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Review Article

Diagnostic value of sputum nucleic acid amplification testing for COVID-19 a meta-analysis

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Abstract: Objective: To systematically evaluate the diagnostic value of nucleic acid test in sputum for COVID-19 and to determine the suitable population for sputum specimens. Methods: PubMed, CNKI, Scopus, Web of Science, medRxiv and bioRxiv databases were searched for the diagnostic value of sputum nucleic acid test for COVID-19 from December 2019 to April 2022. Two researchers independently screened the literature, extracted data, and evaluated the risk of bias with QUADAS-2 in the included studies. We used sensitivity, specificity, AUC and DOR to evaluate the diagnostic value of sputum specimens. Results: A total of 25 studies were included, including 10,731 subjects. Meta-analysis results showed that: The combined sensitivity (SEN), specificity (SPE), diagnostic odds ratio (DOR), and area under operating characteristic curve (AUC) of sputum nucleic acid for the diagnosis of COVID-19 were 89.2% (95% CI, 86.6-91.4), 97.5% (95% CI, 97.2-97.8), 41.4 (95% CI, 11.7-145.9), 0.9474 (95% CI, 0.8964-0.9846). The results of subgroup analysis showed that the Asian group's DOR was 36.835 (95% CI, 10.83-134.570), and the Non-Asian group's DOR was 66.294 (95% CI, 0.719-6109.09). The DOR was 27.207 (95% CI, 2.860-258.780) in the OPS group and 44.165 (95% CI, 4.828-403.970) in the NPS group. DOR of mild patients was 84.255 (95% CI, 9.975-711.690), the DOR of the severe group was 14.216 (95% CI, 3.527-57.142) and was 19.464 (95% CI, 0.724-522.920) in the cured group. Conclusion: Current evidence shows that sputum nucleic acid test is of high diagnostic value for COVID-19. Study area and severity of disease are the influencing factors for the diagnostic accuracy of the sputum nucleic acid test. Due to the limitations on the number and quality of the included studies, the above conclusions need to be verified by more high-quality studies.

Keywords: COVID-19, sputum, diagnostic value, meta-analysis

Introduction

Corona Virus Disease 2019 (COVID-19) is viral pneumonia caused by novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection [1, 2]. Typical laboratory findings in patients with COVID-19 are lymphopenia and an increase in inflammatory factors such as C-reactive protein. However, the total number of white blood cells in patients with mild disease is often within the normal range, and patients with moderate and severe disease will have leukopenia [3]. By April 10, 2022, more than 496 million confirmed cases and more than 6 million deaths have been reported globally, according to the WHO Trend Report. Early detection, isolation, and control of SARS-CoV-2 infected persons are of great significance for controlling the COVID-19 pandemic [3, 4]. Nucleic acid amplification detection has the advantages of strong specificity, high sensitivity and specificity; being simple, rapid, with low contamination and low purity requirement for samples. In the detection of SARS-CoV-19 infection, only a small number of samples need to be taken to obtain more accurate results. At present, the technology for nucleic acid amplification detection is very mature and has become the most widely used nucleic acid detection method in the field of SARS-CoV-2 infection detection. The "New Coronary Virus Pneumonia Diagnosis and Treatment Program (Trial Ninth Edition)" pointed out that when the nucleic acid amplification detection method is used to

detect SARS-CoV-2 nucleic acid, it is mainly used in nasopharyngeal and oropharyngeal swabs, sputum and other low respiratory tract secretions, feces and other specimens. At present, oropharyngeal swabs (OPS), nasopharyngeal swabs (NPS), and sputum specimens are most commonly used for viral nucleic acid detection all over the world [5]. The detection of OPS or NPS in testees usually uses Quantitative Real-time PCR, which is the gold standard for diagnosing COVID-19. However, the collection of common respiratory samples such as NPS and OPS exposes public-health workers to a high risk of infection [6]. Deep throat sputum, also known as lower-respiratory tract specimens, including sputum, tracheal aspirate, and bronchoalveolar lavage are not validated [7]. Sputum specimens have the advantages of being less invasive, having less risk of infection among medical staff, and low technical requirements for operation, and can be self-tested by the testees. Based on these advantages, sputum specimens may be more suitable for large-scale community screening than OPS and NPS. Moreover, sputum specimens have the advantage of maintaining a long positive rate. Therefore, sputum specimens might be used for discharge testing and patient management. However, few studies have analyzed the applicable population and scenarios of sputum samples and the influencing factors of the diagnostic accuracy of sputum samples. Thus, in this study, the diagnostic value of sputum specimens for COVID-19 was comprehensively evaluated by collecting research data on the use of sputum specimens for RT PCR to diagnose COVID-19, to explore the appropriate population and scenario for deep throat sputum specimens and the influencing factors of them.

