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

Pei-Ying Huang[1,2*], Xin Yin[1,3*], Yue-Ting Huang[1,2], Qi-Qing Ye[1,3], Si-Qing Chen[1,2], Xun-Jie Cao[1,4], Tian-Ao Xie[4], and Xu-Guang Guo[1,4,5,6]

[1]Department of Clinical Laboratory Medicine, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou;
[2]Nanshan School, Guangzhou Medical University, Guangzhou;
[3]Pediatrics School, Guangzhou Medical University, Guangzhou;
[4]Department of Clinical Medicine, The Third Clinical School of Guangzhou Medical University, Guangzhou;
[5]Key Laboratory for Major Obstetric Diseases of Guangdong Province, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou;
[6]Key Laboratory of Reproduction and Genetics of Guangdong Higher Education Institutes,
The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, China.

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-

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Corresponding author: Xu-Guang Guo, MD, PhD, Department of Clinical Laboratory Medicine, The Third Affiliated Hospital of Guangzhou Medical University, 63th Road Duobao, Liwan District, Guangzhou 510150, China.
Tel: 86-20-81292492, Fax: 86-20-81292245, E-mail: gysygxg@gmail.com

*Pei-Ying Huang and Xin Yin contributed equally to this work.
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CoV-2 immunoglobulins, respectively.[5] 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.[6-9] However, it requires well-trained personnel and advanced equipment, which limits the application of RT-qPCR, especially in resource-constrained developing countries.[10-12] 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.[13] 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.[4] In addition, asymptomatic patients are considered to play a major role in the spread of the virus.[14] These factors increase the need for effective, cheap, and rapid alternative methods.[4]

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.[15] 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.[16,17] 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.[18-20]

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.[21] 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,[22] 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).[6,10,16,17,23-48] 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

