Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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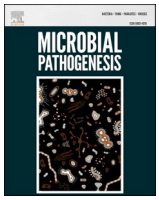

Accuracy of clustered regularly interspaced short palindromic repeats (CRISPR) to diagnose COVID-19, a meta-analysis

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ABSTRACT

Objective: To estimate the accuracy of clustered regularly interspaced short palindromic repeats (CRISPR) in determining coronavirus disease-19 (COVID-19).

Methods: As of January 31, 2022, PubMed, Web of Science, Embase, Science Direct, Wiley and Springer Link were searched. Sensitivity, specificity, likelihood ratio (LR), diagnostic odds ratio (DOR) and area under the summary receiver-operating characteristic (AUC) curve were used to assess the accuracy of CRISPR.

Results: According to the inclusion criteria, 5857 patients from 54 studies were included in this meta-analysis. The pooled sensitivity, specificity and AUC were 0.98, 1.00 and 1.00, respectively. For CRISPR-associated (Cas) proteins-12, the sensitivity, specificity was 0.96, 1.00, respectively. For Cas-13, the sensitivity and specificity were 0.99 and 0.99.

Conclusion: This meta-analysis showed that the diagnostic performance of CRISPR is close to the gold standard, and it is expected to meet the Point of care requirements in resource poor areas.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is infectious in human beings, which is the cause of coronavirus disease 19 (COVID-19) in 2019. It spreads rapidly to all parts of the world through the close interaction of human beings or the respiratory substances (coughing and sneezing) of infected persons [1]. The World Health Organization (WHO) declared that COVID-19 is Public Health Emergency of International Concern (PHEIC) on January 30, 2020 and pandemic on March 11, 2020 [2,3], due to it spread rapidly around the world in a concise period of time. The number of global COVID-19 cases remained at the highest level since the beginning of the pandemic for the second consecutive week, with more than 5.7 million new cases per week [4]. The number of new deaths rose for the seventh consecutive week, with more than 93000 [4]. The end of the epidemic requires diagnostic tools that can handle large numbers of samples and still perform well in order to prevent further spread of the epidemic.

Detection and isolation of asymptomatic carriers has proved to be quite effective in controlling the spread of the virus. Therefore, there is a need for efficient, low-cost detection methods to carry out a wide range of patients and repeated detection. Reverse-Transcription Polymerase Chain Reaction (RT-PCR) has frequently been used as first-line test for COVID-19 as it is fast, sensitive and reliable, and can produce results in 3–4 h [5]. However, RT-PCR requires expensive laboratory instruments and professional technicians, and its availability is limited to public health laboratories [6]. In the environment of backward economy, crowd gathering and poor sanitary conditions, COVID-19 is easy to spread, and the limitations of detection technology hamper the real-time monitoring and detection of highly contagious pathogens. The development of diagnosis system based on clustered regularly interspaced short palindromic repeats (CRISPR) had changed the mode of molecular diagnosis. CRISPR has the advantages of high speed, high sensitivity and high precision, and strong versatility. With the outbreak of global COVID-19, different organizations have begun to design and develop diagnostic methods based on efficient CRISPR system. Based on CRISPR detection, CRISPR-associated (Cas) proteins were used to detect isothermal amplification products, the signal is generated only when the correct sequence exists. Cas12 and Cas13 are outstanding in analytical application, the Cas12 or Cas13 protein are guided by a CRISPR RNA (crRNA) to target a specific nucleic acid sequence, while the single-stranded region of the crRNA is complementary to the target. However, the functions of Cas12 and Cas13 are different, Cas12 targets