Materials and methods

This study followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) reporting guideline for meta-analysis of diagnostic test accuracy. Our study systematically searched four major databases for this meta-analysis (PubMed, Sino Me, CNKI and Web of Science). We also manually searched medRxiv and bioRxiv's preprint archives, and references of included studies to supplement the search results (last updated April 2022). The language of the included studies was limited to English and Chinese.

This study used the following search terms and their variations: "COVID-19", "SARS-COV-2", "novel coronavirus", "2019 novel coronavirus", "new coronavirus", "diagnosis", "diagnostic test", "diagnostic assay", "sputum", "deep throat saliva", "deep throat sputum", and other terms combined with Boolean operators "AND" and "OR".

Inclusion and exclusion criteria

We included studies in this meta-analysis if they met all the following inclusion criteria: (1) Study type was diagnostic accuracy study; (2) Use RT-PCR for nucleic acid detection of sputum samples, the recognized clinical diagnostic standard is used as the gold standard, including the results of OPS and NPS; (3) Patients with COVID-19 confirmed by the gold standard diagnosis, regardless of gender, age, and race; (4) The outcome indicators: sensitivity (SEN), specificity (SPE), positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odd ratio (DOR), summary receiver operating characteristic curves (SROC) area under the curve (AUC).

We excluded studies in this meta-analysis if they met one of the following exclusion criteria: (1) Publications without primary results, such as reviews, conference abstracts, editorials, letters, and reviews; (2) Studies in preprints achievements that do not state COVID-19's definition; (3) Studies with duplicate data select the most recent and complete one; (4) Studies where the four-table information could not be extracted or calculated.

Study selection and data collection

Two authors (Niu and Chen) independently screened studies, extracted data, and cross-checked them. If there was any disagreement, it was resolved through discussion or consultation with a third author (Liu). In study screening, the title and abstract of the article were read first, and after excluding obviously irrelevant studies, the full texts were further read to determine whether to include or not. If necessary, the original study authors were contacted by email or telephone for unreported but impor-

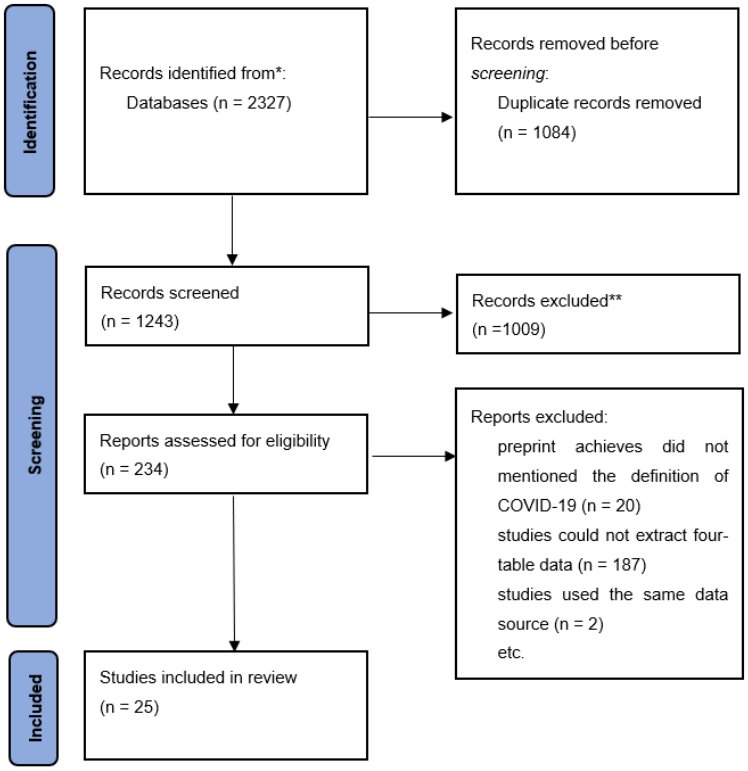

Figure 1. PRISMA flow diagram of sputum diagnostic value analysis from Dec. 2019 to Apr. 2022.

tant information. Data extraction contents include first author, publication year, study area (country), diagnostic critical value, and four-table data.

