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REVIEW

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Diagnostic efficiency of RT-LAMP integrated CRISPR-Cas technique for COVID-19: A systematic review and meta-analysis

Akansha Bhatt[a], Gurvinder Singh Bumbrah[b], Munindra Ruwali[a], Saif Hameed[a] and Zeeshan Fatima[a]

[a]Amity Institute of Biotechnology, Amity University Haryana, Gurugram, India; [b]Department of Forensic Sciences, Amity School of Applied Sciences, Amity University Haryana, Gurugram, India

ABSTRACT

To address the challenges associated with COVID-19 diagnosis, we need a faster, direct, and more versatile detection method for efficient epidemiological management of the COVID-19 pandemic. RT-qPCR (reverse transcription quantitative real-time Polymerase Chain Reaction) although the most popular diagnostic method suffers from a major drawback of equipment dependency and trained molecular biologists that limits rapid and large-scale screening, particularly in low resource regions. Reverse transcription loop-mediated isothermal amplification (RT-LAMP) is a feasible alternative for RT-qPCR; however, it also suffers from the drawback of false-positive issues. Recently, RT-LAMP has been integrated with the CRISPR-Cas technique to take care of the problems associated with RT-LAMP for COVID-19 diagnosis. In this study, a meta-analysis was conducted using three scientific databases considering the PRISMA guidelines to assess the diagnostic efficiency of RT-LAMP integrated CRISPR-Cas technology. Out of a total of 1286 studies on COVID-19, we identified 15 articles that met our eligibility criteria of using simultaneous RT-LAMP and CRISPR-Cas technique. Our meta-analysis of the included studies revealed that most of the studies were conducted in the USA with the N gene as the most common target and fluorescence-based detection method. The meta-analysis results of all included studies have further revealed a pooled sensitivity value of higher than 85% and a pooled specificity value of 80% with the confidence interval of 95%, respectively, as revealed from the forest plot and SROC curve. The accuracy rate of included studies was also calculated which varied from 77.4% to 100%. Furthermore, the precision of included studies varied from 75% to 100%. Lastly, a quality assessment of bias and applicability was performed based on QUADAS-2. Taken together, combined RT-LAMP and CRISPR-Cas technique could be a potential alternative to RT-qPCR particularly in low resource regions having a high demand for rapid testing.

KEYWORDS

COVID-19; SARS-CoV-2; RT-LAMP; CRISPR-Cas; meta-analysis; diagnosis

Introduction

The recent emergence of Coronavirus disease (COVID-19) is caused by the virus known as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). In 2020, the WHO declared COVID-19 a global pandemic which has escalated into a major worldwide health emergency. This pandemic has not only affected the health of the humankind but also the global economy [1, 2]. Person-to-person transmission from infected individuals and asymptomatic patients has been reported [3, 4]. SARS-CoV-2 is fatal and dangerous as it rapidly spreads and robustly mutates into new variants. Due to the fast mutations in the SARS-CoV-2 genome, different variants have emerged, which cause the infection at a very rapid rate in all population groups across the globe (Schermer et al., 2020[5]). This leads to the urgent requirement of rapid and low-cost diagnostic screening of a population at risk to mitigate the sources of infection. Such diagnostic capability also helps policy-makers to decide when and to what extent the restrictions can be eased and restore the economic activities.

The rapid, accessible, and accurate nature of diagnostic tests for coronavirus infections is crucial for patient management and to control the pandemic. Quantitative reverse transcription-polymerase chain reaction (RT-qPCR) is the mostly used detection method for SARS-CoV-2 with the application of Reverse transcription-Loop Associated mediated amplification (RT-LAMP) as a new addition to the diagnosis of the SARS-CoV2 [6, 7]. To fulfill the demand for rapid diagnosis during disease outbreaks, point-of-care tests (POCTs) are needed that are cheaper, faster, and deployable in the field. Various commercially and non-commercially developed tests have been reported since the pandemic started. Most of the commercially available kits are based on RT-qPCR. Non-commercially available tests are based on RT-LAMP, CRISPR, Biosensors, Sequencing-based tests, etc. These tests predominantly target five genes – ORF1 (Open Reading Frame 1), N (Nucleoprotein), E (Envelope), S (Spike), and RdRp (Recombinant dependent RNA polymerase) [8, 9]. Nucleic acid-based detections

CONTACT Zeeshan Fatima drzeeshanfatima@gmail.com; Saif Hameed munindraruwali@gmail.com; Munindra Ruwali saifhameed@yahoo.co.in Amity Institute of Biotechnology, Amity University Haryana, Gurugram (Manesar)-122413, India.

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relying on isothermal amplification such as RT-LAMP and RT-RPA (Reverse Transcription-Recombinase polymerase amplification) obviate the need for a thermal cycler and these methods are also cost-effective, less time-consuming, and realistic [10, 11]. Simple amplification-based assay (SAMBA) uses DNA-dependent RNA polymerase and RNA-dependent DNA polymerase to alternately transcribe and reverse transcribe RNA target. CRISPR diagnosis combines isothermal amplification techniques (such as RT-LAMP and RT-RPA) with specific DNA or RNA targeting ability of crRNA and Cas12 [12, 13] or Cas13 [14, [5]Schermer et al., 2020] enzymes. The outputs of these detection techniques can be coupled with fluorescent or colorimetric reporters as well as lateral flow strip platforms to facilitate readout processes.