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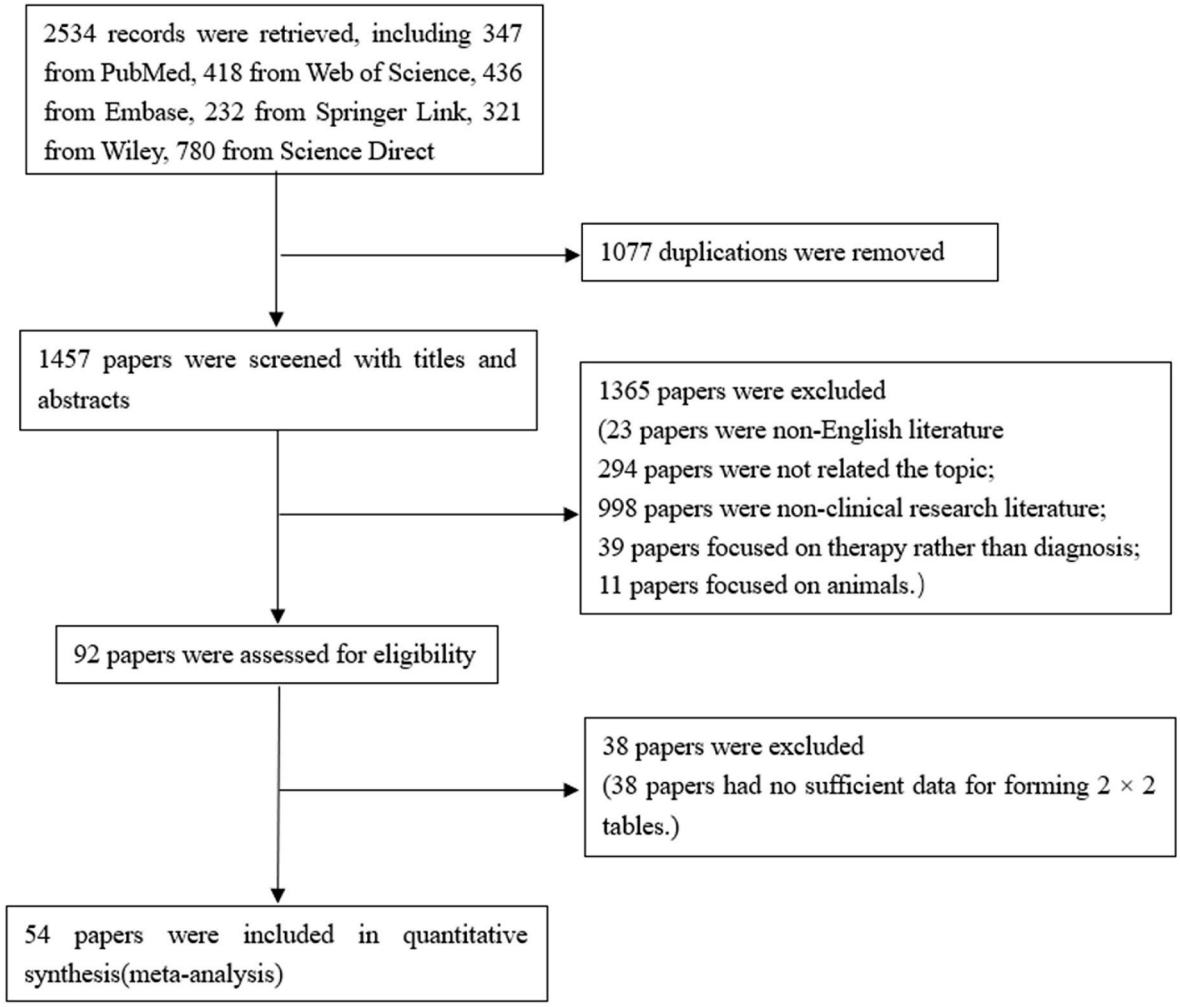

Fig. 1. Flow diagram of the study selection process.

ssDNA, while Cas13 targets ssRNA [7,8]. Timely and correct case management and correct reporting can reduce incidence and mortality of COVID-19. CRISPR system can help develop molecular diagnostic methods with rapid, accurate and point-of-care. Compared with existing RT-PCR based diagnostic methods, CRISPR is convenient to use, portable and time-saving, without needing any technical expertise and expensive equipment in the absence of resources [9,10]. The availability of affordable portable diagnostic systems may facilitate large-scale screening of affected populations to stop the transmission chain of highly infectious viral infections [11].