Risk of bias

Two investigators (Niu and Chen) independently evaluated the risk of bias in the included, studies and cross-checked the results. The QUADAS-2 tool was used to assess the risk of bias [8].

Data analysis

In this study, Meta Disc 1.4 software was used for Meta-analysis in calculating the pooled SEN, SPE, PLR, NLR, DOR, and drawing the SROC curve to calculate the AUC. We used Review Manager 5.3 to evaluate the risk of bias assessment. We also used Stata 17.0 for sensitivity analysis and drawing funnel plots.

The heterogeneity among the results of each study was analyzed by the χ^2 test (the test level was α=0.05), and heterogeneity was quantitatively judged with I^2. If there was no statistical heterogeneity among the study results, a fixed-effect model was used for meta-analysis; if there was statistical heterogeneity among the study results, the source of the heterogeneity was further analyzed to exclude the influence of obvious clinical heterogeneity. Afterward, a meta-analysis was performed using a random-effects model. The test level of the meta-analysis was set to α=0.05. Significant clinical heterogeneity was handled by subgroup analysis and sensitivity analysis.

Results

A total of 2,327 relevant studies were obtained from the initial screening of our research. After removing duplicate studies, we initially retained 1,243 studies for title and abstract. After the tile and abstract review, we retained 234 studies for full text. Twenty-five diagnostic accuracy studies were finally included [9-33], including 10,731 subjects. The literature screening process and results are shown in **Figure 1**.

The basic characteristics of the included studies are shown in **Table 1**, and the results of the risk of bias assessment are shown in **Figures 2** and **3**.

Meta-analysis

A total of 10,731 subjects were included in this study. The SROC curve did not show a typical "shoulder-arm shape". Spearman's rank correlation coefficient was -0.133, P=0.526. Therefore, there was no threshold effect between the included studies. Since Q=153.44, P<0.01, and I^2>90%, suggesting that there is heterogeneity among included studies caused by non-threshold effects. For these reasons, we used the random effects model to analyze heterogeneity. This analysis showed that the area under the curve of 0.9474 (95% CI, 0.90-0.98) (**Figure 4**), pooled sputum sensitivity of 89.2% (95% CI, 86.6-91.4) (**Figure 5**), a pooled sputum specificity of 97.5% (95% CI, 97.2%-97.8%) (**Figure 6**), and a pooled sputum diagnostic odds ratio of

Table 1. Basic characteristics of the included studies of sputum diagnostic value analysis from Dec. 2019 to Apr. 2022

Author	Study Area	Years	Gold Standard	CT Value	TP	FP	FN	TN	No. of testees
Xu [9]	China	2021	OPS	-	6	24	0	36	66
Sharm [11]	India	2020	OPS	-	82	0	12	36	130
Wang [14]	China	2020	NPS	-	8	2	0	2	12
Zheng [15]	China	2020	OPS	-	3	5	0	13	21
Thwe [10]	America	2020	NPS	-	58	0	5	54	117
Rao [12]	England	2021	NPS	38	73	76	11	0	160
Lin [13]	China	2020	OPS	30	19	21	4	8	52
Babad [16]	America	2020	OPS	-	29	6	1	64	100
Burdet [17]	England	2020	NPS	-	73	0	1	221	295
Pasomsub [18]	Thailand	2020	OPS	38	16	2	3	179	200
Garret [19]	America	2020	NPS	-	8	0	10	2	20
Zhang [20]	China	2022	OPS	-	10	2	0	134	144
Pan [21]	China	2021	NPS	38	46	33	13	78	170
Deng [22]	China	2021	OPS	43	5	3	2	0	10
Rong [23]	China	2020	OPS	40	17	8	1	0	26
Feng [34]	China	2020	OPS	40	8	0	1	8492	8500
Zeng [25]	China	2020	OPS	35	19	6	2	309	326
Zha [26]	China	2021	NPS	32	23	17	0	15	55
Lyv [27]	China	2020	NPS	40	36	3	0	39	78
Tang [28]	China	2020	OPS	-	6	1	0	2	9
Jiang [29]	China	2020	NPS	37	2	12	0	12	26
Zeng (b) [30]	China	2020	OPS	-	0	9	3	13	25
Deng (b) [31]	China	2021	OPS	38	11	15	0	1	27
Wu [32]	China	2020	NPS	40	8	0	5	6	19
Suh [33]	Korea	2022	NPS	-	46	1	0	40	87

