Accuracy of the Polymerase Chain Reaction (PCR) test in the diagnosis of acute respiratory syndrome due to coronavirus: a systematic review and meta-analysis

Idevaldo Floriano[1]
Antônio Silvinato[1]
Wanderley M. Bernardo[2]
João C. Reis[1]
Gabriel Soledade[1]

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1. Programa Diretrizes da AMB, São Paulo, SP, Brasil
2. Coordenador do Programa Diretrizes da AMB, São Paulo, SP, Brasil

E-mail: wmbernardo@usp.br

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The Guidelines Project, an initiative of the Brazilian Medical Association, aims to combine information from the medical field in order to standardize producers to assist the reasoning and decision-making of doctors.
The information provided through this project must be assessed and criticized by the physician responsible for the conduct that will be adopted, depending on the conditions and the clinical status of each patient.

INTRODUCTION

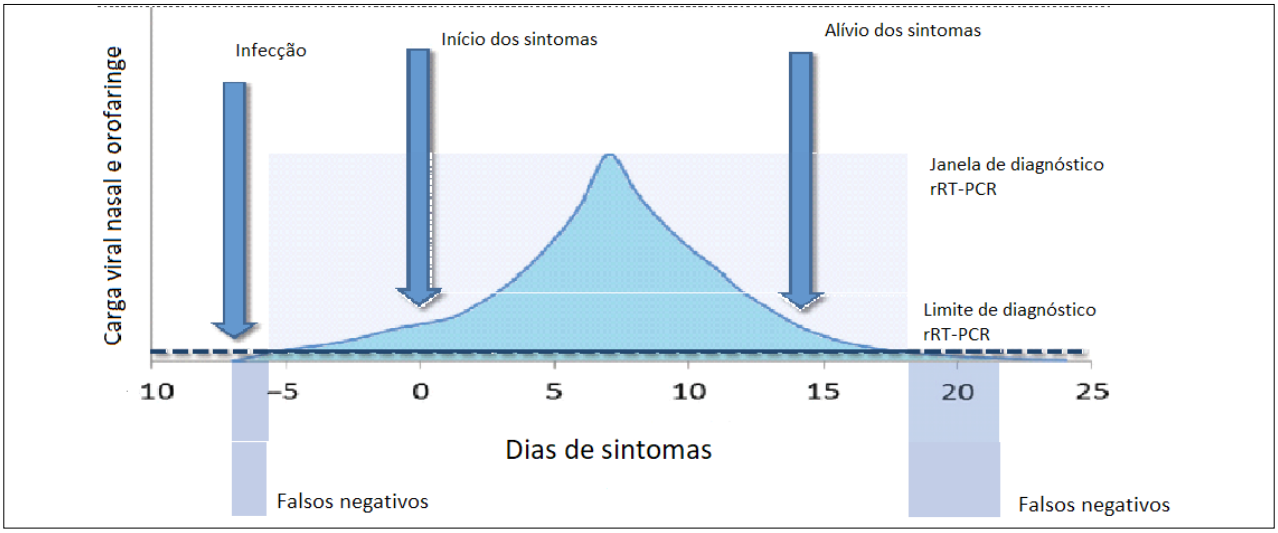
The first case of a patient with a diagnosis of respiratory syndrome due to coronavirus (SARS-COV-2), in the current pandemic, was reported in January 2020, when a patient resident of the city of Wuhan, the Hubei province, in China, was admitted to the central hospital in December 2019[1]. Patients affected by the coronavirus 2019 (COVID-19) can be asymptomatic, present mild symptoms (cough, sore throat, fever, diarrhea, myalgia, anosmia), moderate symptoms (weakness, myalgia, dyspnea), or severe symptoms with acute respiratory insufficiency, acute respiratory distress syndrome, and acute kidney failure[2]. The mortality rate can reach 0.5%[3].

The "novel coronavirus" belongs to the *Coronaviridae* family, whose genetic material is the ribonucleic acid (RNA) and which is known to cause influenza and enteric syndromes since 2003. It is associated with Severe Acute Respiratory Syndrome (SARS)[4] in Asia, with mortality rates of 8.7% (it reached 50% among people aged over 60 years), and in the Middle East (Meridian East Respiratory Syndrome(MERS) in 2013, with 40% of mortality[5].