The number of cases of COVID-19 and its related mortality have caused serious concern all over the world. At present, there is no meta-analysis on the diagnosis of COVID-19 based on CRISPR system. The purpose of this study is to evaluate the diagnostic value of CRISPR for COVID-19.

2. Materials and methods

2.1. Search strategy and study selection

Two independent researchers searched PubMed, Web of Science, Embase, Science Direct, Wiley and Springer Link for all relevant articles published before January 31, 2022, Search terms were defined as follows: ("severe acute respiratory syndrome coronavirus 2″ or “SARS-CoV-2″) and ("coronavirus disease 19″ or "COVID-19″) and ("clustered regularly interspaced short palindromic repeats" or “CRISPR").

2.2. Study selection

All articles were screened according to the inclusion and exclusion criteria by two independent reviewers. The inclusion criteria were as follow: (1) The purpose of research is to evaluate the accuracy of CRISPR diagnostic method; (2) Participants were diagnosed with novel coronavirus pneumonia; (3) The extracted or calculated data could be used to obtain true-positive (TP), false-positive (FP), false-negative (FN), and true-negative (TN) values. The exclusion criteria were as follows: (1) Non-English literature; (2) Non-clinical research literature consisting of conference abstracts, reviews, case reports; (3) The data was not enough to form a 2 × 2 table.

2.3. Data extraction

For each eligible study, the following information was extracted: author, year of publication, parameters such as TP, FP, FN and TN, the type of amplification reaction, the type of Cas protein, reaction steps and results display method. Controversial results were evaluated and a consensus was reached by third-party researchers.

2.4. Quality assessment

To assess the quality of included literature, Quality Assessment of diagnostic Accuracy Studies 2 (QUADAS-2) was conducted. QUADAS-2 was an evidence-based quality assessment tool that consisted of four domains: patient selection, index, test, reference standard, and flow and

Table 1
Major characteristics of included studies.

