



# Utility of Available Methods for Diagnosing SARS-CoV-2 in Clinical Samples

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## Abstract

The laboratory diagnosis of SARS-CoV-2 should be done to confirm coronavirus disease 2019 (COVID-19) in suspected patients. Although several diagnostic methods have been developed in this regard, their accuracy for clinical application is not very clear yet. To compare the diagnostic value of laboratory tests for the detection of COVID-19 infection, this study provides an upcoming review of the newly developed detection methods. Sensitivity, specificity, detection limit, and turn-around-time of these methods are compared and challenges for their application in clinical settings are reviewed. PubMed and Google Scholar web sites were used for the systematic search until April 9, 2020 to identify the published studies based on the following keywords: "Detection", "Coronavirus 2019", "SARS-CoV-2", and "Sensitivity". Out of 526 results, a total of 54 articles, including 46 studies on detection methods, were considered eligible for the review. The results showed that most of the proposed tests focused on molecular methods, while immunological and point-of-care tests were investigated in 13 studies. There were also a few commercial automated methods for the qualitative detection of SARS-CoV-2 in clinical samples, most of which are not examined in the current review, as no data about their sensitivity and specificity were presented. Although the assessment of publication biases showed that 64% sensitivity and nearly 100% specificity for RT-PCR are close to reality, most of the related reports for serological methods are not valid and further studies are needed to confirm their utility in clinical settings. Moreover, the RT-PCR test alone cannot act as a gold standard because of bias in measurements. Therefore, antibody tests and other proposed methods could be used as supplementary diagnostic tests to improve RT-PCR accuracy. Although clinical findings are invaluable, in many cases, they can provide more valuable supportive data than serological tests.

**Keywords:** Novel Coronavirus, SARS-CoV-2, COVID-19, Diagnosis, Sensitivity and Specificity, Diagnostics, Real-time PCR, Serologic Tests

## 1. Context

Several coronaviruses can infect humans to cause mild to fatal respiratory disease. Coronaviruses with the highest fatality risk include the Middle East respiratory syndrome coronavirus (MERS-CoV), severe acute respiratory syndrome coronavirus (SARS-CoV), and the novel coronavirus (2019 novel coronavirus, 2019-nCoV) that was initially characterized in Wuhan, China (1). The latter virus, which is currently named as SARS-CoV-2 by the International Committee of Taxonomy of Viruses (ICTV) (2), can cause coronavirus-associated acute respiratory disease, coron-

avirus disease 19 (COVID-19). Globally 7,120,466 confirmed cases of COVID-19 were reported by 8 June 2020, leading to 406,660 deaths in 215 countries/territories/areas (<http://www.worldometers.info/coronavirus/#countries>). COVID-19 is reported from most of the countries with the highest total deaths in Italy, Spain, the USA, France, the UK, Iran, China, Germany, Netherlands, and Belgium, in sequence. This virus is highly transmissible, probably through mutations in the viral receptor-binding domain (RBD) that can increase its attachment to the host receptor angiotensin-converting enzyme (ACE2), local

transmission by pre-symptomatic people and patients with milder symptoms, and travel of infected passengers to other countries, which could explain the widespread distribution of the disease in these countries (3-5).

The laboratory detection of SARS-CoV-2 in clinical samples of symptomatic patients showed its worldwide explosive spread. However, the underestimation of the cases seems to be seen in most settings, which is mainly due to weakness in laboratory methods and screening of infected cases, especially pre-symptomatic cases or those with milder syndrome (6). A higher fatality risk among cases with laboratory-confirmed tests was reported in China. Based on the last report of the National Institute for Infectious Diseases of Japan, nearly half of the laboratory-confirmed cases were asymptomatic at the time of confirmation, which shows the importance of the selection of appropriate laboratory tests and their impact on the outbreak containment (6).