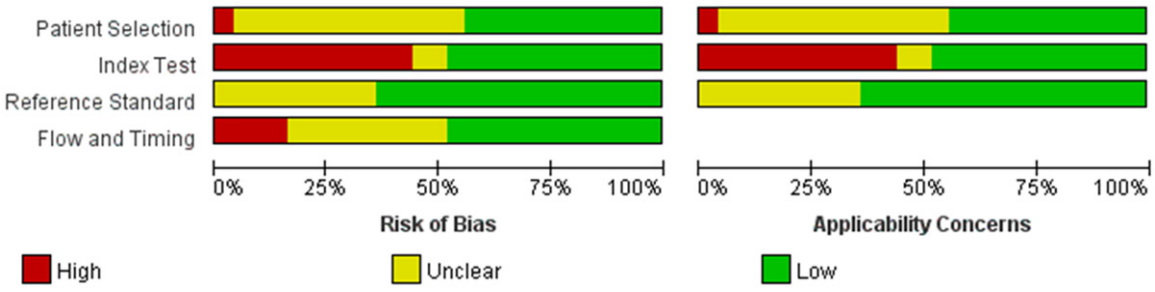

Figure 2. QUADAS-2 study regulation chart of sputum diagnostic value analysis from Dec. 2019 to Apr. 2022.

41.38 (95% CI, 11.73-145.93) (**Figure 7**). With a pooled sputum positive likelihood ratio of 6.10 (95% CI, 3.07-12.11), and a pooled sputum negative likelihood ratio of 0.20 (95% CI, 0.11-0.39). Meta-regression analysis was performed on the included studies, and the results are shown in **Table 2**.

Subgroup analysis

Due to the heterogeneity among different studies, we divided included studies by region (Asian group and Non-Asian group), gold standard (OPS group and NPS group), the severity of symptoms (mild group, severe group, and

Figure 3. QUADAS-2 study quality summary of sputum diagnostic value analysis from Dec. 2019 to Apr. 2022.

cured group) for subgroup analysis, the results are shown in **Table 3**.

Sensitivity analysis

The included studies were excluded one by one, and the remaining studies were recombined for pooled analysis, and to observe the changes in the combined effect size DOR and its 95% confidence interval. As shown in **Figures 8** and **9** that after the study of Mohan Rao (2021) was excluded, the estimate point of the combined effect size fell inside the 95% CI of the total combined effect size. Mohan Rao's study had the greatest impact on the stability of the results. The other studies had a light impact on the combined effect size.

Publication bias assessment

The results of Egger's test showed that the funnel plot was basically symmetrical, and the slope of the 24 studies of sputum specimens was Bias =1.181, P=0.296, indicating that the included studies had no obvious publication bias (**Figure 10**).

Discussion

In addition to diagnostic techniques, the accuracy of COVID-19 diagnosis mainly depends on the samples' quality which mainly depends on the detection site, detection time, sampling method, and other factors. At present, the most commonly used detection samples in the world are NPS. The subgroup analysis in this study also found that NPS is more accurate as the gold standard than OPS. The reason is that the nasopharynx is mostly composed of columnar epithelial cells, and the oropharynx is mostly made of squamous epithelial cells. Therefore, the expression of COVID-19 is different due to the different surface epithelial cells of the nasopharynx and oropharynx [35]. Sputum samples have the advantages of being convenient, rapid, and highly safe. The WHO and