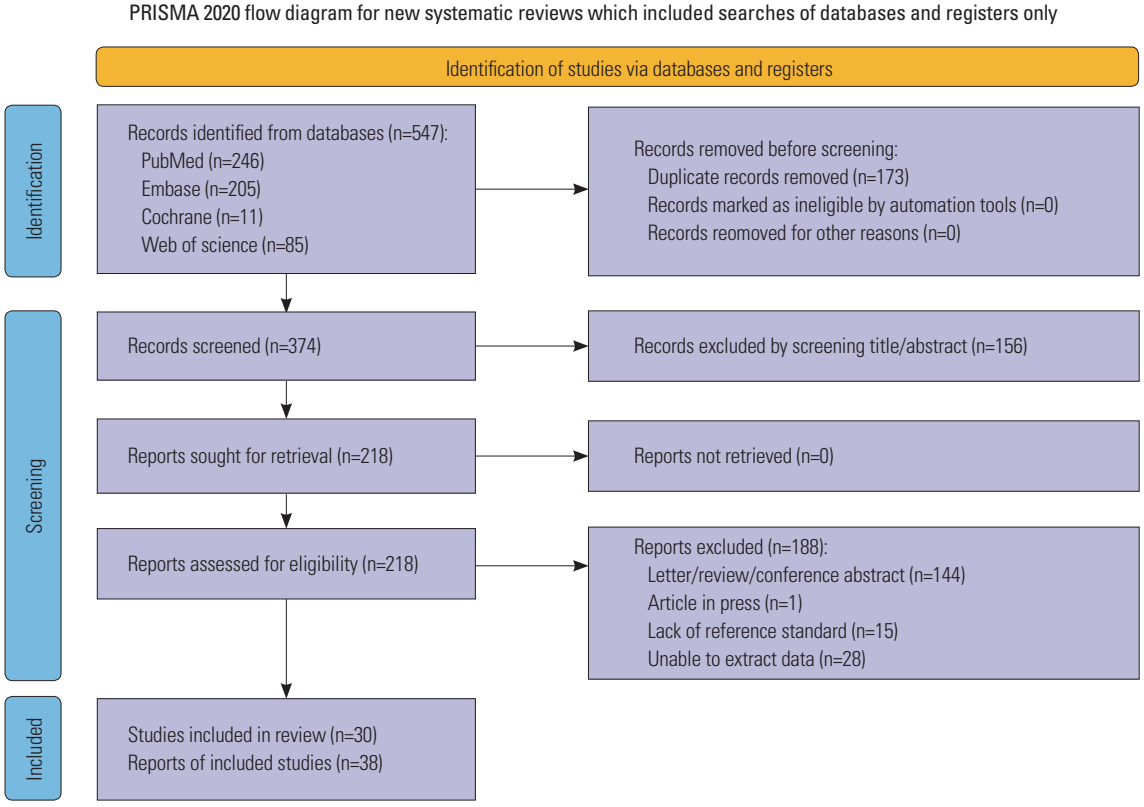

Fig. 1. Flow diagram of study identification and inclusion.

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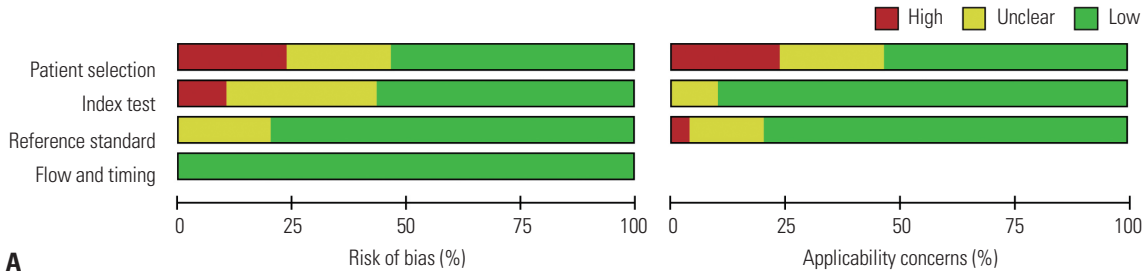
was 0.94 (95% CI 0.93–0.95, I^2=52.8%) (Fig. 3A), and the specificity was 0.98 (95% CI 0.97–0.99, I^2=65.0%) (Fig. 3B). As shown in the chart in Fig. 