Based on the WHO definition in its guidance on 27 February 2020 and 18 May 2020, a confirmed COVID-19 case is a person with laboratory confirmation of COVID-19 infection, irrespective of clinical signs and symptoms (7, 8). This definition emphasizes the usage of laboratory tests with good quality, highest sensitivity and specificity, the lowest limit of detection, and lowest false-positive and false-negative results. Preparedness of referral diagnostic laboratories in each country could help governments to contain the outbreak. The tests used by these laboratories should be done with the lowest turn-around-time and highest accuracy. The application of reliable and rapid methods enables clinicians to better manage milder and probable cases, which will be effective in the containment of the outbreak by limiting its local and global transmission. Several diagnostic tests have been developed by different companies, many of which have been rejected by reference laboratories. The diagnostic methods for the primary screening of SARS-CoV-2 patients could vary in different countries based on their capacity and resources. The selection of a reliable, rapid, simple, and sensitive method could be helpful in regions with lower resources and will reduce the mismanagement of patients and decreases the number of new cases. This is a challenge due to the urgent need for access to laboratory tests for screening and confirmation of the infection, mainly in symptomatic cases. This systematic review describes and compares the developed diagnostic methods for the detection of SARS-CoV-2, based on increasing data on the sequence diversity of this virus and its immunogenicity and pathogenicity.

## 2. Evidence Acquisition

### 2.1. Study Identification and Selection of Data

An initial search using keywords “Detection”, “Coronavirus 2019”, “SARS-CoV-2”, and “Sensitivity” identified a total of 526 articles from PubMed and Google Scholar web sites. The exclusion criteria included duplicate studies and research not aiming to develop or validate diagnostic methods for the detection of SARS-CoV-2 in clinical samples. Newly developed or commercial products that did not present approved documents for the report of sensitivity or specificity of the designed tests were also excluded from the study. Based on differences in gold standards and methodologies of the studies, as well as severe biases among them, a qualitative analysis instead of a meta-analysis was done to find the closest findings to reality, and present more accurate and valuable results, their strengths, and weaknesses.