Isothermal amplification techniques when integrated with CRISPR-Cas increase the specificity and sensitivity of the assay. Techniques based solely on isothermal amplification have their drawbacks such as RT-LAMP which is very prone to false-positive results and the RT-RPA reagents are not readily available in the market [15, Nguyen et al., 2020[16]]. Hence, the RT-LAMP integrated CRISPR techniques can help to achieve the goal of simplifying the assay for POCT. Considering the growing appreciation of many studies reporting the RT-LAMP integrated CRISPR-Cas technique as POCT for SARS-CoV-2 [15,17–19], it is important to systematically evaluate and draw conclusions about the performance and quality of these studies. This current study aims to determine credible evidence for the use of the proposed diagnostic tool, RT-LAMP integrated with CRISPR-Cas and how it can be utilized as a potential alternative to widely used RT-qPCR for addressing the current COVID-19 challenge under high demand and low resource places.

Materials and methods

For identifying relevant studies in this systematic review and meta-analysis, the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009[20]) have been followed.

Search strategy

We have searched different scientific databases i.e. PubMed, BioRxiv, and MedRxiv for studies performed through August 2021. For the literature search in scientific databases, text words related to COVID-19 diagnostics, RT-LAMP, and CRISPR-Cas assays were used in different combinations. The following search terms were included (coronavirus OR COVID-19), (SARS-CoV -2), (RT-LAMP-based assays on COVID-19), (CRISPR-Cas-based assays on COVID-19) and (RT-LAMP integrated CRISPR-Cas assay on COVID-19). The retrieved results were screened for duplication and conformity with the pre-specified eligibility criteria.

Study eligibility criteria

Inclusion criteria- This systematic review and meta-analysis included: (1) both peer-reviewed and preprint original articles on RT-LAMP integrated CRISPR-Cas technology; (2) full-text articles (all English language articles were found); and (3) articles that provided enough information to determine the number of true positive, false positive, false negative and true negative (performed on clinical samples) relative to a standard reference test.

Exclusion criteria- We excluded: (1) studies investigating antibody test, direct antigen tests or non-isothermal nucleic acid test and other isothermal amplification techniques such as RPA, (2) studies based only on either RT-LAMP or CRISPR-Cas assays related to COVID-19, (3) studies in which data is irretrievable, (4) review articles, editorials, commentaries, and proceedings, etc. based on RT-LAMP or CRISPR-Cas assays related to COVID-19.

Data extraction

Data were extracted by one author (AB) while for any ambiguity, two independent authors (GSB and ZF) were consulted. The information related to variables such as authors, year of publication, location of study, sample size, types of specimens, targeted genes, involvement of RNA extraction step, duration of the assay, detection method, limit of detection, and standard reference method were extracted from included studies. The studies were searched for diagnostic assays based on RT-LAMP integrated CRISPR diagnostics performed on the same set of samples or on a different set of samples or the studies in which different variants of the same assays were used (e.g. using crude samples or on purified RNA or using fluorescent readout, lateral flow readouts, and gold-nanoparticle-based detection). All these studies were included separately. The important parameters in this meta-analysis were the diagnostic values of RT-LAMP integrated CRISPR-Cas, consisting of the true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN) of all studies, providing their sensitivity and specificity values. The included studies (n = 15) were then assessed for their methodological quality to reduce systematic biases and inferential errors from the data extracted.

Statistical analysis

Quantitative analysis was conducted on the outcomes of the included studies (n = 15). The values of TP, FP, TN, FN, LoD, and sample size were extracted from the included studies (n = 15). In addition to this, values of sensitivity and specificity were either extracted from

these studies or calculated from the available data. The accuracy and precision were calculated using the formulas: Accuracy = [TP+TN/TP+TN+FP+FN] *100 and Precision = [TP/TP+FP] *100 [21, 22]. Accuracy is the closeness of the measurements to a specific value, while precision is the closeness of the measurements to each other, i.e. reproducibility. In this study, accuracy depicts how correct a diagnostic test gives a result in a given condition. It is the proportion of true results, either true positive or true negative, in a selected population and represents the percentage of correctly identified samples. Precision represents the ratio between the correctly identified samples and the total number of positive samples (both true and false). It is the proportion of true positive results and quantifies the number of correct positive predictions made in a diagnostic test, in a selected population. RT-qPCR was considered as a standard reference test. Forest plot for sensitivity and specificity were plotted using R-software.

Quality assessment

The risk of bias of the included studies (n = 15) was assessed using Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2). QUADAS-2 assesses four areas of bias; patient selection, index test, reference standards, and flow/timing [1, 23, 24]. All studies were subsequently judged to have low, unclear, or high risks of bias. Additionally, the authors also assessed the applicability in terms of three parameters: patient selection, index test, and reference standards, which were then judged to generate low, unclear, or high-risk applicability.

Results

Literature survey

The literature for the present study has been summarized by using PRISMA guidelines (Figure 1). The three search engines viz. PubMed (n = 522), Biorxiv (n = 428), and Medrxiv (n = 336) have been extensively searched and a total of 1286 articles were found based on the combination of search terms described above. Out of the 1286 shortlisted articles, 422 used RT-LAMP-based diagnosis for COVID-19 while 864 used CRISPR-based diagnostic tools. Only 98 articles used RT-LAMP integrated CRISPR technology for the diagnosis of COVID-19. While searching the abstracts and titles of the studies, the review articles, editorials, proceedings, etc. (n = 71) have been excluded from the current systematic review and after removing the duplicated articles (n = 6) we had 21 articles. However, from the 21 included articles which were assessed through full-text assessments, further 6 articles were also eliminated because the TP, FP, TN, and TN values were not specified in these articles. Overall, after applying all the exclusion criteria, 15 articles were included for detailed analysis (Figure 1).