Author	Year	TP	FN	FP	TN	Cas-protein	Amplification	Reaction Steps	Result display
Iqbal Azmi	2021	45	2	0	29	Cas13	RPA	TWO-STEP	Later Flow
Eelke Brandsma	2021	144	10	11	213	Cas12	LAMP	TWO-STEP	–
Daniel J Brogan	2020	12	9	0	21	CasRx	RPA	TWO-STEP	Fluorescence
Lucía Ana Curti	2021	105	0	1	104	Cas12	LAMP	TWO-STEP	Fluorescence
Alfredo Garcia-Venzor	2021	6	0	0	6	Cas12	LAMP	TWO-STEP	Fluorescence
Weiren Huang	2020	20	4	2	238	Cas12	PCR	TWO-STEP	Fluorescence
Tin Marsic	2021	54	0	2	4	Cas9	RPA	TWO-STEP	Later Flow
Maturada Patchsung	2020	78	0	3	73	Cas13	RPA	TWO-STEP	Fluorescence
Ashwin Ramachandran	2020	30	0	2	32	Cas12	LAMP	TWO-STEP	Fluorescence
Dan Xiong	2020	11	0	0	11	Cas12	RPA	TWO-STEP	–
Xiong Zhu	2021	37	0	0	37	Cas12	MCDA	TWO-STEP	Later Flow
Yanju Chen	2020	7	0	0	3	Cas12	LAMP	ONE-STEP	Fluorescence
Shreeya Agrawal	2020	31	2	0	30	Cas13	LAMP	ONE-STEP	Fluorescence
Xiong Ding[1]	2021	12	0	0	23	Cas12	DAMP	ONE-STEP	Fluorescence
Xiong Ding[2]	2020	8	0	0	20	Cas12	RPA	ONE-STEP	Fluorescence
Zahir Ali[1]	2020	18	3	0	3	Cas12	LAMP	ONE-STEP	Fluorescence
Jon Arizti-Sanz	2020	27	3	0	20	Cas13	RPA	ONE-STEP	Fluorescence
Julia Joung	2020	12	0	0	5	Cas12	LAMP	ONE-STEP	Later Flow
Ning, Bo	2021	30	0	0	30	–	RPA	ONE-STEP	Fluorescence
Rui Wang	2021	26	0	0	24	Cas12	LAMP	ONE-STEP	Fluorescence
Zhen Huang	2020	15	4	0	10	Cas12	RPA	–	Fluorescence
Peixiang Ma	2020	13	0	0	11	Cas12	RRA	–	Fluorescence
Tieying Hou	2020	52	0	0	62	Cas13	RPA	–	Fluorescence
James P Broughton	2020	38	2	0	42	Cas12	LAMP	–	Later Flow
Oraphan Mayuramart	2021	51	2	0	111	Cas12	RPA	–	Fluorescence
Pattaraporn Nimsamer	2021	42	2	0	63	Cas12	RPA	–	Fluorescence
Jen-Hui Tsou	2021	10	0	0	12	Cas12	RPA	–	Fluorescence
Yu Wang	2021	58	5	0	57	Cas12	RPA	–	Fluorescence
Erhu Xiong	2021	34	0	1	29	Cas9	RPA	–	Later Flow
Roberto Alcántara	2021	40	10	0	50	Cas12	PCR	ONE-STEP	Fluorescence
Zahir Ali[2]	2021	74	0	3	17	Cas9	RPA	–	Later Flow
Azhar, M.	2021	14	1	0	32	Cas9	RPA	ONE-STEP	Later Flow
Cao, Y.	2021	25	2	0	27	Cas12	LAMP	–	–
Chen, F. E.	2021	11	0	0	16	Cas12	RPA	ONE-STEP	Later Flow
de Puig, H.	2021	26	1	1	20	Cas12	RPA	ONE-STEP	Fluorescence
Ding, Xiong[3]	2022	10	1	0	9	–	DAMP	ONE-STEP	Fluorescence
Feng, W.	2021	18	3	0	25	Cas12	RPA	ONE-STEP	Fluorescence
Zhen Huang	2021	31	3	1	124	Cas12	PCR	–	Fluorescence
Jiang, Yongzhong	2021	19	2	0	20	Cas12	RPA	–	–
Khan, W. A.	2021	43	0	0	17	Cas13	LAMP	–	Fluorescence
Lee, C. Y.	2021	10	0	0	10	Cas12	–	ONE-STEP	Fluorescence
Hao Li	2021	243	3	25	378	Cas13	RAA	–	Later Flow
Long Ma	2022	20	0	0	30	Cas12	PCR	TWO-STEP	Fluorescence
Ahmed Mahas	2021	38	0	1	2	Cas13	LAMP	–	Fluorescence
Jeong Moon	2021	30	0	0	30	Cas13	LAMP	–	Fluorescence
Jennifer N Rauch[1]	2021	8	1	0	1067	Cas13	PCR	–	Fluorescence
Jennifer N Rauch[2]	2021	63	3	2	150	Cas13	PCR	–	Fluorescence
Chandana S. Talwar	2021	12	0	0	8	Cas12	RPA	–	–
Zhang, Qin	2022	62	0	0	25	Cas13	RAA	–	Fluorescence
Zhao, Xiangxiang	2021	30	0	0	30	Cas12	–	–	–
Jiajie Liang	2021	32	0	0	80	Cas12	–	–	Fluorescence
Shijun Li[a]	2021	38	14	0	80	Cas12	–	–	Fluorescence
Shijun Li**	2021	35	17	0	80	Cas12	–	–	Later Flow
Zhijian Yi	2021	38	2	0	20	Cas12	LAMP	–	Fluorescence
Brendan J Manning	2021	65	0	2	90	Cas13	LAMP		Fluorescence

TP: True positive. FN: False negative; FP: False positive; TN: True negative; RPA: Recombinase polymerase amplification; LAMP: Loop-mediated isothermal amplification; PCR: Polymerase chain reaction; MCDA: Multiple cross displacement amplification; DAMP: Dual-priming isothermal amplification; 1, 2, 3: Multiple sets of valid data for the different article of same author, the same for the following included articles.