3C, the AUC was 0.9894. The PLR was 34.03 (95% CI 20.81–55.66, I^2=66.0%) (Fig. 4A), and the NLR was 0.08 (95% CI 0.06–0.10, I^2=14.0%) (Fig. 4B). The value of the pooled DOR was 575.74 (95% CI 382.36–866.95) (Fig. 4C).

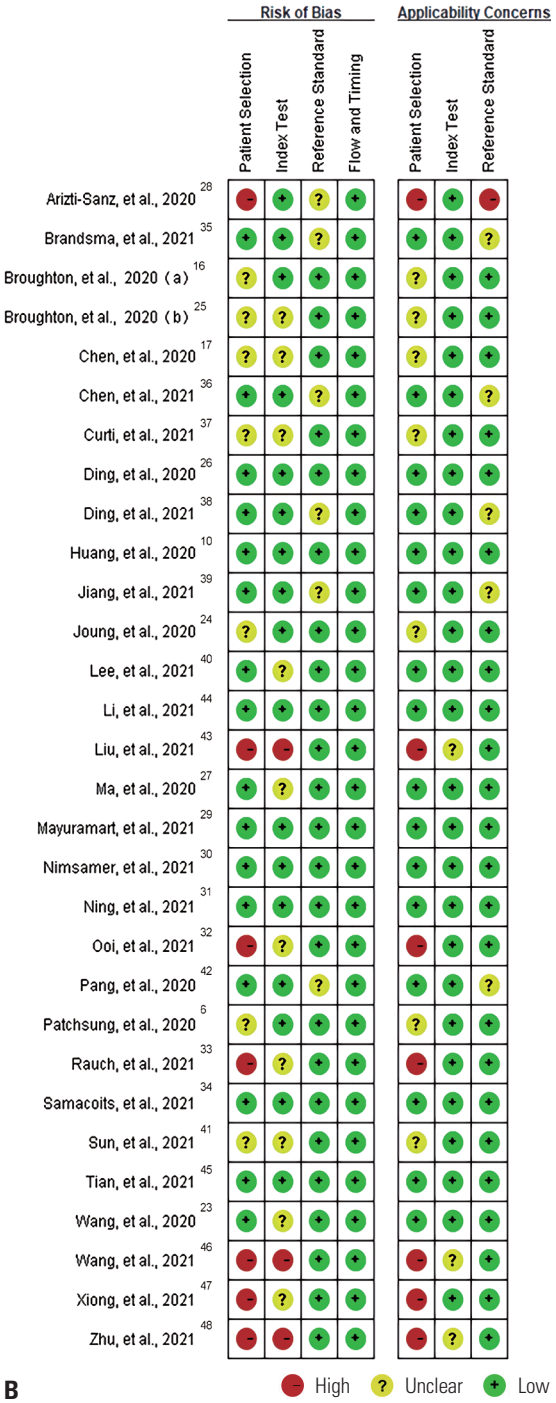
Table 1. Characteristics of Included Studies about CRISPR Detection of SARS-CoV-2