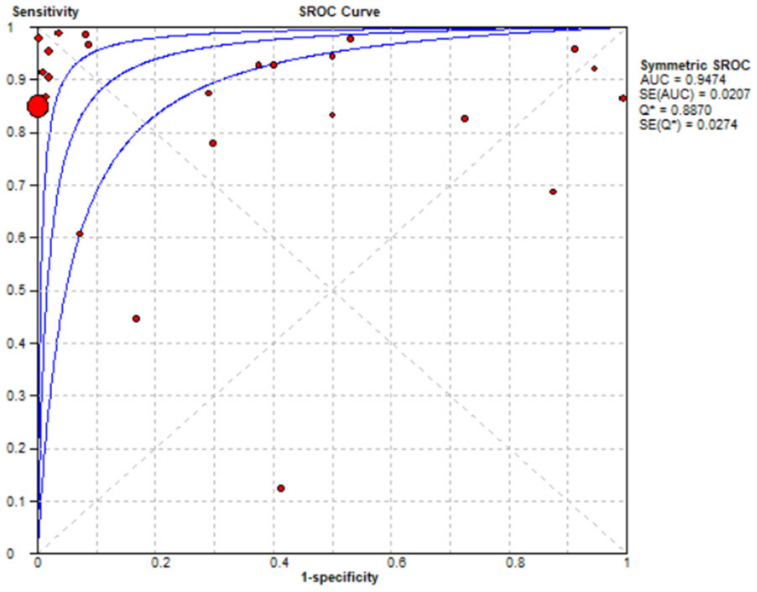

Figure 4. ROC plane of sputum diagnostic value analysis from Dec. 2019 to Apr. 2022.

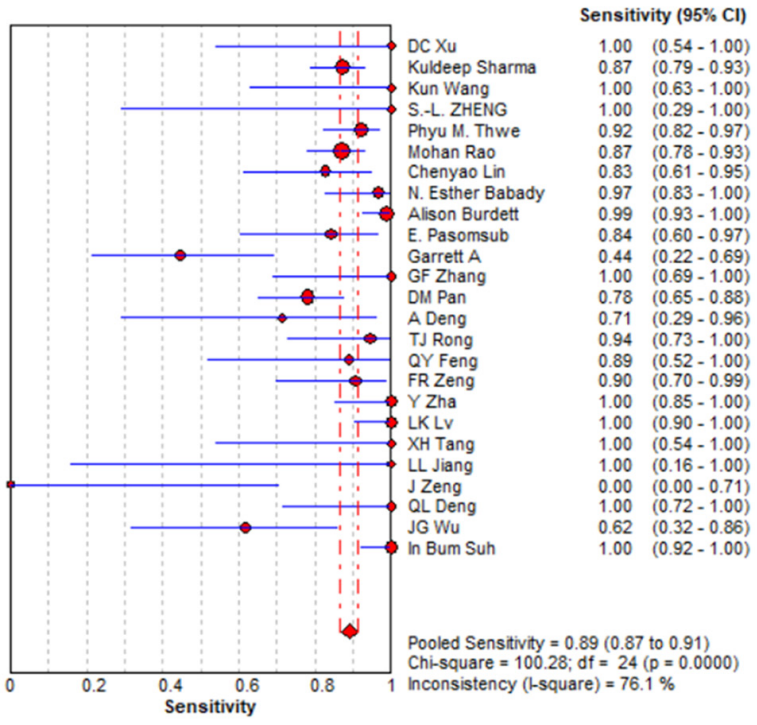

Figure 5. Sensitivity graph of sputum diagnostic value analysis from Dec. 2019 to Apr. 2022.

CDC have recommended the lower respiratory tract sputum as a diagnostic specimen to detect SARS-CoV-2 nucleic acid. The sampling of sputum samples is not invasive, and the patient suffers little physiological distress. Since nucleic acid testing needs to be performed frequently, the smaller physiological burden is of great significance to reduce the suffering of the tested person. For children who have difficulty cooperating with nasopharyngeal swab sampling or patients with contraindications, the question arises, is it appropriate to use sputum samples for SARS-CoV-19 nucleic acid testing? The sampling of sputum samples is very convenient and can be performed by the patients themselves. Medical staff do not need to have any contact with the patient during the sampling process, and the infection problem of medical staff during the sampling process is effectively avoided. Most importantly, the diagnostic preciseness of sputum samples for COVID-19 is pretty high. The sensitivity of the sputum samples of this study is 89.2% (95% CI, 86.6-91.4), 97.5% (95% CI, 97.2-97.8). This result is consistent with the majority of current studies, which can be seen that sputum specimens have high diagnostic efficacy for COVID-19. Guillaume et al. found that the use of sputum samples for COVID-19 screening can save about 40% of the cost and 20% of the personnel compared with the current [36].