The etiological diagnosis of SARS-COV-2 he is currently carried out using the Polymerase Chain Reaction (PCR) technique to detect viral RNA in the sample; enzyme-linked immunosorbent assay (ELIZA) to detect the presence of antibodies in serum (rapid tests to detect antibodies or antigens),[6] and computed tomography[7]. The PCR technique provides better accuracy when carried out between 2 and 5 days after the onset of symptoms, with the collection of material via oral/nasal swab or sputum[8-11]; serological tests may be collected starting at the seventh day. (Figura1).

The evolution of the PCR technique resulted in a reduction in the time for executing the examination and in quantification. The real-time polymerase chain reaction (RT-PCR) uses *primers* that target the upE and ORF1a areas of the coronavirus genome[12-13]. During the PCR technique, reverse transcription can monitor the progress of the process as it takes place (in

FIGURE 1. CORRESPONDENCE BETWEEN THE VIRAL LOAD AND INFECTION BY THE CORONAVIRUS 2 (SARS-COV-2), CLINICAL SYMPTOMS, AND POSITIVE RRT-PCR.



Source: Adapted from Lippi (2020, p 4).

real-time), and the data are collected throughout the examination. The "TaqMan System®" uses a fluorescent probe for quantification and the "SYBR Green I System® uses a dye that binds specifically to DNA and accumulates during cycles for quantification. Currently, there are other enhancements to the PCR technique that aim to decrease costs and facilitate the execution of the technique.

OBJECTIVE

The objective of this review is to identify the efficacy of the PCR test in the diagnosis of patients with coronavirus.

METHODS

The clinical question is: What is the efficacy of the PCR test in the coronavirus diagnosis?

Eligibility criteria:

- Patients with a suspicion of coronavirus infection;
- Coronaviruses diagnosis by PCR;
- Collection of nasopharyngeal (NF) and/or oropharyngeal (OF) swab samples;
- Studies on the diagnosis of SARS, MERS, and SARS-COV-2;
- Clinical trials with better evidence and quality;
- No time or language restrictions;
- Full texts available for access, with results on PCR sensitivity and specificity;
- Studies with incomplete data for specificity and sensitivity and viral panel for etiologic diagnosis of respiratory tract infection will be excluded.

The search for evidence will be conducted on the following virtual scientific information databases, using the search strategies:

MEDLINE/PUBMED: ((COVID OR COV OR nCOV OR CORONAVIRUS) AND (PCR OR Polymerase Chain Reaction OR Nucleic Acid Amplification OR Nucleic Acid Amplification Techniques OR Reverse Transcriptase Polymerase Chain Reaction) AND (diagnosis/broad[filter])), date 04/2020.

CENTRAL COCHRANE: (COVID OR COV OR nCOV OR CORONAVIRUS) AND (PCR OR Polymerase Chain Reaction OR Nucleic Acid Amplification OR Nucleic Acid Amplification Techniques OR Reverse Transcriptase Polymerase Chain Reaction), date 04/2020.

The information obtained from the characteristics of the studies selected were: author's name and year of the study, study design, number of patients, population, type of test, and comparison, described in Table 1.

Data from the results will be collected in absolute numbers provided directly or by information inferred from what is reported in the text. The results from the studies will be placed in a 2x2 table, where true positive, false positive, true negative, and false negative results will be compiled. The data collection and meta-analysis process will be completed by two independent authors and revised by all authors. Disagreements will be resolved by consensus and discussion between all authors.

Bias assessment and quality of evidence

The methodology used to assess the quality of the studies was the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) [14] tool, which was applied by two independent authors. Disagreements were resolved by consulting with a third independent author.

Data Analysis

The data will be extracted for the primary outcome of accuracy of the test RT-PCR for coronavirus diagnosis. The data collected will be true positive, false positive, true negative, and false negative results, sensitivity, and specificity, which will be analyzed in a 2x2 Table using the Catmaker Tables [15] software.

The results of the studies included may be aggregated and meta-analyzed using the Meta-Disc software Version 1.4 [16], through which results on the sensitivity, specificity, and positive likelihood ratio, negative likelihood ratio, and SROC curve will be obtained.

RESULTS

In the search for evidence, we recovered 1260 studies, of which 107 were selected based on their titles, 6 based on the abstract, 28 were excluded, and 22 were evaluated in full. Of the 22 studies, 9 were excluded and 13 [17-29] were selected to support this assessment; the grounds for exclusion and list of studies excluded are available in the references, Table 1, and Figure 7, in the Annexes.

The characteristics of the populations included and results extracted are summarized in Tables 2 and 3, in the Annexes.

The thirteen studies included in this review were effectively cross-sectional, with no sample size calculation, conducted in a single institution, including a total of 6295 samples taken through nasal and/or oropharynx swab.

