%) (Fig. 3B). As shown in the chart in Fig. 3C, the AUC was 0.9894. The PLR was 34.03 (95% CI 20.81–55.66, I²=66.0%) (Fig. 4A), and the NLR was 0.08 (95% CI 0.06–0.10, I²=14.0%) (Fig. 4B). The value of the pooled DOR was 575.74 (95% CI 382.36–866.95) (Fig. 4C).

Table 1. Characteristics of Included Studies about CRISPR Detection of SARS-CoV-2

| Author | Year | Geographical distribution of virus | Patients (n) | Sample source | Type of cas enzyme | Gene site | Readout mode | TP | FP | FN | TN |
|--------|------|-----------------------------------|-------------|---------------|-------------------|-----------|--------------|----|----|----|----|
| Patchung, et al. (1) | 2020 | Thailand | 154 | Nasopharyngeal swabs | Cas13a | N | Lateral flow assays | 71 | 0 | 10 | 73 |
| Patchung, et al. (2) | 2020 | Thailand | 154 | Nasopharyngeal swabs | Cas13a | N | Fluorescence reader | 78 | 3 | 73 |
| Huang, et al. | 2020 | America | 29 | Nasal swabs | Cas12a | N | Fluorescence reader | 15 | 4 | 0 | 10 |
| Wang, et al. | 2020 | China | 31 | Nasal swabs | Cas12a | E | Fluorescence reader | 16 | 0 | 0 | 15 |
| Joung, et al. | 2020 | America | 402 | Nasal swabs | Cas12b | N | Fluorescence reader | 188 | 3 | 14 | 197 |
| Broughton, et al. (a1) | 2020 | America | 82 | Nasopharyngeal swabs | Cas12 | N | Lateral flow assays | 9 | 0 | 1 | 12 |
| Broughton, et al. (a2) | 2020 | America | 82 | Nasopharyngeal swabs | Cas12 | N | Fluorescence reader | 37 | 0 | 3 | 42 |
| Broughton, et al. (b1) | 2020 | America | 21 | Nasopharyngeal swabs | Cas12 | E | Fluorescence reader | 10 | 0 | 0 | 11 |
| Broughton, et al. (b2) | 2020 | America | 21 | Nasopharyngeal swabs | Cas12 | N | Fluorescence reader | 9 | 0 | 1 | 11 |
| Chen, et al. | 2020 | China | 10 | Respiratory swabs | Cas12a | N | Lateral flow assays | 7 | 0 | 0 | 3 |
| Ding, et al. | 2020 | America | 28 | Respiratory swabs | Cas12a | N | Fluorescence reader | 8 | 0 | 0 | 20 |
| Ma, et al. | 2020 | China | 24 | Nasopharyngeal swabs | Cas12a | E | Fluorescence reader | 13 | 0 | 0 | 11 |
| Ariti-San, et al. | 2020 | America | 50 | Nasopharyngeal swabs | Cas13 | N | Fluorescence reader | 27 | 0 | 3 | 20 |
| Mayuramart, et al. | 2021 | Thailand | 164 | Nasopharyngeal and/or throat swabs | Cas12a | S | Fluorescence reader | 51 | 0 | 2 | 111 |
| Nimsamer, et al. (1) | 2021 | Thailand | 107 | Nasopharyngeal and/or throat swab | Cas12a | N1 | Fluorescence reader | 41 | 0 | 3 | 63 |
| Nimsamer, et al. (2) | 2021 | Thailand | 107 | Nasopharyngeal and/or throat swab | Cas12a | N2 | Fluorescence reader | 42 | 6 | 2 | 57 |
| Nimsamer, et al. (3) | 2021 | Thailand | 107 | Nasopharyngeal and/or throat swab | Cas12a | E | Fluorescence reader | 43 | 10 | 1 | 53 |
| Nimsamer, et al. (4) | 2021 | Thailand | 107 | Nasopharyngeal and/or throat swab | Cas12a | S | Fluorescence reader | 42 | 0 | 2 | 63 |
| Ning, et al. (1) | 2021 | America | 103 | Nasal swabs | Cas12a | O | Fluorescence reader | 27 | 0 | 0 | 76 |
| Ning, et al. (2) | 2021 | America | 103 | Nasal swabs | Cas12a | O | Fluorescence reader | 27 | 1 | 0 | 75 |
| Ooi, et al. | 2021 | Singapore | 75 | Nasopharyngeal swabs | Cas12a | S | Lateral flow assays | 37 | 0 | 8 | 30 |
| Rauch, et al. | 2021 | America | 218 | Nasopharyngeal swabs | Cas13 | N | Fluorescence reader | 63 | 3 | 2 | 150 |
| Samacoits, et al. | 2021 | Thailand | 115 | Nasal swabs | Cas12a | N | Fluorescence reader | 45 | 5 | 7 | 58 |
| Brandsma, et al. | 2021 | Netherlands | 378 | Nasopharyngeal swabs, bronchoalveolar lavage and sputum | Cas12 | N | Lateral flow assays | 144 | 10 | 11 | 213 |
| Chen, et al. | 2021 | America | 27 | Nasopharyngeal swabs | Cas12a | N | Fluorescence reader | 11 | 0 | 0 | 16 |
| Curti, et al. | 2021 | Argentina | 210 | Nasopharyngeal swabs | Cas12 | N | Fluorescence reader | 106 | 1 | 0 | 104 |
| Ding, et al. | 2021 | America | 32 | Clinical swabs | Cas12a | N1 | Fluorescence reader | 12 | 0 | 0 | 20 |
| Jiang, et al. (1) | 2021 | China | 41 | Nasopharyngeal and throat swabs | Cas12a | N | Colorimetric analysis | 21 | 0 | 0 | 20 |
| Jiang, et al. (2) | 2021 | China | 41 | Nasopharyngeal and throat swabs | Cas12a | O | Colorimetric analysis | 21 | 0 | 0 | 20 |
| Lee, et al. | 2021 | Korea | 20 | Nasopharyngeal and oropharyngeal swabs and sputum | Cas12a | N | Fluorescence reader | 10 | 0 | 0 | 10 |
| Sun, et al. | 2021 | China | 54 | Pharyngeal swabs | Cas12a | O | Fluorescence reader | 6 | 0 | 0 | 48 |
| Pang, et al. | 2020 | Canada | 100 | Respiratory swabs | Cas12a | NE | Fluorescence reader | 47 | 0 | 3 | 50 |
| Liu, et al. | 2021 | China | 25 | Nasal swabs | Cas12a | O/N | Fluorescence reader | 20 | 0 | 0 | 5 |
| Li, et al. | 2021 | China | 649 | Oropharyngeal and sputum swabs | Cas13a | N | Lateral flow assays | 243 | 3 | 25 | 378 |
| Tian, et al. | 2021 | China | 40 | Nasopharyngeal swabs | Cas13a | O/N | Fluorescence reader | 20 | 0 | 0 | 20 |
| Wang, et al. | 2021 | China | 50 | Respiratory swabs | Cas12a | S | Fluorescence reader | 26 | 0 | 0 | 24 |
| Xiong, et al. | 2021 | China | 64 | Nasopharyngeal swabs | Cas9 | E/O | Lateral flow assays | 34 | 0 | 1 | 29 |
| Zhu, et al. | 2021 | China | 114 | Respiratory swabs | Cas12a | O/N | Lateral flow assays | 37 | 0 | 0 | 77 |