Author	Year	Geographical distribution of virus	Patients (n)	Sample source	Type of cas enzyme	Gene site	Readout mode	TP	FP	FN	TN
Patchsung, et al. (1)[6]	2020	Thailand	154	Nasopharyngeal swabs	Cas13a	N	Lateral flow assays	71	0	10	73
Patchsung, et al. (2)[6]	2020	Thailand	154	Nasopharyngeal swabs	Cas13a	N	Fluorescence reader	78	0	3	73
Huang, et al.[10]	2020	America	29	Nasal swabs	Cas12a	N	Fluorescence reader	15	4	0	10
Wang, et al.[23]	2020	China	31	Nasal swabs	Cas12a	E	Fluorescence reader	16	0	0	15
Joung, et al.[24]	2020	America	402	Nasal swabs	Cas12b	N	Fluorescence reader	188	3	14	197
Broughton, et al. (a1)[16]	2020	America	82	Nasopharyngeal swabs	Cas12	N\E	Lateral flow assays	9	0	1	12
Broughton, et al. (a2)[16]	2020	America	82	Nasopharyngeal swabs	Cas12	N\E	Fluorescence reader	37	0	3	42
Broughton, et al. (b1)[25]	2020	America	21	Nasopharyngeal swabs	Cas12	E	Fluorescence reader	10	0	0	11
Broughton, et al. (b2)[25]	2020	America	21	Nasopharyngeal swabs	Cas12	N	Fluorescence reader	9	0	1	11
Chen, et al.[17]	2020	China	10	Respiratory swabs	Cas12a	N\E	Lateral flow assays\ Fluorescence reader	7	0	0	3
Ding, et al.[26]	2020	America	28	Respiratory swabs	Cas12a	N	Fluorescence reader	8	0	0	20
Ma, et al.[27]	2020	China	24	Nasopharyngeal swabs	Cas12a	E	Fluorescence reader	13	0	0	11
Arizti-San, et al.[28]	2020	America	50	Nasopharyngeal swabs	Cas13	N	Fluorescence reader	27	0	3	20
Mayuramart, et al.[29]	2021	Thailand	164	Nasopharyngeal and/or throat swabs	Cas12a	S	Fluorescence reader	51	0	2	111
Nimsamer, et al. (1)[30]	2021	Thailand	107	Nasopharyngeal and/or throat swab	Cas12a	N1	Fluorescence reader	41	0	3	63
Nimsamer, et al. (2)[30]	2021	Thailand	107	Nasopharyngeal and/or throat swab	Cas12a	N2	Fluorescence reader	42	6	2	57
Nimsamer, et al. (3)[30]	2021	Thailand	107	Nasopharyngeal and/or throat swab	Cas12a	E	Fluorescence reader	43	10	1	53
Nimsamer, et al. (4)[30]	2021	Thailand	107	Nasopharyngeal and/or throat swab	Cas12a	S	Fluorescence reader	42	0	2	63
Ning, et al. (1)[31]	2021	America	103	Nasal swabs	Cas12a	O	Fluorescence reader (Smartphone)	27	0	0	76
Ning, et al. (2)[31]	2021	America	103	Nasal swabs	Cas12a	O	Fluorescence reader (Plate reader)	27	1	0	75
Ooi, et al.[32]	2021	Singapore	75	Nasopharyngeal swabs	Cas12a	S	Lateral flow assays	37	0	8	30
Rauch, et al.[33]	2021	America	218	Nasopharyngeal swabs	Cas13	N	Fluorescence reader	63	3	2	150
Samacoits, et al.[34]	2021	Thailand	115	Nasal swabs	Cas12a	N	Fluorescence reader	45	5	7	58
Brandsma, et al.[35]	2021	Netherlands	378	Nasopharyngeal swabs, bronchoalveolar lavage and sputum	Cas12	N	Lateral flow assays	144	10	11	213
Chen, et al.[36]	2021	America	27	Nasopharyngeal swabs	Cas12a	N	Fluorescence reader	11	0	0	16
Curti, et al.[37]	2021	Argentina	210	Nasopharyngeal swabs	Cas12	N	Fluorescence reader	105	1	0	104
Ding, et al.[38]	2021	America	32	Clinical swabs	Cas12a	N1	Fluorescence reader	12	0	0	20
Jiang, et al. (1)[39]	2021	China	41	Nasopharyngeal and throat swabs	Cas12a	N	Colorimetric analysis	21	0	0	20
Jiang, et al. (2)[39]	2021	China	41	Nasopharyngeal and throat swabs	Cas12a	O	Colorimetric analysis	21	0	0	20
Lee, et al.[40]	2021	Korea	20	Nasopharyngeal and oropharyngeal swabs and sputum	Cas12a	N	Fluorescence reader	10	0	0	10
Sun, et al.[41]	2021	China	54	Pharyngeal swabs	Cas12a	O	Fluorescence reader	6	0	0	48
Pang, et al.[42]	2020	Canada	100	Respiratory swabs	Cas12a	N\E	Fluorescence reader	47	0	3	50
Liu, et al.[43]	2021	China	25	Nasal swabs	Cas12a	O\N	Fluorescence reader	20	0	0	5
Li, et al.[44]	2021	China	649	Oropharyngeal and sputum swabs	Cas13a	N	Lateral flow assays	243	3	25	378
Tian, et al.[45]	2021	China	40	Nasopharyngeal swabs	Cas13a	O\N	Fluorescence reader	20	0	0	20
Wang, et al.[46]	2021	China	50	Respiratory swabs	Cas12a	S	Fluorescence reader	26	0	0	24
Xiong, et al.[47]	2021	China	64	Nasopharyngeal swabs	Cas9	E\O	Lateral flow assays	34	0	1	29
Zhu, et al.[48]	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; O, open reading frame 1 ab.
*The reference standard of the included studies was reverse transcription-quantitative PCR.