[a] Multiple sets of valid data for the same article.

timing [12].

2.5. Statistical analysis

Heterogeneity from threshold and non-threshold effects were reflected by the spearman correlation coefficient, Cochran' s-Q and I^2 tests, respectively. If the *P*-value of spearman correlation coefficient was more than 0.05, there was no threshold effect, whether a heterogeneity caused by non-threshold effect existed was further analyzed by Meta-regression. The pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR) and diagnostic odds ratio (DOR), along with their 95% confidence intervals (CIs) were computed and graphically displayed using forest plots. A summary receiver operating characteristic (sROC) curve was constructed to access the diagnostic accuracy for CRISPR, and area under the curve (AUC) was calculated. The higher the AUC was, the higher the diagnostic value was. Publication bias was assessed by Deeks' funnel plot asymmetry test, Begg's test and Egger's test [13]. The meta-analysis was undertaken using STATA15.0 (StataCorp, College Station, TX, USA) and Meta-Disc 1.4 (Unit of Clinical Biostatistics, Ramo e Cajal Hospital, Madrid, Spain). Quality assessments of included studies were carried out with RevMan 5.3 (RevMan, Cochrane Collaboration). $P < 0.05$ was considered of statistical significance.

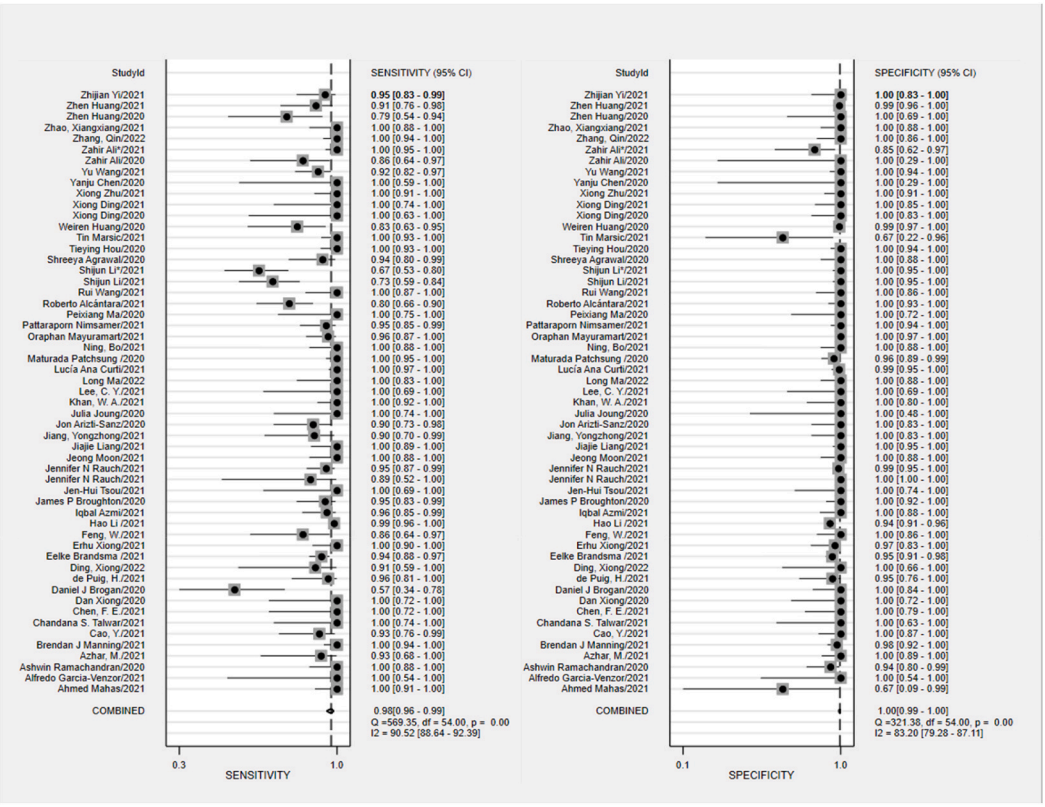

Fig. 2. Forest plots of pooled sensitivity and specificity.