Characteristics and meta-analysis of the study

Information regarding 15 identified articles (Table 1) describes the authors, year of publication, location of study, sample size, types of specimens, target genes, involvement of RNA extraction step, duration of the assay, detection method, limit of detection and standard reference method. The 15 articles included consist of studies from different countries around the world (Figure 2). It was observed that 40% of the studies were conducted in the USA only [14, 15, 25–28], 13% in Canada [29, 17] and China [2, 30] each. Apart from this, some studies have been conducted in Germany [31], Singapore (Ooi et al., 2021[32]), Netherlands [18], Japan [33], and Saudi Arabia [34], representing 7% each from included studies (Figure 2). Patient's details have not been specified in any of the articles, although the type of specimen taken for most of the studies was nasopharyngeal swab (n = 10) [14, 25, 26, 2, 13, 17, 18, 27, 28, 31]. Some studies have tested on both nasopharyngeal and oropharyngeal swab (n = 3) [15, 33, 34] and only a few studies (n = 2) [29, 30] have used the respiratory throat swab samples for their experiments (Table 1). In terms of applicability of crude patient sample or RNA extraction from patient samples, it has been observed that most of the studies (n = 12) showed the RNA extraction involvement as the initial step for the RT-LAMP integrated CRISPR technology, while 3 articles showed the applicability of RT-LAMP integrated CRISPR technology on crude samples (Table 1).

Furthermore, the standard quantitative RT-qPCR assay was used as a reference standard in all included studies (n = 15). However, in addition to RT-qPCR, the RT-LAMP DETECTR was also used as a reference standard for validating the RT-LAMP integrated CRISPR results (Table 1). Next, we analyzed the type of target gene(s) chosen for the studies. In the included 15 studies, we found E (Envelope), N (Nucleoprotein), ORF1 (Open reading frame 1), and S (Spike protein) genes were used as target genes (Figure 3). N gene was most commonly used in 86.67% of included studies followed by E-gene (53.33%), ORF1 (20.00%), and S-gene (13.33%) (Figure 3). Additionally, from the perspective of the type of detection method used for these 15 studies, the fluorescence method (n = 7) [2, 14, 17, 18, 27, 28, 30] was most frequently used while gold nanoparticle (n = 1) based detection method was used in one study [29]. In five studies, the combination of fluorescence and lateral flow assay was used for the viral detection [13, 15, 25, 26, 34] while the lateral flow assay was used in two studies [31, 33] (Figure 4).

