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. 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. The mortality rate can reach 0.5%.

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) in Asia, with mortality rates of 8.7% (it reached 50% among people aged over 60 years), and in the Middle East Meridien East Respiratory Syndrome (MERS) in 2013, with 40% of mortality.

The etiological diagnosis of SARS-COV-2 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), and computed tomography. 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; serological tests may be collected starting at the seventh day (Figure 1).

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. During the PCR technique, reverse transcription can monitor the progress of the process as it takes place (in

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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 (NP) and/or oropharyngeal (OP) 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) 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 software.

The results of the studies included may be aggregated and meta-analyzed using the Meta-Disc software Version 1.4, 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 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 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 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%); I² = 85%.

The estimate of specificity calculated for the studies (Figure 3) was 96% (95% CI = 94 to 97%); I² = 0 %.

The results for a positive likelihood ratio (Figure 4) was 18.8 (95% CI = 14.5 to 24.3); I² = 0%

The results for a negative likelihood ratio (Figure 5) was 0.13 (95% CI = 0.1 to 0.19); I² = 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
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. 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  | 26203985   | Absent specificity data            |
| 6. Shirato K 2014 | 25103205 | Absent specificity data            |
| 7. Cho CH 2017  | 246582583  | 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, 725 NF, 35 OP, 62 NF and OP, 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                                   | Magna Pure 96 RNA extraction kit                |
| 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                                   | Conventional PCR                                |
| Yam WC 2005  | 15797361   | Cross-sectional   | Patients with clinical suspicion of SARS. 54 NF samples collected and 10 OF by swab | rRT-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 nasofaringeos 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                          |
### TABLE 3. RESULTS EXTRACTED FROM THE STUDIES INCLUDED.

| 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 WC 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.

### FIGURE 7. Flowchart – The selection of retrieved from the virtual databases of scientific information is detailed in the flowchart below:

- **Identification**
  - Papers identified during the search (n = 1280)
- **Selection**
  - Papers evaluated based on the title and abstract (n = 107)
  - Papers selected with full-text (n = 22)
  - Full texts accessed for eligibility (n = 13)
- **Eligibility**
  - Studies included in the quantitative and qualitative analysis (n = 13)
- **Excluded**
  - Total of papers excluded (n = 101)
  - Do not correspond to the clinical question
  - 5 papers did calculate the specificity. 2 papers compared PCR techniques. 2 papers compared CT and PCR
- **Included**
  - Total papers excluded (n = 9)

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| Criteria of biases ↓ | Papers → | Go 2017 | Lee 2017 | Huh HJ 2017(1) | Huh HJ 2017(2) | Yam 2005 | Wang 2004 | Poon 2004 | Mahony 2004 | Emery 2004 | Poon 2003(1) | Yam 2003 | Wu X 2003 | Poon LL 2003(2) |
|----------------------|-----------|--------|--------|----------------|----------------|---------|---------|---------|-----------|------------|-------------|---------|--------|----------------|
| **Patient selection** | Guiding questions | Was there consecutive or random sampling? | yes | yes | yes | yes | yes | yes | yes | yes | no | low | low | low | low | uncertain | uncertain |
|                     |                        | Was a case-control design avoided? | yes | yes | 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 | yes | yes |
| **Risk of bias**     | Is it possible that patient selection introduced a bias? | low | low | low | low | low | low | low | low | low | low | uncertain | uncertain | uncertain | uncertain | uncertain | uncertain |
|                     | 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 | low | low | low |
| **Test evaluated**   | 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 | no | no | yes | no |
|                     |                        | If a threshold was used, was it predetermined? | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes |
| **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 | uncertain | High | High | High | High | High |
| **Concerns about the applicability** | Were there concerns that the conduction or interpretation of the index test differ from the review question? | low | low | low | high | low | low | low | low | low | low | uncertain | Low | Low | Low | Low | Low |
| **Reference 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 | uncertain | uncertain | uncertain | uncertain | uncertain |
|                     |                        | 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 | uncertain | no | no | no | no |
| **Risk of bias**     | Could the reference standard, its conduction or interpretation, introduce a bias? | low | low | low | Low | low | low | low | low | low | low | low | uncertain | High | High | High | 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 | uncertain | uncertain | uncertain | uncertain | uncertain | uncertain |
| **Flow and time**    | Guiding questions | Was there an appropriate interval between the index and the reference tests? | yes | yes | yes | l | yes | yes | yes | yes | yes | yes | uncertain | uncertain | uncertain | uncertain | uncertain | uncertain |
|                     |                        | Did all patients receive a reference standard? | yes | yes | yes | no | yes | yes | yes | yes | yes | yes | uncertain | uncertain | uncertain | uncertain | uncertain | uncertain |
|                     |                        | Did all patients receive the same reference test? | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | uncertain | uncertain | uncertain | uncertain | uncertain | uncertain |
| **Risk of bias**     | Were all patients included in the analysis? | yes | yes | yes | no | yes | yes | yes | yes | yes | yes | yes | uncertain | uncertain | uncertain | uncertain | uncertain | uncertain |
|                     | Is it possible that patient flow introduced a bias? | low | low | low | low | low | low | low | low | low | low | low | uncertain | uncertain | uncertain | uncertain | uncertain | uncertain |
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