CRISPR, clustered regularly interspaced short palindromic repeats; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; Cas, CRISPR-associated proteins; FN, false negative; FP, false positive; TN, true negative; TP, true positive; N, nucleocapsid protein gene; E, envelope protein gene; S, spike protein gene. *The reference standard of the included studies was reverse transcription-quantitative PCR.
Fig. 2. Quality evaluation results for each study included in the meta-analysis. (A) Risk of bias and applicability concerns summary. (B) Quality evaluation of the included studies.
Analysis of threshold effect
In the threshold effect analysis, the Spearman correlation coefficient was 0.024, and the p-value was 0.888 (p>0.05). Moreover, the SROC curve (Fig. 3C) did not have a “shoulder arm” distribution. Therefore, we concluded that there was no threshold effect in the included studies.

Fig. 3. Forest plots for CRISPR-based SARS-CoV-2 detection methods. (A) Forest plots for combined sensitivity. (B) Forest plots for combined specificity. (C) The SROC of SARS-CoV-2 infections detected by CRISPR. CRISPR, clustered regularly interspaced short palindromic repeats; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; CI, confidence interval; SROC, summary receiver operating characteristic; AUC, area under the curve; SE, standard error.
Heterogeneity analysis of non-threshold effect

A forest map was used to plot the ratio following a random pattern. The heterogeneity in non-threshold effects was low (Fig. 4C): Cochran-Q=38.80, p=0.3884 (>0.05), inconsistency=4.6% (inconsistency<50%).