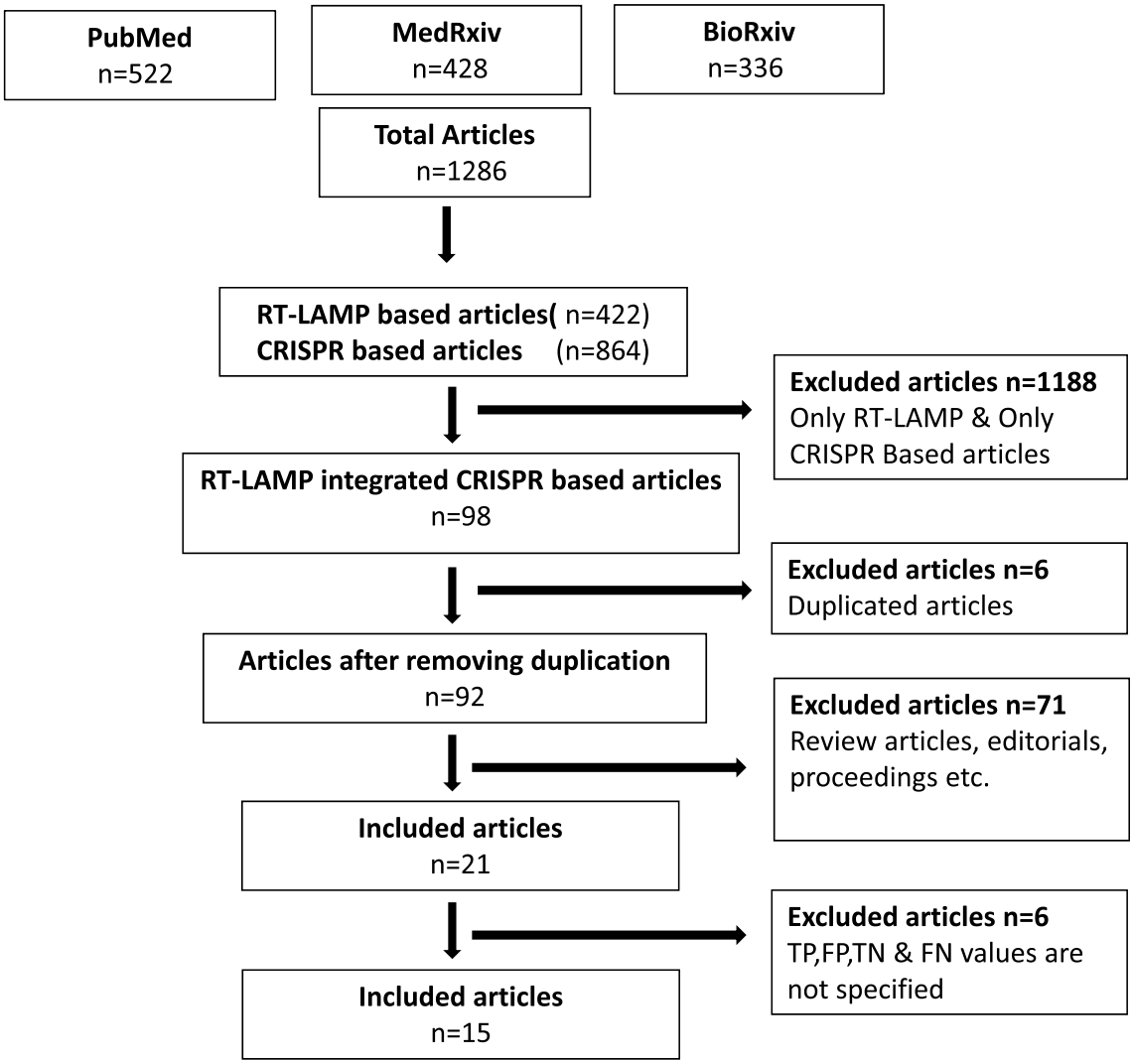


Figure 1. PRISMA flowchart depicts search of the literature and screening strategy for meta-analysis.

Detection time was another parameter included for all 15 articles and ranges between 30 and 60 minutes approximately (Table 1).

The sensitivity and specificity values for each study were calculated using TP, FP, TN, and FN values. The sensitivity of RT-LAMP integrated CRISPR varies in the range from 72% to 100%, while its specificity varies in the range from 89.2% to 100% (Table 1). Out of all 15 studies, 7 studies showed sensitivity values higher than 90%, while 4 studies showed sensitivity values of 100%. In terms of specificity, 14 studies showed a specificity value of higher than 90% while 12 studies showed a specificity value of 100%. Further, we analyzed the sensitivity and specificity of the included studies (n = 15) using a forest plot (Figure 5). These sensitivity and specificity values were found to be at the confidence interval (CI) of 95%. The sensitivity value at 95% CI was found to vary from 0.73 to 1.00. The specificity value at 95% CI was found to vary between 0.51 and 1.00 (Figure 5).

The summary receiver operating characteristic (SROC) plot between sensitivity and false-positive rate was analyzed by using R-software (Figure 6). All studies showed pooled sensitivity higher than 85% except one study which reported a sensitivity value of 73% [31]. In terms of specificity, all studies showed pooled specificity of 80% or higher except a study by Steens et al. (2021 [18]), who reported a specificity value of 51%. Data on the limit of detection (LoD) for each of the studies were also extracted from these studies which range from 2 RNA copies per microliters to 1000 RNA copies per microliters. However, one study (Joung et al., 2020 [25],) reported the LoD value as 33 copies/ml (Table 1). The accuracy rate of included studies was also calculated which varied from 77.4% to 100%; 11 out of 15 studies showed an accuracy rate of more than 90.0%. However, five studies showed an accuracy rate of 100% (Table 2). Furthermore, the precision of included studies varied from 75% to 100%; 12 out of 15 studies showed a precision of 90% or more. However, six studies showed a precision of 100%. On intra-comparison of accuracy with precision, it was observed only in three studies, the accuracy rate is lower than their corresponding precision values. However, in six studies, the accuracy rate is higher than their corresponding precision values and in the other six studies, the accuracy rate equals to precision value for the same study (Table 2).

Table 1. Characteristics and outscomes of included studies (n = 15).

S. No.	First author & year of publication	Country	Type of specimen	Target genes	Sample size	TP	FP	TN	FN	RNA extraction involvement	Detection time	Detection method	Sensitivity	Specificity	LoD	Reference standard
1	35	China	Nasopharyngeal swab samples	S gene	50	26	0	24	0	Yes	45 min.	Fluorescence	100%	100%	5 copies / ul	quantitative RT-PCR assay
2	30	China	Respiratory throat swab samples	ORF1a,N & E	10	7	0	3	0	Yes	40 min.	Fluorescence	100%	100%	2 copies/ ul	quantitative RT-PCR assay
3	34	Saudi Arabia	Orophrayngeal and nasopharyngeal swabs	N& E	24	18	0	3	3	Yes	60 min.	Fluorescence & lateral flow readouts	86%	100%	10 copies/ ul	quantitative RT-PCR assay
4	15	USA	Orophrayngeal and nasopharyngeal swabs	N& E	24	10	2	12	0	Yes	45 min.	Fluorescence & lateral flow readouts	90%	100%	10 copies/ ul	quantitative RT-PCR assay
5	17	Canada	Nasopharyngeal swabs	N& E	100	47	3	50	0	Yes	40 min.	Fluorescence	94%	100%	30 copies / ul	quantitative RT-PCR assay
6	31(RT-LAMP +SHERLOCK	Germany	Nasopharyngeal swabs	ORF1a & N	102	54	3	25	20	Yes	N/A	lateral flow readouts	72.90%	89.20%	N/A	quantitative RT-PCR assay
7	13	Singapore	Nasopharyngeal swabs	N & S gene	129	72	0	57	0	Yes	30 min.	Fluorescence & lateral flow readouts	72%	100%	2 copies/ ul	quantitative RT-PCR assay
8	28	USA	Nasopharyngeal swabs	N gene	56	46	0	10	0	Yes	30 min.	Fluorescence	100%	100%	200 copies/ ul	quantitative RT-PCR assay
9	25	USA	Nasopharyngeal swabs	N gene	402	188	14	197	3	Yes	45 min.	Fluorescence & lateral flow readouts	93.1%	98.5%	33 copies / ml	quantitative RT-PCR assay
10	18	Netherlands	Nasopharyngeal swabs	E gene	101	62	19	20	0	No	35 min.	Fluorescence	76%	100%	1000 copies/ ul	quantitative RT-PCR assay
11	27	USA	Nasopharyngeal swabs	N& E	8	3	1	4	0	No	30 min.	Fluorescence	75%	100%	10 copies/ ul	quantitative RT-PCR assay
12	26	USA	Nasopharyngeal swabs	N& E	62	30	1	30	1	Yes	50 min.	Fluorescence & lateral flow readouts	96.70%	100%	200 copies / ul	quantitative RT-PCR assay
13	14	USA	Nasopharyngeal swabs	N & ORF1a	50	33	0	17	0	Yes	50 min.	Fluorescence	100%	100%	6.75 copies/ ul	quantitative RT-PCR assay
14	33	Japan	Orophrayngeal and nasopharyngeal swabs	N	31	9	1	20	1	Yes	40 min.	Lateral Flow assay	90%	95%	<100 copies/ ul	RT-LAMP-DETECTR & quantitative RT-PCR assay
15	29	Canada	Respiratory swab samples	N& E gene	54	25	2	27	0	No	45 min.	Gold nanoparticle aggultination	96.20%	100%	75 copies/ ul	quantitative RT-PCR assay