3. Results

3.1. Study selection

After PubMed, Web of Science, Embase, Science Direct, Wiley and Springer Link were comprehensive searched, 2534 literature was found. Among these articles, 1077 duplications were removed. By browsing literature abstracts and titles, 23 articles were non-English literature; 294 articles were excluded because those were not related to topic; 998 articles were non-clinical research literature; 39 articles focused on therapy rather than diagnosis; 11 articles focused on animals. After further browsing, 38 articles had no sufficient data for forming 2 × 2 tables. Finally, 54 eligible articles were included (Fig. 1) [14–23; 24–34; 35–45; 46–56; 57–67].

3.2. Study characteristics

The study included 5857 peoples who underwent CRISPR. The characteristics of each included study were summarized in Table 1. The flowchart of the study selection process was shown in Fig. 1.

3.3. Risk of bias and quality assessment

Deeks' funnel chart was used to analyze publication bias. The funnel chart was symmetrical and $P > 0.05$, suggesting no significant publication bias. Begg's test and egger's test reached the same conclusion (Supplementary Fig. 1). The results of QUADAS-2 showed that in terms of risk of bias, 3 studies were considered to have high risk of bias, 13 studies had uncertain risk, and the rest were low-risk bias. In terms of applicability concerns, 3 studies had high risks, 13 studies were considered uncertain risks and 38 studies were low risks. The result means that the overall quality of the included studies is high.

3.4. The diagnostic effect of CRISPR

The pooled sensitivity and specificity of CRISPR for the diagnosis of COVID-19 were 0.98 (0.96–0.99) and 1.00 (0.99–1.00), respectively. The detailed results were shown in Fig. 2. The pooled PLR and NLR were 217.17 (77.40–609.33) and 0.02 (0.01–0.04), respectively, as shown in Supplementary Fig. 3. The pooled DOR was 8971.74 (2962.16–27173.46), as shown in Supplementary Fig. 4. The AUC was 1.00 (0.99–1.00), as shown in Fig. 3.

3.5. Meta-regression

From the threshold analysis, the spearman correlation coefficient was 0.075 and $P = 0.586 > 0.05$, indicating that there was no threshold effect. But the results showed that there was heterogeneity caused by non-threshold effects. As shown in Fig. 2, the sensitivity of I^2 was 90.52, and the specificity of I^2 was83.20, indicating that there was overall heterogeneity. And meta-regression was conducted to perform sources of heterogeneity. Finally, amplification (Loop-mediated isothermal amplification; Recombinase polymerase amplification), results display (fluorescence/later flow) and reaction steps (one-step/two-step) were not potential sources of heterogeneity in sensitivity ($P < 0.05$). But P-value of Cas-protein was less than 0.05 indicating that it might be a significant source of heterogeneity in sensitivity. About specificity, reaction steps might be a significant source of heterogeneity ($P < 0.05$) (Table 2).

4. Discussion

The global epidemic situation continues to be tense, and local medical systems have collapsed in some regions. The disaster caused by the SARS-CoV-2 is incalculable. Although some countries have developed SARS-CoV-2 vaccines, the vaccination rate is not ideal. At the same time, more countries have not entered the process of vaccine research