A



B

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.

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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.

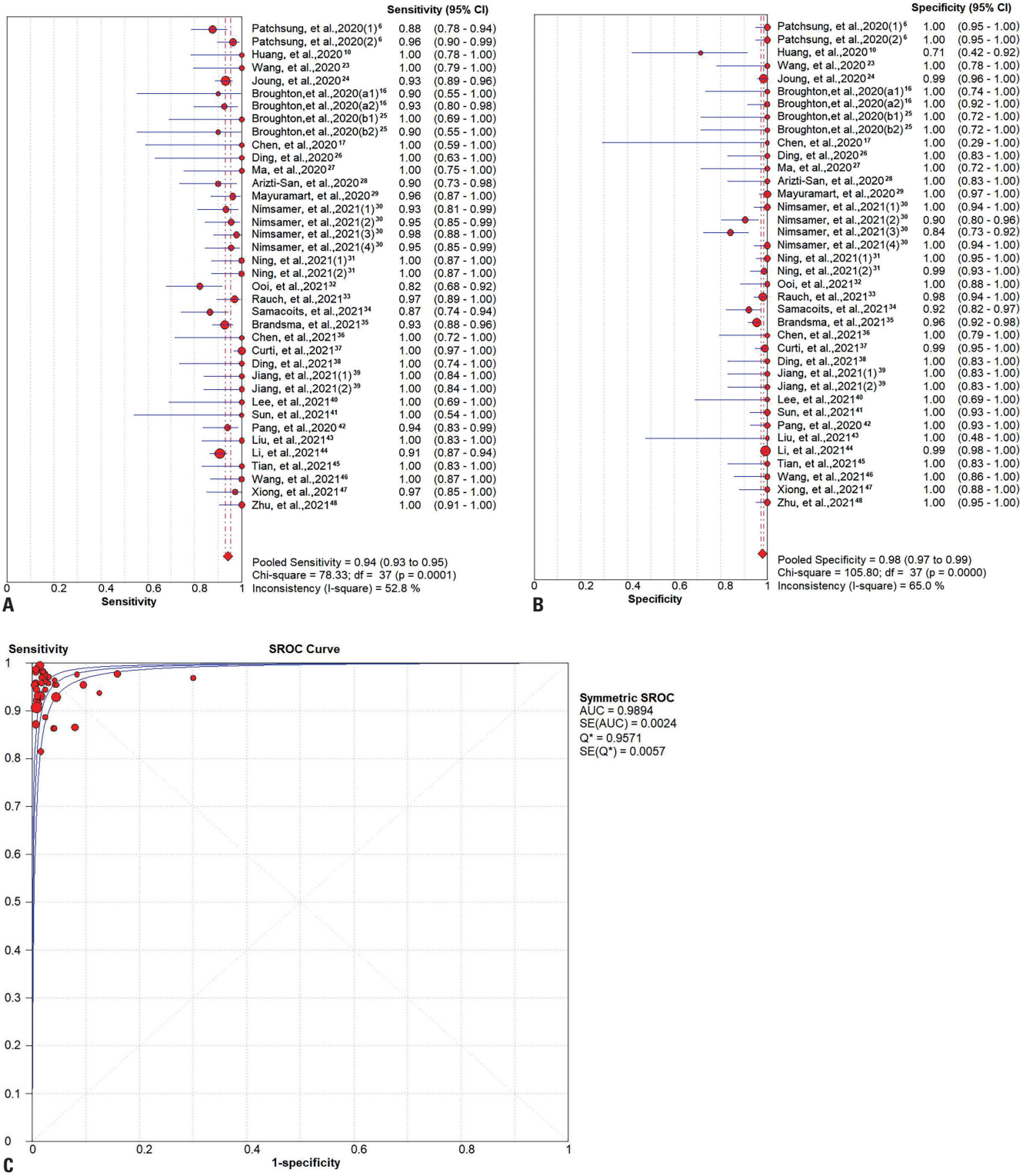

Fig. 3. Forest plots for CRISPR-based SARS-CoV-2 detection methods. (A) Forest plots for combined sensitivity. (B) Forest plots for combined specificity. (C) The SROC of SARS-CoV-2 infections detected by CRISPR. CRISPR, clustered regularly interspaced short palindromic repeats; SARS-CoV-2, severe acute 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 (p>0.05), inconsistency=4.6% (inconsistency<50%).