Quality assessment

Based on QUADAS criteria, in general, studies were at higher risk of bias than at risk of poor applicability (Figure 7). Patient selection procedures in all 15 studies were at high risk of bias, considering that most studies selected patients nonrandomly. The index tests were also at high risk of bias since it was usually clear that the index tests were interpreted with the knowledge of the results of the reference standard, and the detection methods are qualitative as they did not specify detection thresholds. As shown in Tables 1, 14 studies have used RT-qPCR as a reference standard test for the interpretation of results and only one study has used RT-LAMP DETECTR as well as RT-qPCR test [33], due to which reference standards were also at high risk of biasness. The flow and timing, as shown in Figure 7, were unclear mainly in five studies due to unclear intervals between the index test and the reference standard [14, 15, 28, 29, 34]. Although in 10 studies, it was observed that the reference standard test has been simultaneously performed along with RT-LAMP integrated CRISPR test, suggesting a low risk of biasness (Figure 7).

Discussion

This present study endorses a method for detection of SARS-CoV-2 in the human body that involves RT-LAMP integrated CRISPR technique that can visually be detected with the unaided eye. In this systematic review and meta-analysis, we have included only those articles which were solely based on RT-LAMP integrated CRISPR technique used for COVID-19 diagnosis. Typically, a newly developed or introduced diagnostic test was compared with the commonly used reference standard test. After the implementation of several inclusion and exclusion criteria as reported above, finally, 15 articles have been thoroughly assessed and selected for further analysis (Figure 1). Most of the studies have shown more than 90% sensitivity and 100% specificity, as compared to RT-qPCR. Four of the studies showed sensitivity in the range of 70% to 76% and only one study showed the sensitivity of 86% (Table 1). Although, in terms of specificity, all the studies showed a specificity of around 100%, except two studies which reported 89.2% and 95% specificity. Overall, 12 out of 15 studies endorsed the RT-LAMP integrated CRISPR technique as an alternative for RT-qPCR. Our meta-analysis results have also revealed a pooled sensitivity value higher than 85% and a pooled specificity value of 80% with the CI of 95%, respectively (Figures 5 and 6).

Notably, all the 15 studies have a predominantly similar experimental design, i.e. the amplification of viral RNA from RT-LAMP either using crude samples or by involving RNA extraction step from respiratory swab samples either nasopharyngeal swab, oropharyngeal swab, or throat swab followed by detection by CRISPR-Cas technology. Hence, the nasopharyngeal

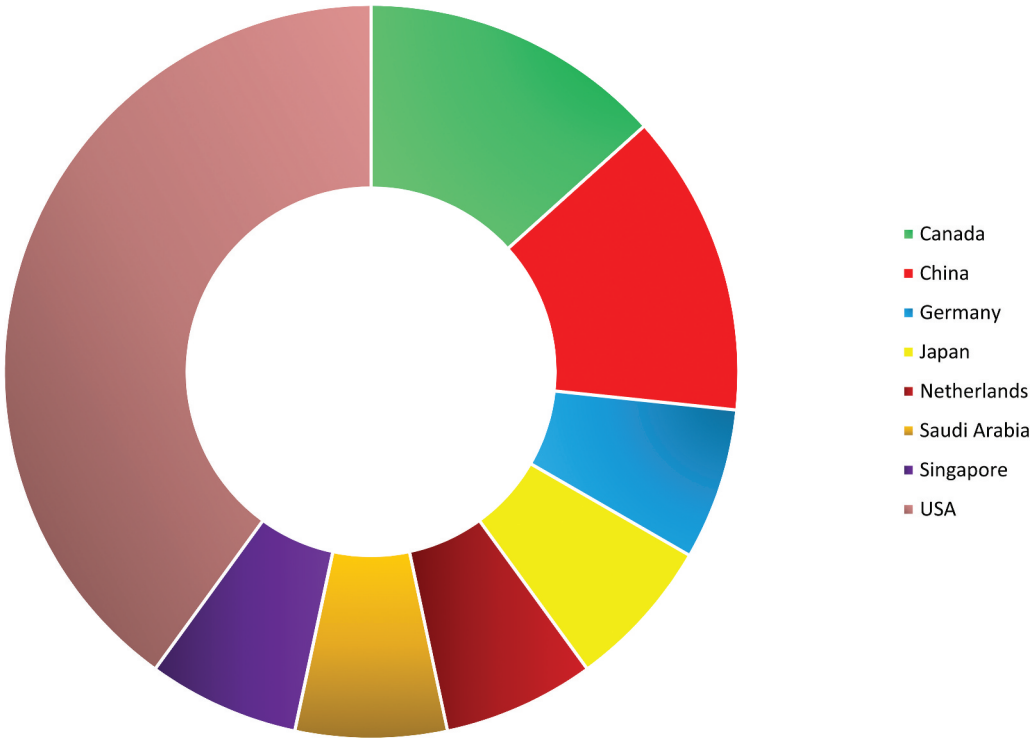


Figure 2. Country-wise distribution of included studies (n = 15) reported in the present investigation.