Sampling and testing time have an important influence on sample quality. According to the results of meta-regression, the severity of symptoms is the dominating factor to influence the accuracy of sputum samples. A number of studies have shown that the viral load of SARS-CoV-2

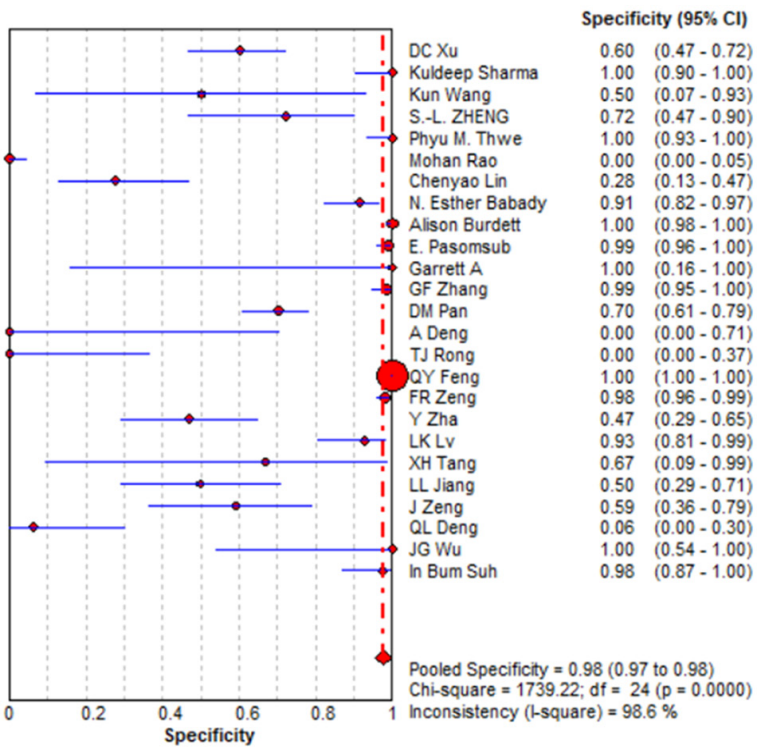

Figure 6. Specificity graph of sputum diagnostic value analysis from Dec. 2019 to Apr. 2022.

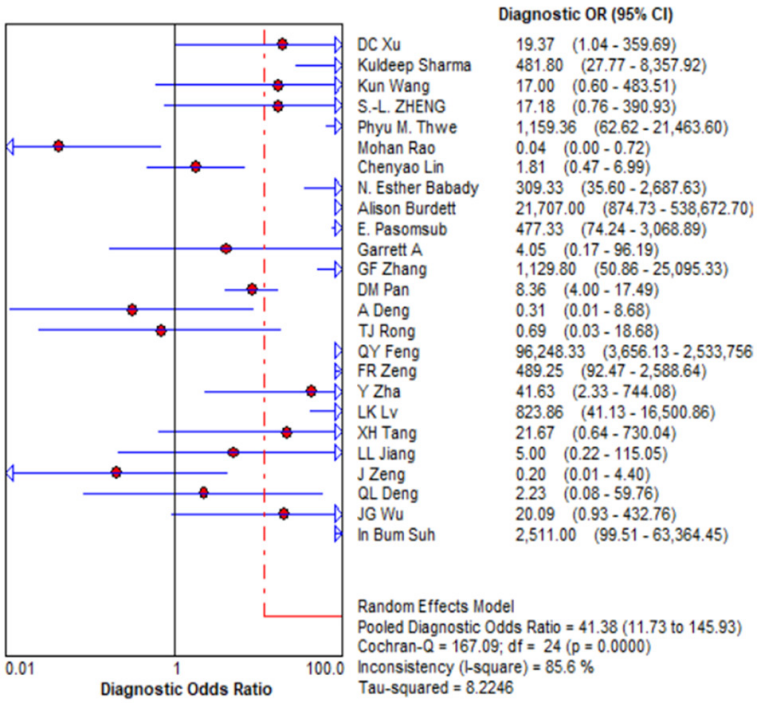

Figure 7. DOR graph of sputum diagnostic value analysis from Dec. 2019 to Apr. 2022.

increases gradually in the early stages, reaches the peak, and then decreases gradually, and the severity of the disease also has an impact on the change of viral load. The study of Li JB et al. showed that the different stages of RNA level of patients at the time of detection would also have an impact on the detection results [37]. The strength of this study is that the sputum diagnostic accuracy was compared at different stages by classifying the subjects into mild or asymptomatic patients, severe patients, and recovered patients. According to the results of subgroup analysis, the sensitivity of sputum samples was 88.7% (95% CI=85.0%-91.4%) for mild or asymptomatic patients and 95.6% (95% CI=87.65-99.1%) for patients in the recovery stage. It can be seen that sputum samples have good diagnostic capabilities on mild or asymptomatic patients and patients in the recovery stage, which is consistent with the results of Kazem Khiabani [38]. According to the sensitivity analysis results of this study, Monhan Rao's study significantly exceeded the upper limit of the 95% confidence interval. The possible reason is that only asymptomatic patients were included in this study, which further indicated that sputum samples had higher diagnostic efficiency for mild or asymptomatic patients. Yasutaka Okita et al. demonstrated that sputum samples could maintain a longer positive rate than OPS and NPS, which also explained the high diagnostic efficiency of sputum samples for patients in the recovery period [39]. In