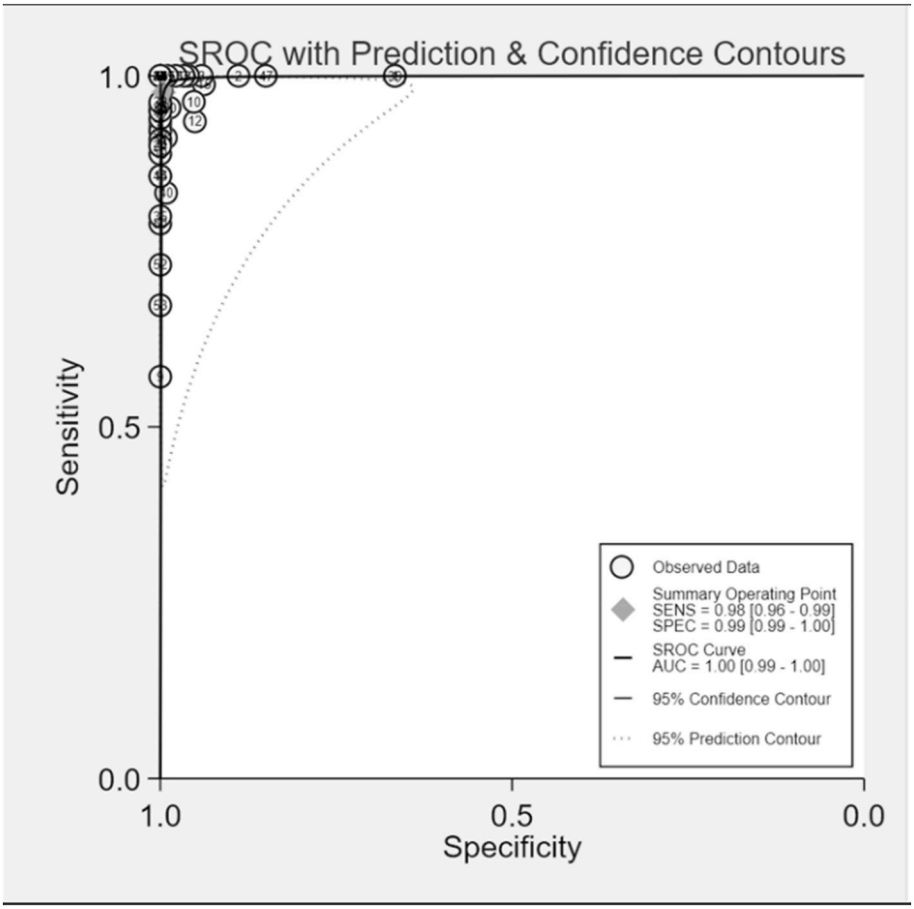

Fig. 3. SROC curve of CRISPR for the diagnosis of COVID-19.

Table 2
Meta-regression.

Covariate	Number of studies	Sensitivity (95%CI)	P-value	Specificity (95%CI)	P-value
Cas-protein					
Cas12	35	0.96 (0.94–0.99)	<0.05	1.00 (0.99–1.00)	0.24
Cas13	13	0.99 (0.97–1.00)		0.99 (0.98–1.00)	
Amplification					
LAMP	16	0.99 (0.97–1.00)	0.46	0.99 (0.97–1.00)	0.52
RPA	22	0.98 (0.95–1.00)		1.00 (0.99–1.00)	
Results display					
Fluorescence	38	0.98 (0.96–0.99)	0.09	1.00 (0.99–1.00)	0.27
Later Flow	11	0.98 (0.97–1.00)		0.98 (0.96–1.00)	
Reaction dish					
One-Step	16	0.97 (0.94–1.00)	0.44	1.00 (0.99–1.00)	<0.05
Two-Step	12	0.98(0.96-1.00)		0.98 (0.96–1.00)	

LAMP: Loop-mediated isothermal amplification; RPA: Recombinase polymerase amplification.

and development. Vaccine control in the world is far away. We still have to face the challenge of virus diagnosis.