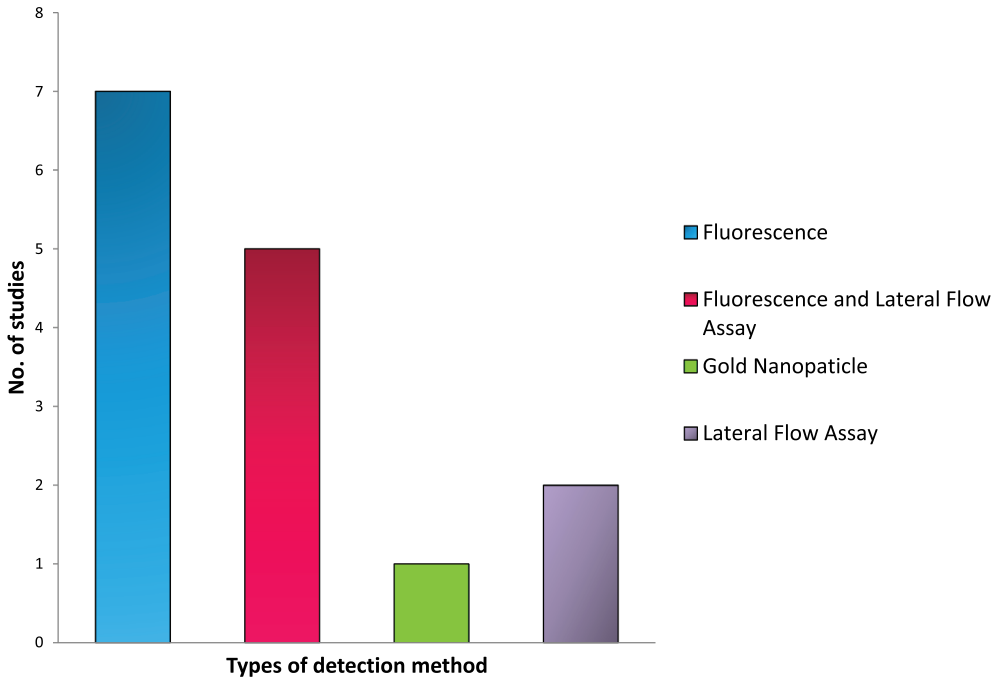


Figure 3. Percentage distribution of target genes reported in the included studies (n = 15).

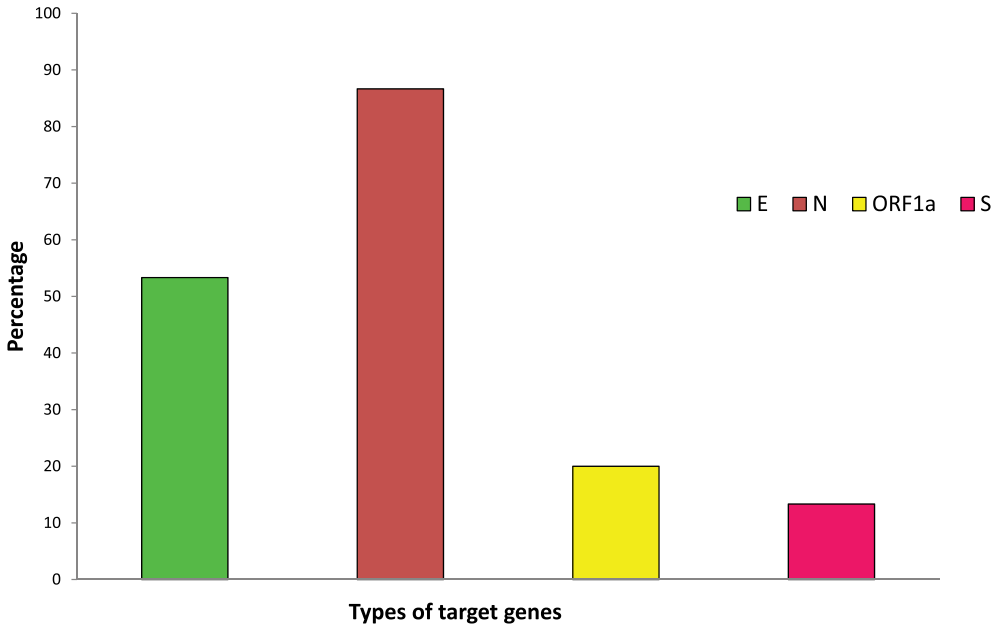


Figure 4. Distribution of type of detection method for SARS-CoV-2 in the included studies (n = 15).