Bias assessment and quality of evidence

We used the QUADAS-2 [14] tool to assess the quality of the thirteen studies included in this review (Figure 4). In the selection of patients, we found a low risk of bias in 12 studies (92%) and low-medium risk in one (8%). In the evaluation of the index tests, we found ten studies (77%) with low risk of bias, two studies (15%) with low-moderate risk, and one with moderate risk (8%). In comparison to the test considered the gold standard (reference), we found twelve studies (92%) with low risk of bias, and one with moderate risk. Regarding flow and time biases, eleven studies (85%) had a low risk, and two (15%) moderate risk.

Meta-analysis

Thirteen studies [17-29] presented data possible to be meta-analyzed. The sensitivity (Figure 2) of the PCR technique for coronavirus diagnosis was 86% (95% CI = 84 to 88%); I^2 = 85%.

The estimate of specificity calculated for the studies (Figure 3) was 96% (95% CI = 94 to 97%); I^2 = 0 %.

The results for a positive likelihood ratio (Figure 4) was 18.8 (95% CI = 14.5 to 24.3); I^2 = 0%.

The results for a negative likelihood ratio (Figure 5) was 0.13 (95% CI = 0.1 to 0.19); I^2 = 83.6%.

Analyzing the SROC curve (Figure 6), we estimated the value of the area under the curve (AUC) as 0.977 and Q = 0.93.

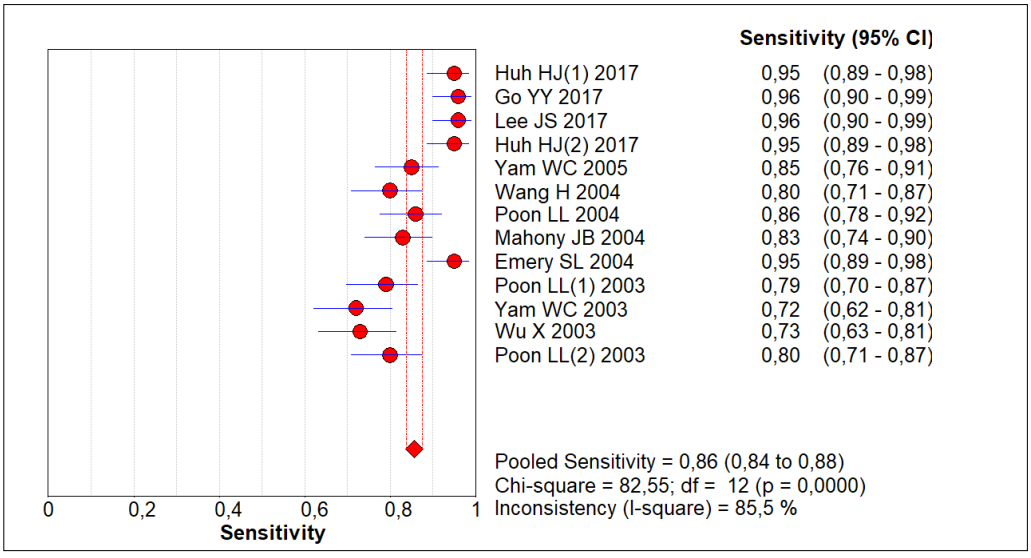


FIGURE 2. FOREST PLOT OF SENSITIVITY ESTIMATE IN THE CORONAVIRUS DIAGNOSIS BY PCR.