Fig. 4. Forest plots for CRISPR-based SARS-CoV-2 detection methods. (A) Forest plots for combined positive likelihood ratio. (B) Forest plots for combined negative likelihood ratio. (C) Forest plots for combined diagnostic OR. CRISPR, clustered regularly interspaced short palindromic repeats; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; LR, likelihood ratio; df, degree of freedom; CI, confidence interval; OR, odds ratio.
Several studies have evaluated the accuracy of immunochromatographic analysis and ELISA for SARS-CoV-2 detection. A test strip for the detection of SARS-CoV-2 IgG/IgM-combined antibody based on immunochromatography has been developed by Liao, et al., with a sensitivity of 92.9% and a specificity of 98.7%. However, the antibody-positive rate in the first week of infection was only 77.3% and reached 100% on day 9. Another study reported by Beavis, et al. evaluated an ELISA assay to detect SARS-CoV-2 IgA and IgG antibodies. The sensitivity of IgA ELISA was 82.9% and the specificity was 88.4%, while the sensitivity of IgG ELISA was 67.1% and the specificity was 97.7%. Although these assays are fast and easy to operate compared to CRISPR, infection-generated antibodies are detectable at later stages in the disease, which is not conducive to early disease screening. In addition, if the sample is heat-inactivated, the effective concentration of the antibody would decrease and probably give false-negative results. Meanwhile, according to Beavis, et al., ELISA assay tended to have a lower sensitivity and specificity compared to CRISPR. Therefore, CRISPR is a valid and appropriate instrument for detecting SARS-CoV-2.

Furthermore, to minimize the sources of heterogeneity, this study implemented strict criteria for the inclusion and exclusion of the studies. In the threshold effect analysis, the Spearman correlation coefficient was found to be 0.024 (<0.6) and the $p$-value was 0.888 ($p>0.05$), which indicated the lack of threshold effect in the included studies. However, $F$ values of pooled sensitivity (52.8%), specificity (65.0%), PLR (66.0%), which exceeded 50%, suggested the presence of heterogeneity from non-threshold effects. Subgroup analysis was performed to investigate the heterogeneity caused by different types of Cas enzyme used, Cas12 and Cas13, but no statistically significant results were obtained. Instead, we found that gene targets and readout modes might be the possible sources of underlying heterogeneity. Moreover, the Deeks’ funnel plot ($p=0.457>0.1$) indicated that no publication bias was possibly present.

The present systematic review and meta-analysis also had several limitations. First, we only extracted data from the literature published in the four select English databases, and ignored some negative results without statistical significance or unpublished data. This may lead to defects in the comprehensiveness of the current study and more publication bias. Second, the detection capability of the reference methods may not necessarily be more reliable than that of CRISPR. The reference methods could also provide false-positive results, thereby leading to underestimation of the specificity of the CRISPR method. Finally, there were no remarkable changes in subgroup analyses. This study can be improved with the accumulation of more clinical data in the future. With more COVID-19 cases being reported every day worldwide, there may be more studies supporting our theory and, at the same time, having important implications for the diagnosis of COVID-19.

In summary, CRISPR has proven to be a rapid, sensitive, and specific method to detect SARS-CoV-2. It can provide reliable information for clinical laboratory tests and contribute to point-of-care diagnostics where simplicity and cost-effectiveness are needed. This technology is expected to become the major auxiliary diagnostic method for COVID-19 in the near future.
DATA AVAILABILITY
All data generated or analyzed during this study are included in this published article and its supplementary information files.

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AUTHOR CONTRIBUTIONS
Conceptualization: Xu-Guang Guo. Data curation: Pei-Ying Huang. Formal analysis: Pei-Ying Huang and Xin Yin. Investigation: Pei-Ying Huang, Xin Yin, Yue-Ting Huang, and Qi-Qing Ye. Methodology: Xu-Guang Guo. Project administration: Pei-Ying Huang. Resources: Xu-Guang Guo. Software: Pei-Ying Huang, Xun-Jie Cao, and Tian-Ao Xie. Supervision: Pei-Ying Huang. Validation: Pei-Ying Huang and Xin Yin. Visualization: Pei-Ying Huang and Xin Yin. Writing—original draft: all authors. Writing—review & editing: Pei-Ying Huang, Xun-Jie Cao, and Tian-Ao Xie. Data curation: Pei-Ying Huang and Xin Yin. Approval of final manuscript: all authors.