swab sample could be considered as the best recommended sample for the detection of COVID-19. On the basis of included studies, we observed that the step of RNA extraction increases the sensitivity and more reliability of the diagnostic performances of the test, when compared to crude sample. However, developing the test on crude sample will definitely make the diagnostic test easier, faster and cost efficient [18, 27, 29]. As most of the included studies were using Cas12 enzyme for detection purposes, RT-LAMP integrated Cas12-based test can be recommended best for the diagnostic purpose. However, among other four studies, two studies used Cas13, one study used Cas10 and used Cas3, but the detection method was different for most of the studies (Figure 4). Cas13 directly acts on the genetic material of the coronavirus, i.e., RNA so using Cas13-based test will definitely reduce the time of detection [14, Schermer et al., 2021]. Overall, Cas12 or Cas13 proteins have been used as the most common CRISPR effectors in SARS-CoV-2 detection methods. The basis for selecting the detection test was not mentioned in any of the research; nonetheless, the majority of the studies used fluorescence-based as well as lateral flow assays [13, 15, 25, 26, 34]. The

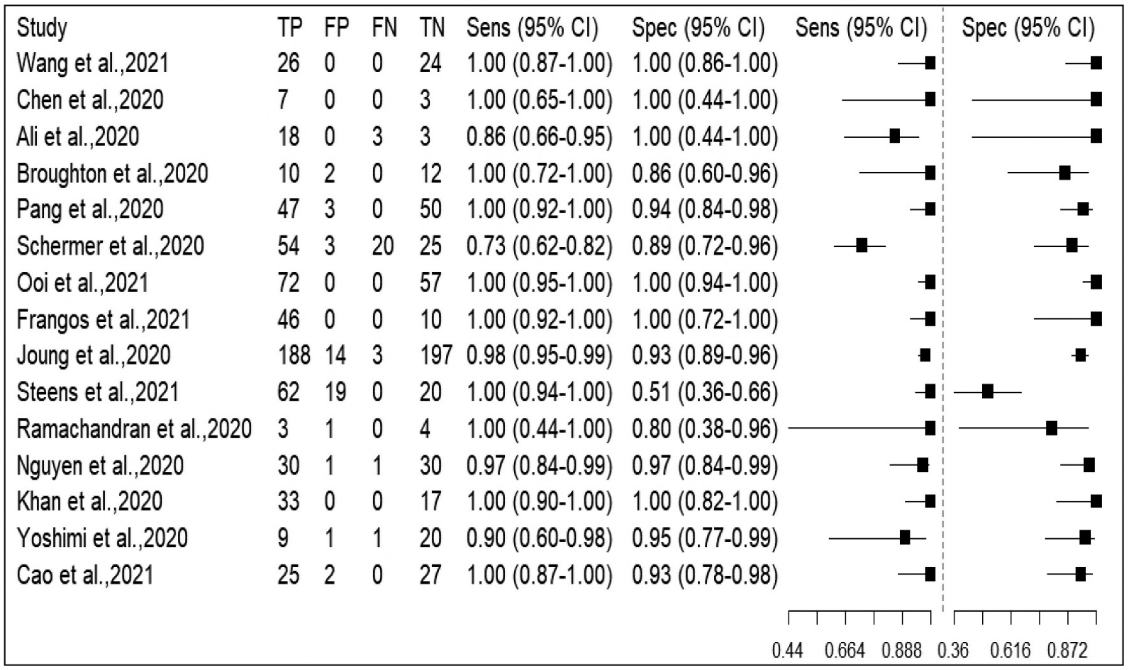


Figure 5. The Forest plot of sensitivity and specificity of included studies (n = 15) on the diagnostic performance of RT- LAMP integrated CRISPR technique.

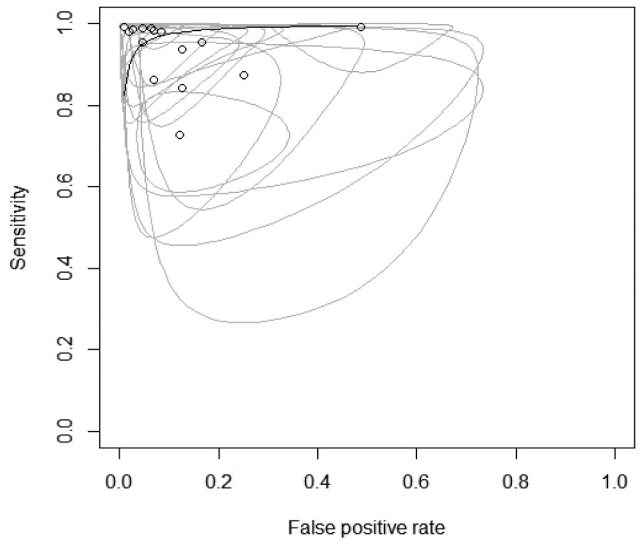


Figure 6. Summary Receiver Operating Characteristic (SROC) depicts RT-LAMP integrated CRISPR diagnostic performance as compared to RT-PCR in COVID-19 diagnosis.

main reason for developing fluorescence-based tests could be to increase sensitivity for the detection limit. However, the lateral flow-based tests would be more appropriate for the market application purpose. Only [29] have tried to use gold-nanoparticle agglutination for the faster detection time with no difference in the detection limit. When compared with RT-qPCR results, most of the studies have reported more than 90% sensitivity and 100% specificity in their results. The diagnostic performance of RT-LAMP integrated CRISPR is very much comparable with RT-qPCR diagnostic performance.