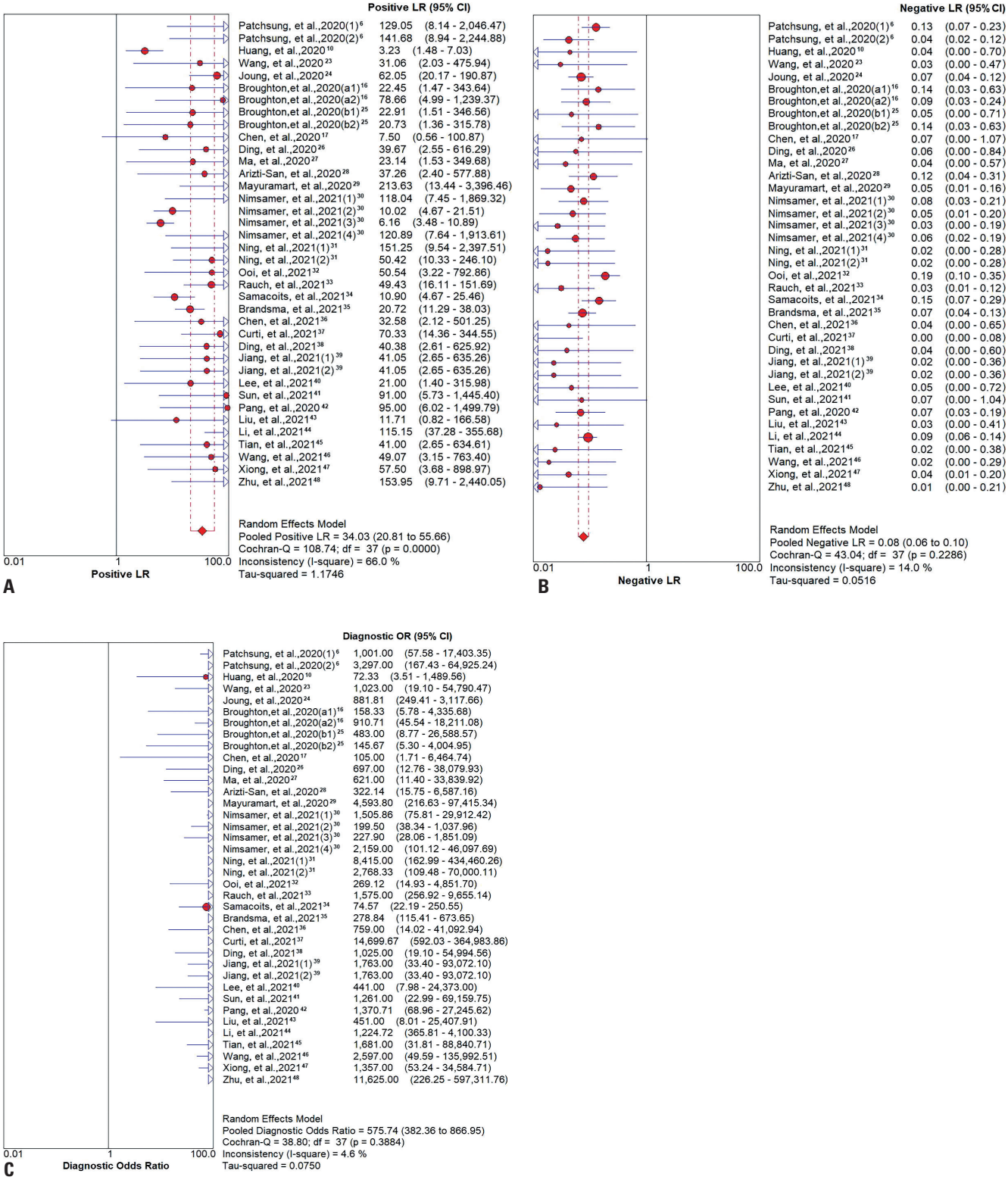

Fig. 4. Forest plots for CRISPR-based SARS-CoV-2 detection methods. (A) Forest plots for combined positive likelihood ratio. (B) Forest plots for combined negative likelihood ratio. (C) Forest plots for combined diagnostic OR. CRISPR, clustered regularly interspaced short palindromic repeats; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; LR, likelihood ratio; df, degree of freedom; CI, confidence interval; OR, odds ratio.