Table 2. Meta-regression of sputum diagnostic value analysis from Dec. 2019 to Apr. 2022

Independent variable	RDOR (95% CI)	*P*
Region (Asian and Non-Asian)	1.56 (0.09-27.27)	0.7512
Gold Standard (NPS and OPS)	1.36 (0.12-14.90)	0.7928
Symptom Severity (Mild, Severe and cured)	0.70 (0.15-3.31)	0.6343

Table 3. Subgroup analysis of sputum diagnostic value analysis from Dec. 2019 to Apr. 2022

Groups	Included studies	Sensitivity (95% CI)	Specificity (95% CI)	DOR (95%)	AUC
Asian	20	0.890 (0.856-0.918)	0.983 (0.980-0.985)	36.835 (10.83-134.570)	0.9582
		I^2=70.7%	I^2=98.3%	I^2=83.6%	
Non-Asian	5	0.896 (0.853-0.930)	0.983 (0.980-0.985)	66.294 (0.719-6109.09)	0.9618
		I^2=88.7%	I^2=98.4%	I^2=92.3%	
OPS	16	0.868 (0.828-0.902)	0.747 (0.707-0.784)	27.207 (2.860-258.780)	0.9356
		I^2=58.6%	I^2=98.2%	I^2=85.4%	
NPS	9	0.884 (0.848-0.914)	0.764 (0.726-0.799)	44.165 (4.828-403.970)	0.9553
		I^2=87.4%	I^2=98.0%	I^2=82.6%	
Mild	14	0.897 (0.867-0.922)	0.986 (0.984-0.989)	84.255 (9.975-711.690)	0.9582
		I^2=75.5%	I^2=99.0%	I^2=90.5%	
Serve	7	0.881 (0.759-0.896)	0.798 (0.749-0.842)	14.216 (3.527-57.142)	0.8962
		I^2=60.2%	I^2=93.5%	I^2=51.3%	
Cured	4	0.956 (0.876-0.991)	0.660 (0.580-0.734)	19.464 (0.724-522.920)	0.7410
		I^2=87.8%	I^2=87.3%	I^2=79.7%	

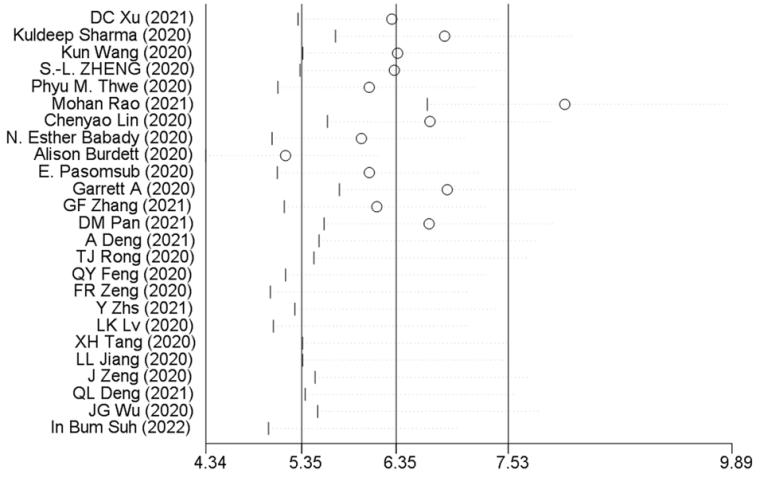

Figure 8. Sensitivity analysis of sputum diagnostic value analysis from Dec. 2019 to Apr. 2022 (before excluding).

addition, since mild and asymptomatic patients have a low viral load and a fast viral clearance rate, effective detection methods in the early stage of patients can effectively improve the detection rate of patients. Therefore, the application of sputum samples is of great significance for early large-scale community screening, patient discharge testing, and patient management.