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REFERENCES
1. Trougakos IP, Stamatopoulou K, Terpos E, Tsitsilonis OE, Aivalioti E, Paraskevis D, et al. Insights to SARS-CoV-2 life cycle, pathophysiology, and rationalized treatments that target COVID-19 clinical complications. J Biomed Sci 2021;28:9.
2. Atzrodt CL, Maknojia J, McCarthy RDP, Oldfield TM, Po J, Ta KTL, et al. A guide to COVID-19: a global pandemic caused by the novel coronavirus SARS-CoV-2. FEBS J 2020;287:3633-50.
3. Piroth L, Cotenet J, Mariet AS, Bonniald P, Blot M, Tubert-Bitter P, et al. Comparison of the characteristics, morbidity, and mortality of COVID-19 and seasonal influenza: a nationwide, population-based retrospective cohort study. Lancet Respir Med 2021;9:251-9.
4. Yiße M, Filiztekin E, Özkaya KG. COVID-19 diagnosis—A review of current methods. Biosens Bioelectron 2021;172:112752.
5. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): a review. JAMA 2020;324:782-93.
6. Patchsung M, Jantarak K, Pattama A, Aphichko K, Suraritdechaclai S, Meesawat P, et al. Clinical validation of a Cas13-based assay for the detection of SARS-CoV-2 RNA. Nat Biomed Eng 2020;4:1140-9.
7. Vogels CBF, Brito AF, Wylie AL, Fauver JR, Ot Im, Kalinich CC, et al. Analytical sensitivity and efficiency comparisons of SARS-CoV-2 RT-qPCR primer-probe sets. Nat Microbiol 2020;5:1299-305.
8. Wu J, Liu J, Li S, Peng Z, Xiao Z, Wang X, et al. Detection and analysis of nucleic acid in various biological samples of COVID-19 patients. Travel Med Infect Dis 2020;37:101673.
9. Jin YH, Cai L, Cheng ZS, Cheng H, Deng T, Fan YP, et al. A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version). Mil Med Res 2020;7:4.
10. Huang Z, Tian D, Liu Y, Lin Z, Lyon CJ, Lai W, et al. Ultra-sensitive and high-throughput CRISPR-p overed COVID-19 diagnosis. Biosens Bioelectron 2020;164:112316.
11. Bachman J. Reverse-transcription PCR (RT-PCR). Methods Enzymol 2013;530:67-74.
12. Wong ML, Medrano JF. Real-time PCR for mRNA quantitation. Biotechniques 2005;39:75-85.
13. Mostafa HH, Fissel JA, Fanelli B, Bergman Y, Gniazdzowski V, Dadlani M, et al. Metagenomic next-generation sequencing of nasopharyngeal specimens collected from confirmed and suspect COVID-19 patients. mBio 2020;11:e01969-20.
14. Long QX, Tang XJ, Shi QL, Li Q, Deng HJ, Yuan J, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med 2020;26:1200-4.
15. Winichakoon P, Chaiwarith R, Liewsrisakun C, Salee P, Gonnana A, Limskukan A, et al. Negative nasopharyngeal and oropharyngeal swabs do not rule out COVID-19. J Clin Microbiol 2020;58:e00297-20.
16. Broughton JP, Deng X, Yu G, Fasching CL, Servellita V, Singh J, et al. CRISPR-Cas12-based detection of SARS-CoV-2. Nat Biotechnol 2020;38:870-4.
17. Chen Y, Shi Y, Chen Y, Yang Z, Wu H, Zhou Z, 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.
18. Ali Z, Aman R, Mahas A, Rao GS, Tehseen M, Marsic T, et al. iSCAN: an RT-LAMP-coupled CRISPR-Cas12 module for rapid, sensitive detection of SARS-CoV-2. Virus Res 2020;288:198129.
19. Wang F, Wang L, Zou X, Duan S, Li Z, Deng Z, et al. Advances in CRISPR-Cas systems for RNA targeting, tracking and editing. Biotechnol Adv 2019;37:708-29.
20. Gootenberg JS, Abudayeh OO, Lee JW, Esletzbichler P, Dy AJ, Joung J, et al. Nucleic acid detection with CRISPR-Cas13a/C2c2. Science 2017;356:438-42.
21. McInnes MDF, Moher D, Thombs BD, McGrath TA, Bossuyt PM, Clifford T, et al. Preferred reporting items for a systematic review and meta-analysis of diagnostic test accuracy studies: the PRISMA-DTA statement. JAMA 2018;319:388-96.
22. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reits MA-DTA statement. JAMA 2018;319:388-96.
23. Yüce M, Filiztekin E, Özkaya KG. COVID-19 diagnosis—A review of current methods. Biosens Bioelectron 2021;172:112752.
24. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): a review. JAMA 2020;324:782-93.
25. Patchsung M, Jantarak K, Pattama A, Aphichko K, Suraritdechaclai S, Meesawat P, et al. Clinical validation of a Cas13-based assay for the detection of SARS-CoV-2 RNA. Nat Biomed Eng 2020;4:1140-9.
26. Ding X, Yin K, Li Z, Lalla RV, Ballesteros E, Séir MM, et al. Ultra-
sensitive and visual detection of SARS-CoV-2 using all-in-one dual CRISPR-Cas12a assay. Nat Commun 2020;11:4711.