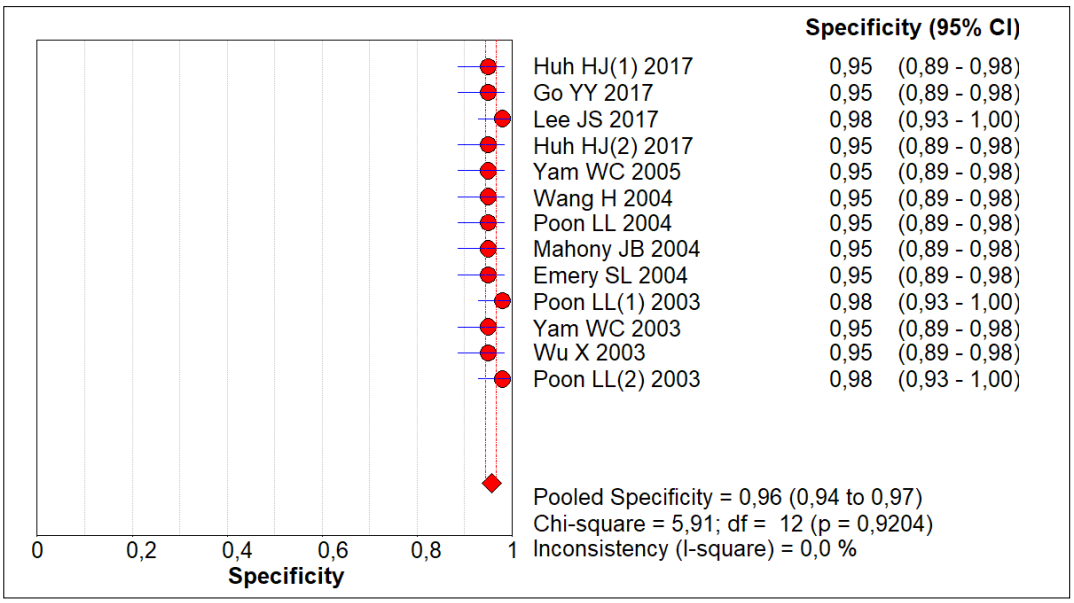


FIGURE 3.
FOREST PLOT OF SPECIFICITY ESTIMATE IN THE CORONAVIRUS DIAGNOSIS BY PCR

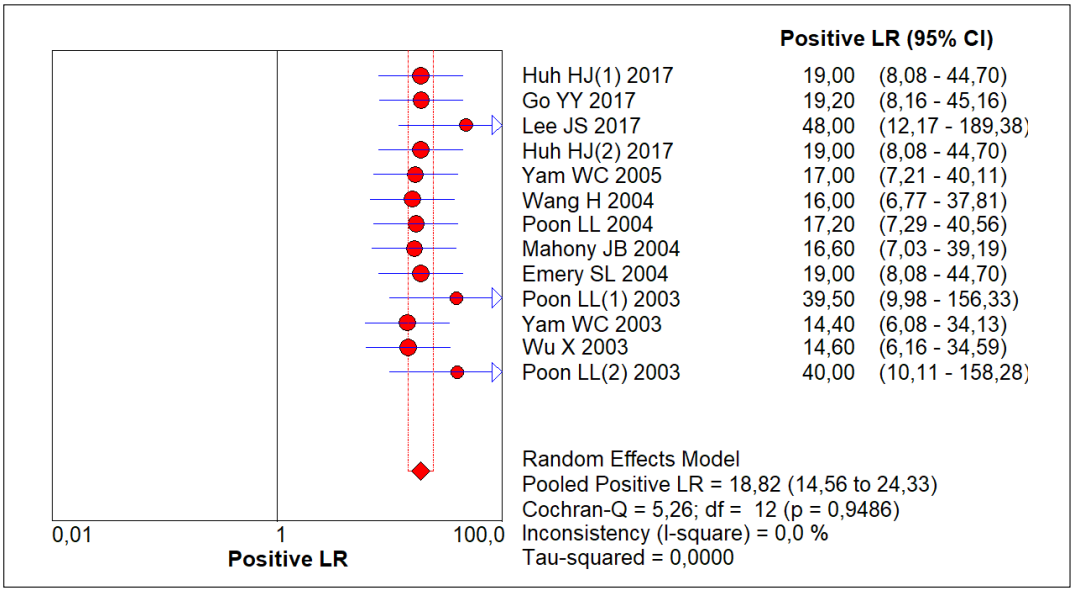


FIGURE 4.
FOREST PLOT OF THE POSITIVE LIKELIHOOD RATIO ESTIMATE IN THE CORONAVIRUS DIAGNOSIS BY PCR

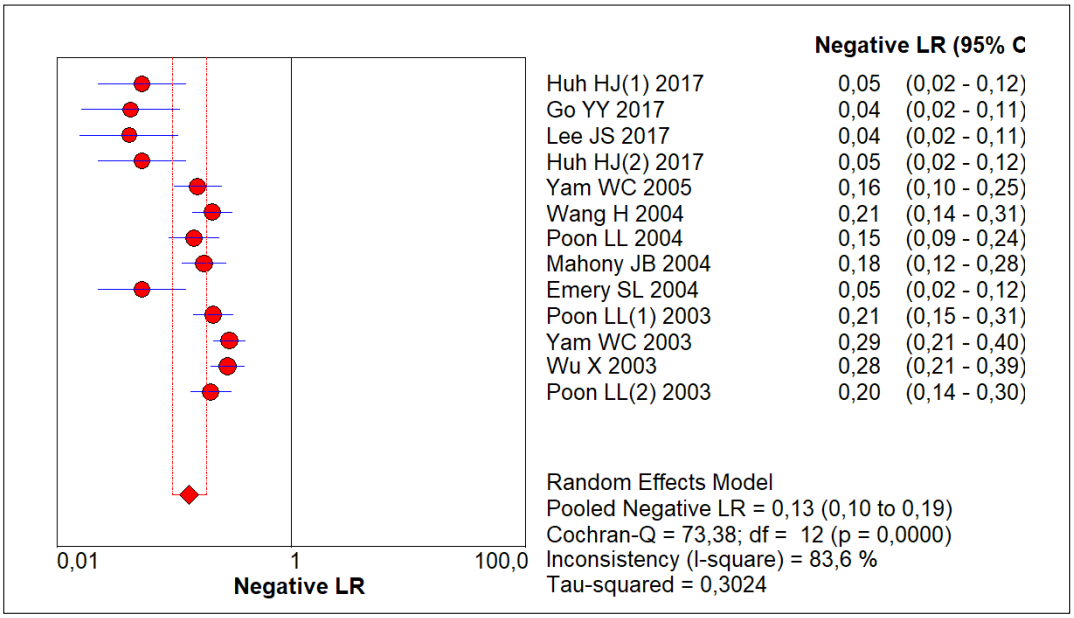


FIGURE 5.
FOREST PLOT OF THE NEGATIVE LIKELIHOOD RATIO IN THE CORONAVIRUS DIAGNOSIS BY PCR

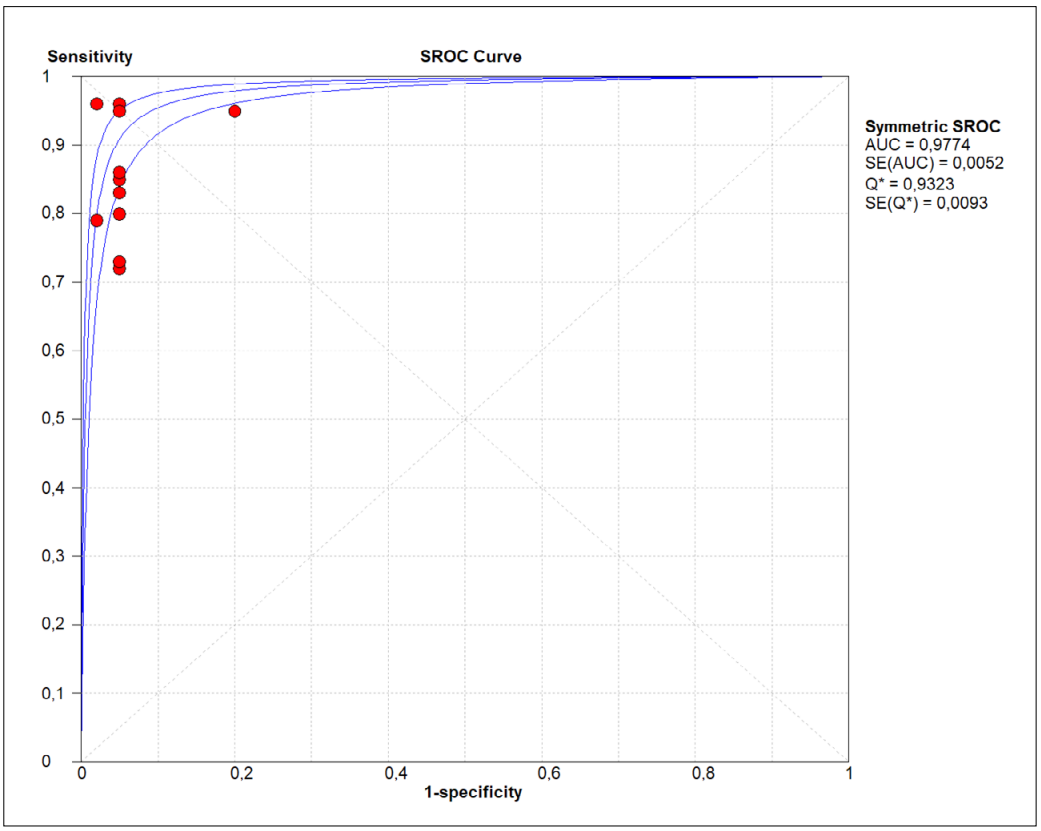


FIGURE 6. SUMMARY RECEIVER OPERATING CHARACTERISTIC CURVE (SROC) IN THE DIAGNOSIS OF CORONAVIRUS BY PCR

DISCUSSION

Since 2003, there have been cases of respiratory syndromes whose etiology is due to a coronavirus infection, with two previous epidemic outbreaks (SRAS-VOC, and MERS). In November 2019, a new outbreak began, of pandemic proportions, which has spread throughout the world at great speed, causing a large number of deaths and morbidities. At the same speed as the virus propagation, research was carried out for diagnosis and treatment of the pathology.