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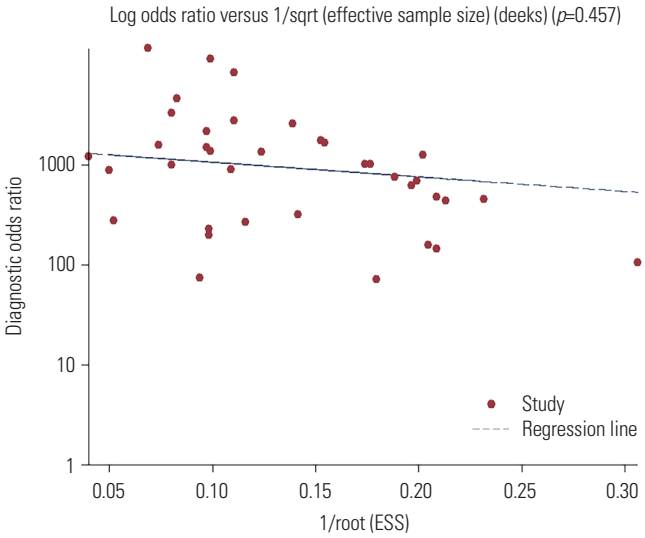

Fig. 5. Deeks' funnel plot asymmetry test to evaluate publication bias of CRISPR. CRISPR, clustered regularly interspaced short palindromic repeats; ESS, effective sample size.

Publication bias

The Deeks' funnel plot (Fig. 5) was made using the Stata 15.0 software to identify publication bias in the included publications (p=0.457>0.1), and it showed no potential publication bias for the included studies.

DISCUSSION

Currently, RT-qPCR assays are the recommended molecular diagnostic tools to detect COVID-19 infection.[7,8] However, they come with a high demand for equipment and skillful lab technicians.[10] In contrast, the CRISPR-based nucleic acid detection platforms have the combined advantages of conventional RNA-targeting technologies, and a fluorescence readout or a lateral-flow readout can be used to analyze the results in an hour, with a setup time of less than 15 min.[49] The simplicity of operation, remarkable specificity, and high efficiency make CRISPR-based diagnostics the new avenues for sensitive, robust, and rapid detection of viral pathogens.

In this study, we performed a pooled analysis, and the results of the systematic review and meta-analysis indicated that CRISPR had an overall sensitivity of 0.94 (95% CI 0.93–0.95) and an overall specificity of 0.98 (95% CI 0.97–0.99) in detecting SARS-CoV-2. The value of the pooled DOR was 575.74. The AUC was 0.9894 and was close to 1. Based on these results, we can infer that the diagnosis of COVID-19 by using CRISPR was highly accurate.

Several studies have evaluated the accuracy of immunochromatographic analysis and ELISA for SARS-CoV-2 detection.[50] A test strip for the detection of SARS-CoV-2 IgG/IgM-combined antibody based on immunochromatography has been developed by Liao, et al.,[51] 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.[52] 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[50,53] compared to CRISPR, infection-generated antibodies are detectable at later stages in the disease, which is not conducive to early disease screening.[51,54] In addition, if the sample is heat-inactivated, the effective concentration of the antibody would decrease and probably give false-negative results.[55] Meanwhile, according to Beavis, et al.,[52] 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, I^2 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.[6,10,23] Moreover, the Deeks' funnel plot (p=0.457 >0.1) indicated that no publication bias was possibly subsistent.

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, Xin Yin, and Si-Qing Chen. Approval of final manuscript: all authors.

ORCID iDs

Pei-Ying Huang	https://orcid.org/0000-0002-6814-0611
Xin Yin	https://orcid.org/0000-0002-6200-2111
Yue-Ting Huang	https://orcid.org/0000-0002-2954-6483
Qi-Qing Ye	https://orcid.org/0000-0001-6292-6210
Si-Qing Chen	https://orcid.org/0000-0002-0480-5012
Xun-Jie Cao	https://orcid.org/0000-0001-5084-9692
Tian-Ao Xie	https://orcid.org/0000-0003-1738-4840
Xu-Guang Guo	https://orcid.org/0000-0003-1302-5234

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