RT-LAMP integrated CRISPR could be considered as a novel, rapid and cost-effective approach for the diagnosis of COVID-19. This technique removes the barrier of the requirement of RT-PCR equipment and trained professionals for performing the assay to screen the population. As reported (Table 1), detection time in all the studies was around 30–60 minutes, making this technique more robust and ease-effective for solving the purpose of screening the large population in a short time (Table 1). All included studies echoed the same view on the potential of RT-LAMP

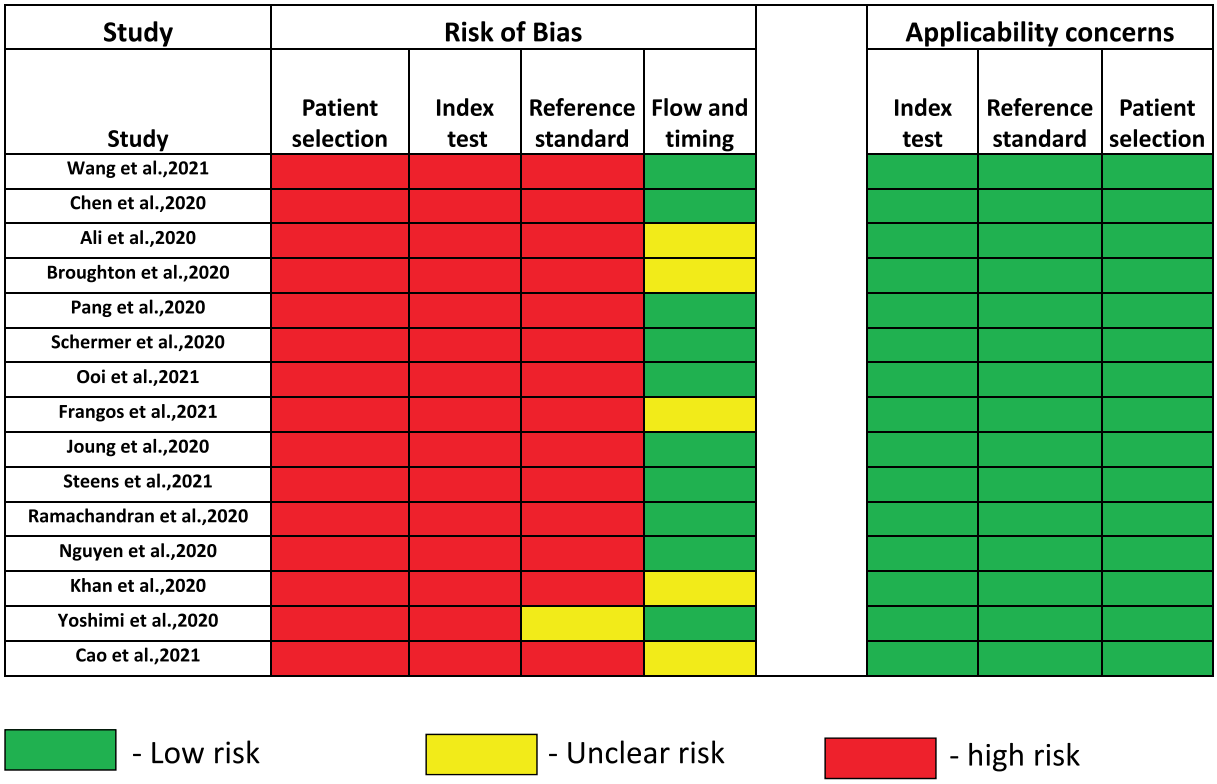


Figure 7. Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) summary of items for risk of bias and applicability in included studies (n = 15). Green color depicts the low risk of biasedness, yellow color depicts the unclear risk of biasedness and red color depicts the high risk of biasedness.

Table 2. Accuracy and precision of the included studies (n = 15).

S.No.	First author & year of publication	Accuracy	Precision
1	35	100	100
]2	30	100	100
3	34	87.5	100
4	15	91.666667	83.333333
5	17	97	94
6	31	77.45098	94.736842
7	13	100	100
8	28	100	100
9	25	95.771144	93.069307
10	18	81.188119	76.54321
11	27	87.5	75
12	26	96.774194	96.774194
13	14	100	100
14	33	93.548387	90
15	29	96.296296	92.592593

integrated CRISPR-Cas technique. This study identified both relevant peer-reviewed studies and preprints for illustrating better scientific conclusions in the diagnosis of COVID-19 promptly with few limitations. First, almost half of the included studies had a high risk of patient selection bias or index test bias (Figure 7). Such bias could lead to over-estimation of diagnosis performance. Future studies should focus on the use of un-bias patient cohorts, double-blinded index test and detection assays that do not require RNA extraction. Secondly, some studies have reported the highest performance with 100% sensitivity and 100% specificity with narrow 95% CIs (Figure 6). Hence, these were also the ones with the lowest QUADAS risk and concerns in all domains. Other limitations highlighted by the authors include the use of solely peer-reviewed English language research and the lack of subgroup analysis. This will pave way for further studies and reviews on the same topics with improvement.

Conclusions

The use of RT-LAMP integrated CRISPR-Cas in COVID-19 diagnosis has made significant impact that should be endorsed for further optimization and improvement. Particularly in resource-limiting regions, patients can get their COVID-19 testing reports in considerably lesser time and at reasonable cost which is vital during outbreaks. Even onsite screening can also be applied for prevention of asymptomatic carriers to transmit infection unintentionally. Following necessary improvisations, maybe it can offer making diagnostic kits for home usage. Thus, it can be safely concluded that the RT-LAMP integrated CRISPR performs well and has a high potential to be used as an alternative for

RT-qPCR in the diagnosis of COVID-19 that will be easier to use to facilitate their applicability as POCT.

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Disclosure statement

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Author contributions

AB, GSB, ZF: search, data extraction, validation. AB and GSB: data analysis. MR, SH and ZF: supervision. AB, GSB and MR: writing, original draft. SH and ZF contributed to the conception and design of the study and review and editing of the manuscript.

Declaration of data availability

All the data related to the study are available within the manuscript.

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