In this review, we looked for scientific studies of the best quality available to evaluate the accuracy of the PCR test for coronavirus diagnosis.

During our search, we retrieved only cross-sectional observational studies to support the evidence of the review, which provided us moderate quality and a low risk of bias. However, Deeks JJ, et al.[30] found a high risk of bias and low quality when they evaluated, through a systematic review, the serologic diagnosis test for COVID-19. This result was probably due to the methodological rigor applied in the assessment using the QUADAS-2 tool.

In this review, we searched for studies with suspected or diagnosed respiratory infection by the coronavirus in human patients. The PCR technique was adopted in all studies, with minor variations that do not interfere in their accuracy.

The values obtained through the meta-analysis were: sensitivity (86%), specificity (96%), positive likelihood ratio (18.82), a negative likelihood ratio (0.13), and area under the curve (AUC) (0.97).

The accuracy of the PCR test for coronavirus diagnosis can change according to the prevalence of the disease.

We can simulate 3 situations:

- With a prevalence of 50%, common among health professionals with respiratory symptoms, we found a post-test probability of 96%.
- With a prevalence of 20%, the post-test probability was 84%.
- With a prevalence of 5%, there is a 55% post-test probability.

As we can observe, even with high sensitivity and specificity of the PCR test for coronavirus diagnosis, we can obtain different results regarding its effectiveness.

We can interpret that when the test is applied in conditions of low prevalence of the disease, it allows a precise diagnosis in 55% of the cases.

Hypothetically, when carrying out a second consecutive test in the same patient, considering a prevalence of 96% (post-test probability of the first test with an

initial prevalence of 50%), there is a post-test probability of approximately 100% (diagnostic accuracy).

We should also point out the factors that can influence the results of the examination, thus producing false negative results, such as: technique and place of collection, time of onset of symptoms, storage and transportation of the sample to the location of the examination.

Synthesis of evidence

The PCR technique for coronavirus diagnosis provides a sensitivity of 86% and specificity of 96%; however, it should be applied in contexts of a high prevalence of coronavirus infection (not specific of SARS-Cov-2). When there is uncertainty regarding the diagnosis, a second sample collection can be indicated to confirm the diagnosis. Moderate quality of evidence.

ANNEXES

TABLE 1. STUDIES EXCLUDED AND REASON

Study and year	PMID	Reason for exclusion
1. Long C 2020	32229322	Comparison between CT and RT-PCR
2. Yan C 2020	32276116	Comparison between PCR techniques
3. Fang Y 2020	32073353	Comparison between CT and RT-PCR
4. Shirato k 2018	29763640	Comparison between PCR techniques
5. Pas SD 2015	26209385	Absent specificity data
6. Shirato K 2014	25103205	Absent specificity data
7. Cho CH 2014	24582583	Absent specificity data
8. Cho CH 2013	23743345	Absent specificity data
9. Corman VM 2012	23041020	Absent specificity data

TABLE 2. DESCRIPTION OF THE CLINICAL CHARACTERISTICS OF THE STUDIES INCLUDED.

Studies	PMID	DESIGN	POPULATION	TEST	COMPARISON
Huh HJ 2017	28840986	Cross-sectional	100 samples were analyzed (90 sputum, 10 NF swabs), collected from 100 different patients between June and July 2015. 50 samples were from patients with clinical suspicion of SARS and 50 asymptomatic ones.	rRT-PCR	Nested RT-PCR and sequencing of the RNA polymerase gene (RdRp) and N.
Huh HJ 2017	27834073	Cross-sectional	5,330 samples of 3,484 patients with suspected SARS-CoV were analyzed (4291 sputum, 145 AT, 732 NF, 35 OF, 62 NF and OF, and 65 others).	Real-time RT-PCR upE and ORF1a	Different locations of sample collection
Go YY 2017	28807812	Cross-sectional	Total of 55 samples collected from 20 patients positive for MERS, in 2015. Sputum collection. 48 samples of control individuals. Sensitivity analysis of the ORF1a and upE gene sequence.	RT-qPCR	RT-qPCR
Lee JS 2017	28566313	Cross-sectional	Total of 55 samples collected from 20 patients positive for MERS, in 2015. Sputum collection. 48 samples of control individuals. Sensitivity analysis of the ORF1a and upE gene sequence.	rRT-PCR	MagNA Pure 96 RNA extraction kit
Yam WC 2005	15797361	Cross-sectional	Patients with clinical suspicion of SARS. 54 NF samples collected and 10 OF by swab	rRT-PCR	Conventional PCR
Wang H 2004	15229153	Cross-sectional	44 patients with SARS admitted and diagnosed based on the WHO definition were selected	RT-PCR	Serological conversion
Poon LL 2004	15135737	Cross-sectional	Extraído 86 amostras de aspirados nasofaríngeos de pacientes que apresentaram diagnóstico clínico de SARS, com evidência sorológica de infecção por SARS-CoV	1- One step quantitative RT-PCR (monoplex)	Serological conversion