The meta-analysis included 54 studies. The evaluation results of QUADAS-2 tool showed that the included studies were of high quality and could provide reliable results. The pooled sensitivity and specificity of CRISPR in the diagnosis of COVID-19 were 0.98 (0.96–0.99) and 1.00 (0.99–1.00), respectively, and the area under the curve (AUC) was 1.00 (0.99–1.00). AUC, as a measure, reflects the effect of the test in distinguishing patients with and without disease [68]. The results show that CRISPR has good diagnostic performance and high diagnostic value. In subgroup analysis, the pooled sensitivity and specificity of lateral flow-based were 0.98 and 0.98, respectively, and the pooled sensitivity and specificity of fluorescence-based were 0.98 and 1.00, respectively. The performance of the two methods is almost the same, but Jon arizti Sanz thinks that the lateral flow-based is not suitable for testing a large number of samples at the same time, and introduces the potential risk of sample cross contamination [30]. In another subgroup, the pooled sensitivity and specificity of one-step reaction method were 0.97 and 1.00 respectively, while two-step method were 0.98 and 0.98 respectively. Meta-regression showed that the reaction steps was a source of heterogeneity in specificity between one-step and two-step ($P < 0.05$). And, Julia joung and Xiong Ding believed that one-step reaction method could prevent external pollution in the test environment and avoid false positive results [27,31]. The reason why the results are not different might be the included studies belong to the verification stage, not the population-based experiments. In the face of small samples, the operator had patience to control the risk of cross contamination and external contamination of samples, and did not verify in the ward, so the defect of two-step reaction method was not exposed. Besides, considering the application of cas13 and RPA in Zika virus and dengue virus, future studies may be able to combine them with CRISPR [69,70].

The control of epidemic situation in low and middle income countries is always a concern. Taking into account the speed of COVID-19's dissemination, it is hoped that a cost-effective diagnosis system can be established in areas with limited resources. At present, two common

diagnostic tools have been developed based on CRISPR diagnostic methods: specific high sensitivity enzymatic reporter unlocking (SHERLOCK) and DNA endonuclease targeted CRISPR trans reporter (DETECTR) [71], these two methods have comparable diagnostic performance with RT-PCR. At the same time, it can meet the requirements of the ability to identify infectious disease pathogens without complex processing and the field deployable diagnostic tools with affordable cost. Considering the polluted environment, all in one dual CRISPR-Cas12a (AIOD-CRISPR) can effectively solve this problem without increasing too much cost, which can meet the application of point of care diagnosis [28].

CRISPR diagnostic technology has the advantages of fast, accurate, low cost, no need of laboratory, and has a broad development prospect. The included studies show that this diagnostic method is feasible, and there is still room for further improvement of these tools, especially when there is no instrument or amplification step at all. A recent study provides an improved version of CRISPR diagnosis, which bypasses the amplification step [72], but needs more optimization to provide a completely instrument free diagnostic platform. Importantly, CRISPR diagnostics has the function of detecting single nucleotide mismatch, so it will be used as a rapid diagnostic tool to detect any new mutation in the SARS-CoV-2 genome [73]. Some of these mutations have been shown to spread at a higher rate, it is necessary to adopt and deploy CRISPR based diagnostic methods to widely screen the spread of these new SARS-CoV-2 strains.

This study also has some limitations. First of all, most of the included studies are only in the primary stage, and more are carried out in the laboratory, which can't accurately verify their effectiveness in the actual situation, but can still provide direction for future research. Second, the results showed that there was heterogeneity, Meta regression also found the source of heterogeneity, but further confirmation is needed.

5. Conclusion

The results of this meta-analysis showed that CRISPR had good accuracy in diagnosing COVID-19. It might provide a reference for the selection of rapid diagnostic tests for patients of COVID-19.

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CRediT authorship contribution statement

Song Wang: Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Jiayi Hu:** Writing – original draft, Data curation. **Chuanying Sui:** Validation, Formal analysis. **Guangliang He:** Validation, Data curation. **Zihan Qu:** Validation, Data curation. **Xiaofei Chen:** Visualization, Investigation. **Yashan Wang:** Validation, Data curation. **Dingjie Guo:** Visualization, Investigation. **Xin Liu:** Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.micpath.2022.105498.

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