27. Ma P, Meng Q, Sun B, Zhao B, Dang L, Zhong M, et al. MeCas12a, a highly sensitive and specific system for COVID-19 detection. Adv Sci (Weinh) 2020;7:2001300.

28. Azizti-Sanz J, Freije CA, Stanton AC, Petros BA, Boehm CK, Siddiqui S, et al. Streamlined inactivation, amplification, and Cas13-based detection of SARS-CoV-2. Nat Commun 2020;11:5921.

29. Maynaramat O, Nimsamer P, Rattanaburi S, Chantaravisoot N, Khongnomman K, Chansaenroj J, et al. Detection of severe acute respiratory syndrome coronavirus 2 and influenza viruses based on CRISPR-Cas12a. Exp Biol Med (Maywood) 2021;246:400-5.

30. Nimsamer P, Maynaramat O, Rattanaburi S, Chantaravisoot N, Saengchoosong S, Puempa J, et al. Comparative performance of CRISPR-Cas12a assays for SARS-CoV-2 detection tested with RNA extracted from clinical specimens. J Virol Methods 2021;290:114092.

31. Ning B, Yu T, Zhang S, Huang Z, Tian D, Lin Z, et al. A smartphone-read ultrasensitive and quantitative saliva test for COVID-19. Sci Adv 2021;7:eabe3703.

32. Ooi KH, Liu MM, Tay JWY, Teo SY, Kaewsapak P, Jin S, et al. An engineered CRISPR-Cas12a variant and DNA-RNA hybrid guides enable robust and rapid COVID-19 testing. Nat Commun 2021;12:1739.

33. Rauch JN, Valois E, Solley SC, Braig F, Lach RS, Audouard M, et al. A scalable, easy-to-deploy protocol for Cas13-based detection of SARS-CoV-2 genetic material. J Clin Microbiol 2021;59:e02402-20.

34. Samacoits A, Nimsamer P, Maynaramat O, Chantaravisoot N, Siththi-Amorn P, Nakakhes C, et al. Machine learning-driven and smartphone-based fluorescence detection for CRISPR diagnostic of SARS-CoV-2. ACS Omega 2021;6:2727-33.

35. Brandsma E, Verhagen HJMP, van de Laar TJW, Claas EJC, Cornellissen M, van den Akker E. Rapid, sensitive, and specific severe acute respiratory syndrome coronavirus 2 detection: a multicenter comparison between standard quantitative reverse-transcriptase polymerase chain reaction and CRISPR-based DETECTR. J Infect Dis 2021;223:206-13.

36. Chen FE, Lee PW, Trick AY, Park JS, Chen L, Shah K, et al. Point-of-care CRISPR-Cas-assisted SARS-CoV-2 detection in an automated and portable droplet magnetofluidic device. Biosens Bioelectron 2021;190:113390.

37. Curti LA, Primost I, Valla S, Ibáñez Alegre D, Olguín Pergilone C, Repizo GD, et al. Evaluation of a lyophilized CRISPR-Cas12 assay for a sensitive, specific, and rapid detection of SARS-CoV-2. Viruses 2021;13:420.