Studies	PMID	DESIGN	POPULATION	TEST	COMPARISON
Mahony JB 2004	15070991	Cross-sectional	17 NF/OF samples collected from patients with probable SARS between March and April 2003, in Toronto, Canada.	Seven types of reverse transcription-PCR (RT-PCR) tests - 3 conventional and 4 real-time	Culture of virus
Emery SL 2004	15030703	Cross-sectional	Total of 340 samples by nasal and oral swab, from 246 people with confirmed or suspected infection by SARS-CoV.	TaqMan real-time RT-PCR	Culture of virus
Poon LL 2003	12765993	Cross-sectional	29 patients selected (29 samples) with SARS and infections confirmed clinically and serologically, in Hong Kong, between February and March 2003.	Conventional RT-PCR	Patients with clinical and serological diagnosis
Yam WC 2003	14532176	Cross-sectional	124 NF and 65 OF samples collected from 163 patients hospitalized, in Hong Kong, between February and April 2003, with clinical suspicion of SARS, based on the WHO criteria.	RT-PCR - Evaluating two first-generation reverse transcription tests (WHO-HKU and WHO-Hamburg RT-PCR assays)	Serological conversion
Wu X 2003	12890368	Cross-sectional	97 samples (67 from patients with SARS e 30 from healthy individuals)	RT-PCR	Healthy vs. diseased samples
Poon LL 2003	14522060	Cross-sectional	50 patients with a clinical diagnosis of SARS were included, based on the WHO criteria, with a subsequent serological confirmation. 50 NF samples collected 1-3 days after symptom onset	Real-time RT-PCR in serum and nasopharyngeal aspirate samples	NF samples from healthy individuals and patients who presented other viruses were considered negative controls.

NF = nasopharynx, OF = oropharynx, PCR = polymerase chain reaction, RT-PCR= real-time PCR, WHO= World Health Organization.

TABLE 3. RESULTS EXTRACTED FROM THE STUDIES INCLUDED.

RT-PCR results - COVID				
Studies	PMID	DESIGN	Sensit.	Specif.
Huh HJ 2017	28840986	Cross-sectional	0.95	0.95
Go YY 2017	28807812	Cross-sectional	0.96	0.95
Lee JS 2017	28566313	Cross-sectional	0.96	0.98
Huh HJ 2017	27834073	Cross-sectional	0.95	0.95
Yam WC 2005	15797361	Cross-sectional	0.85	0.95
Wang H 2004	15229153	Cross-sectional	0.80	0.95
Poon LL 2004	15135737	Cross-sectional	0.86	0.95
Mahony JB 2004	15070991	Cross-sectional	0.83	0.94
Emery SL 2004	15030703	Cross-sectional	0.95	0.95
Poon LL 2003	12765993	Cross-sectional	0.79	0.98
Yam WC 2003	14532176	Cross-sectional	0.72	0.95
Wu X 2003	12890368	Cross-sectional	0.73	0.95
Poon LL 2003	14522060	Cross-sectional	0.80	0.98

FIGURE 7. FLOWCHART – THE SELECTION OF RETRIEVED FROM THE VIRTUAL DATABASES OF SCIENTIFIC INFORMATION IS DETAILED IN THE FLOWCHART BELOW:

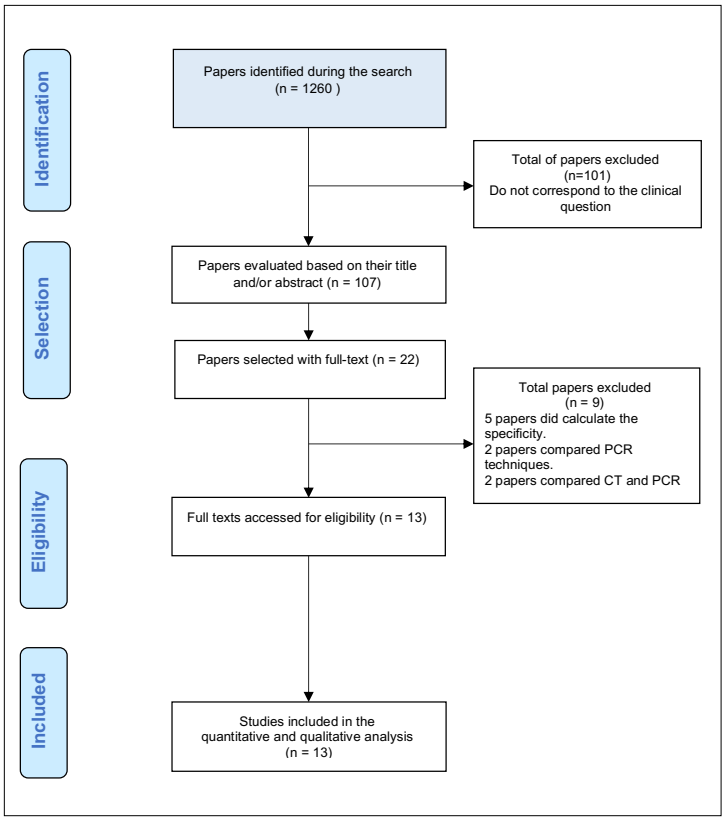


TABLE 4. RISK OF BIAS ASSESSED BY QUADAS-2

		Papers →	Go 2017	Lee 2017	Huh HJ 2017(1)	Huh HJ 2017(2)	Yam 2005	Wang 2004	Poon 2004	Ma-hony 2004	Emery 2004	Poon 2003(1)	Yam 2003	Wu X 2003	Poon LL 2003(2)
	Criteria of biases ↓														
Patient selection	Guiding questions	Was there consecutive or random sampling?	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	uncertain	uncer-tain
		Was a case-control design avoided?	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
		Were inappropriate exclusions avoided?	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
	Risk of bias	Is it possible that patient selection introduced a bias?	low	low	low	low	low	low	low	low	low	low	low	low	Uncer-tain
		Are there concerns that the patients included do not correspond to the question of the review?	low	low	low	low	low	low	low	low	low	low	low	low	low
Test evalu-ated	Guiding questions	Were the index test results interpreted without knowledge of the results of the reference test?	yes	yes	yes	yes	yes	yes	yes	yes	no	no	no	yes	no
		If a threshold was used, was it predetermined?	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	no
	Risk of bias	Is it possible that the conduction or interpretation of the reference test introduced a bias?	low	low	low	Low	low	low	low	low	low	low	low	uncertain	High
	Concerns about the applicability	Are there concerns that the conduction or interpreta-tion of the index test differ from the review question?	low	low	low	high	low	low	low	low	low	low	low	low	Low
Refer-ence test	Guiding questions	Does the reference test likely correctly classifies the target clinical condition?	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	uncer-tain
		Were the reference test results interpreted without knowledge of the results of the index test?	yes	yes	yes	no	yes	no	no	no	no	no	no	uncertain	no
	Risk of bias	Could the reference standard, its conduction or inter-pretation, introduce a bias?	low	low	low	Low	low	low	low	low	low	low	low	low	High
	Concerns about the applicability	Is there concern that the target condition, as defined by the reference test, does not correspond to the definition in the research question?	low	low	low	Low	low	low	low	low	low	low	low	low	uncer-tain
Flow and time	Guiding questions	Was there an appropriate interval between the index and the reference tests?	yes	yes	yes	I	yes	yes	yes	yes	yes	yes	yes	uncertain	uncer-tain
		Did all patients receive a reference standard?	yes	yes	yes	no	yes	yes	yes	yes	yes	yes	yes	uncertain	yes
		Did all patients receive the same reference test?	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	uncertain	uncer-tain
		Were all patients included in the analysis?	yes	yes	yes	no	yes	yes	yes	yes	yes	yes	yes	yes	yes
	Risk of bias	Is it possible that patient flow introduced a bias?	low	low	low	low	low	low	low	low	low	low	low	low	uncer-tain

Huh HJ 2017(1) PMID 28840986, Huh HJ 2017(2) PMID 27834073, Poon LL (1) PMID 12765993, Poon LL (2) PMID 14522060

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