Limitations

According to the results of the heterogeneity test and publication bias assessment, there was high heterogeneity in this study. The possible reasons lie at the source of the test, the test method, the selection of the kit, the sampling method, and so on. In addition, the quality of sputum samples varies at different times of the day, and the quality of sputum samples is the best after morning [34]. However, most of the included studies do not indicate the specific time of sampling, so the inconsistencies in the detection time and stage of the included studies may affect the results of the study. This is consistent with the result of Beatriz Boger et al. [40]. The high heterogeneity suggests that large-scale prospective experiments are needed to verify the feasibility and

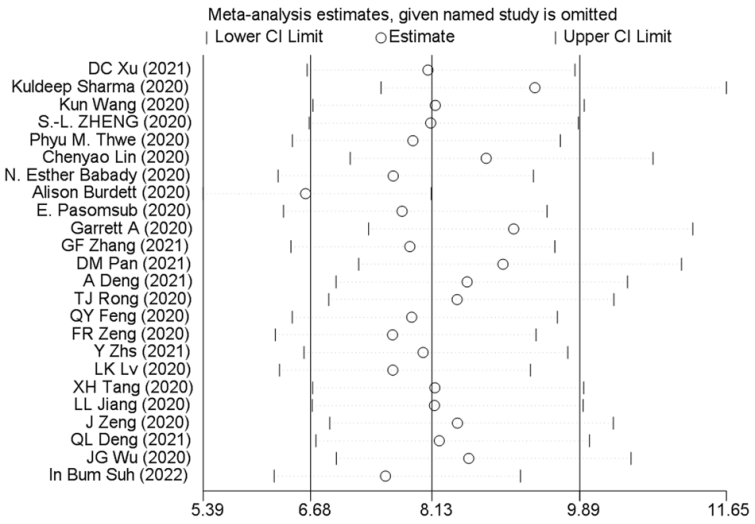

Figure 9. Sensitivity analysis of sputum diagnostic value analysis from Dec. 2019 to Apr. 2022 (after excluding).

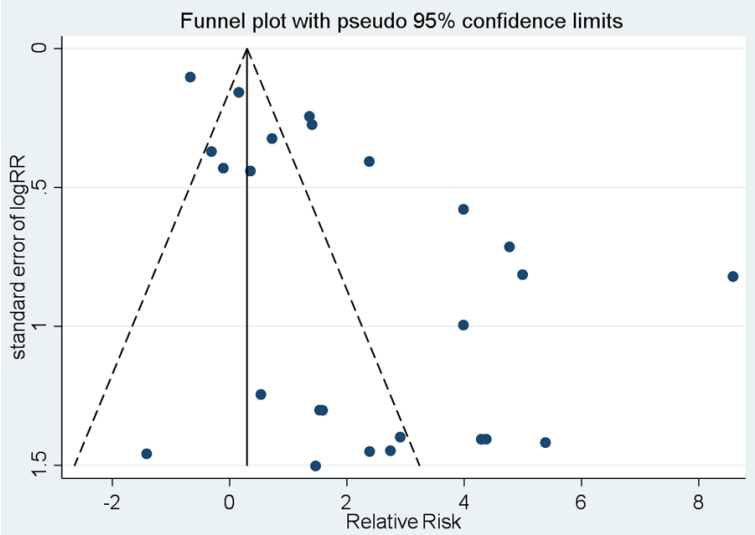

Figure 10. Funnel plot of sputum diagnostic value analysis from Dec. 2019 to Apr. 2022.

optimize the test process before the application of sputum samples, so as to form a more standardized test process. In addition, most of the prediction intervals obtained in this study were wide because most of the included studies did not provide details of patients' symptoms, and the specific clinical symptoms of COVID-19 patients included in the study, such as respiratory disease or critical illness, were still unclear. It may be necessary to select a specific clinical symptom model of the subject to obtain the best diagnostic accuracy. In addition, most of the included studies did not evaluate the quality of sputum specimens by means of microscopic observation, so the quality of sputum specimens could not be determined.

Conclusion

Nucleic acid detection of sputum samples has a high diagnostic value for COVID-19, especially for patients with mild symptoms, and it can be used as an auxiliary diagnostic method for COVID-19 in large-scale screening and patient management. The symptom severity may be an influencing factor in the diagnostic accuracy of nucleic acid in sputum samples, and a prospective study with large samples is needed to verify this.

Disclosure of conflict of interest

None.

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