38. Ding X, Yin K, Li Z, Sfeir MM, Liu C. Sensitive quantitative detection of SARS-CoV-2 in clinical samples using digital warm-start CRISPR assay. Biosens Bioelectron 2021;184:113218.

39. Jiang Y, Hu M, Liu AA, Lin Y, Liu L, Yu B, et al. Detection of SARS-CoV-2 by CRISPR/Cas12a-enhanced colorimetry. ACS Sens 2021;6:1086-93.

40. Lee CY, Degani I, Cheong J, Lee JH, Choi HJ, Cheon J, et al. Fluorescence polarization system for rapid COVID-19 diagnosis. Biosens Bioelectron 2021;178:113049.

41. Sun Y, Yu L, Liu C, Ye S, Chen W, Li D, et al. One-tube SARS-CoV-2 detection platform based on RT-RPA and CRISPR/Cas12a. J Transl Med 2021;19:74.

42. Pang B, Xu J, Liu Y, Peng H, Feng W, Cao Y, et al. Isothermal amplification and ambient visualization in a single tube for the detection of SARS-CoV-2 using loop-mediated amplification and CRISPR technology. Anal Chem 2020;92:16204-12.

43. Liu S, Huang M, Xi Y, Kang J, Ye S, Liu S, et al. CRISPR/Cas12a technology combined with RT-ERA for rapid and portable SARS-CoV-2 detection. Virol Sin 2021;36:1083-7.

44. Li H, Dong X, Wang Y, Yang L, Cai K, Zhang X, et al. Sensitive and easy-read CRISPR strip for COVID-19 rapid point-of-care testing. CRISPR J 2021;4:392-9.

45. Tian T, Shu B, Jiang Y, Ye M, Liu L, Guo Z, et al. An ultralocalized Cas13a assay enables universal and nucleic acid amplification-free single-molecule RNA diagnostics. ACS Nano 2021;15:1167-78.

46. Wang B, Qian C, Pang Y, Li M, Yang Y, Ma H, et al. opvCRISPR: one-pot visual RT-LAMP-CRISPR platform for SARS-cov-2 detection. Biosens Bioelectron 2021;172:112766.

47. Xiong F, Jiang L, Tian H, Mu Y, Yue H, Huang M, et al. Simultaneous dual-gene diagnosis of SARS-CoV-2 based on CRISPR/Cas9-mediated lateral flow assay. Angew Chem Int Ed Engl 2021;133:5367-75.

48. Zhu X, Wang X, Li S, Luo W, Zhang X, Wang C, et al. Rapid, ultrasensitive, and highly specific diagnosis of COVID-19 by CRISPR-based detection. ACS Sens 2021;6:881-8.

49. Kellner MJ, Koob JG, Gootenberg JS, Abudayyeh OO, Zhang F. SHERLOCK: nucleic acid detection with CRISPR nucleases. Nat Protoc 2019;14:2986-3012.

50. Oliveira BA, Oliveira LC, Sabino EC, Okay TS. SARS-CoV-2 and the COVID-19 disease: a mini review on diagnostic methods. Rev Inst Med Trop Sao Paulo 2020;62:e44.

51. Liao M, Yan J, Wang X, Qian H, Wang C, Xu D, et al. Development and clinical application of a rapid SARS-CoV-2 antibody test strip: a multi-center assessment across China. J Clin Lab Anal 2021;35:e23619.

52. Beavis KG, Matushek SM, Abelede APF, Bethel C, Hunt C, Gillen S, et al. Evaluation of the EUROIMMUN anti-SARS-CoV-2 ELISA assay for detection of IgA and IgG antibodies. J Clin Virol 2020;129:104468.

53. Li Z, Yi Y, Luo X, Xiong N, Liu Y, Li S, et al. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. J Med Virol 2021;93:1518-24.

54. Pang B, Qian C, Pang Y, Li M, Yang Y, Ma H, et al. Evaluation of the EUROIMMUN anti-SARS-CoV-2 ELISA assay for detection of IgA and IgG antibodies. J Clin Virol 2020;129:104468.

55. Li Z, Yi Y, Luo X, Xiong N, Liu Y, Li S, et al. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. J Med Virol 2021;93:1518-24.