## 3. Results

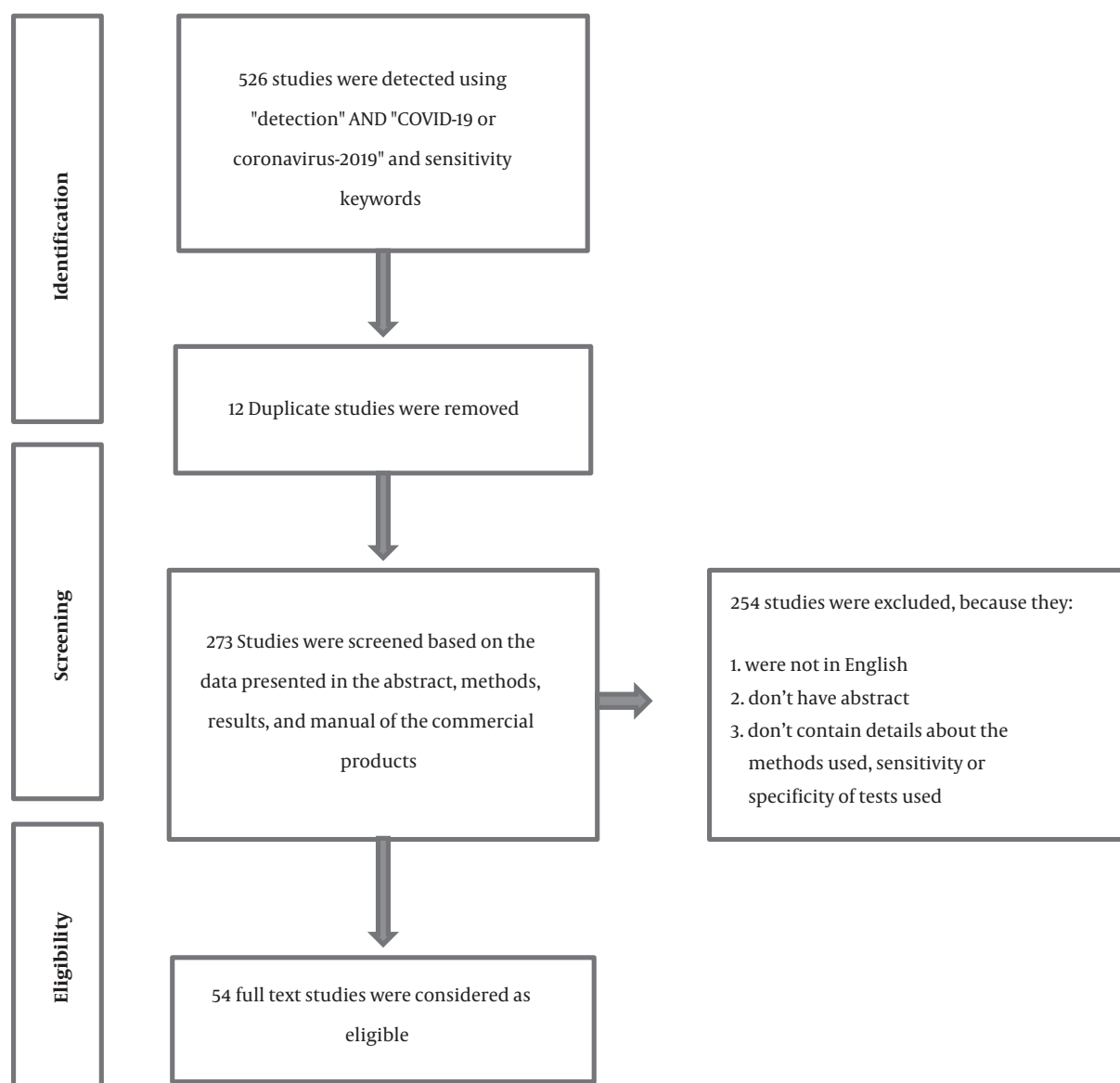
### 3.1. Search Results

A total of 526 articles were obtained. After removing duplicate and irrelevant results based on the exclusion criteria, 54 studies were selected for the full review (Figure 1 and Table 1). Our searches in general web sites showed that a high number of commercial kits were approved or recorded as in-development for the detection of SARS-CoV-2 (commercial kits for molecular diagnosis and serological tests) by different companies. However, most of them did not provide the sensitivity and specificity of their products. Among the reviewed methods, the applications of serological tests and immunohistochemistry were described in 13 studies, while CRISPR-Cas and target-based amplification methods, including real-time PCR, were presented in 33 studies. The detection limits 1 to 105 copies/ $\mu$ L were reported for the diagnostic methods, irrespective of types of samples used, and disease stages.

### 3.2. Diagnostic Methods for SARS-CoV-2

#### 3.2.1. Serology

Research studies on infected patients with SARS-CoV-2 showed the immunogenic nature of the virus in different populations. Increases in IgM, IgA, and IgG antibody titers were confirmed by the newly developed serological tests, as these assays could provide valuable data about the epidemiology of the virus. Serological assays could be used for the detection of infected symptomatic and even asymptomatic cases at the end of the disease course and thereafter. Although no universal serological tests are now



**Figure 1.** Flow diagram of the search strategy used for the review of SARS-CoV-2 detection methods in clinical samples

available for the detection of active infection, the application of such tests can be useful for the detection of prior infection among symptomatic and asymptomatic people in different populations. The drawback of SARS-CoV-2 antibody tests for the diagnosis of COVID-19 infection was also presented in the latest update of the WHO guideline (8). In spite of this shortcoming, ELISA and other antibody-based immunoassay techniques, such as immune colloidal gold detection technique, immunohistochemistry, sandwich ELISA quantum dots (QD) Immunochromatographic technology, point-of-care lateral flow immunoassay, and

peptide-based luminescent immunoassay, are among the developed immunoassay methods for the direct or indirect detection of SARS-CoV-2 in human sera, plasma, or whole blood samples (9, 10, 12, 13, 15, 18, 53). Currently, more than 170 immunoassay methods are available, only one of which, named “qSARS-CoV-2 IgG/IgM Rapid test” by Cellex Inc., has been approved by the Food and Drug Administration (FDA). Rapid chromatographic immunoassay kits, such as COVID-19 Ag GICA kit, rapid IgM-IgG combined antibody test, and qSARS-CoV-2 IgG/IgM Rapid Test, can detect SARS-CoV-2 antigen or antibody in nasopharyngeal swabs

or blood samples and their results are accessible within 10 - 30 min. The point-of-care lateral flow immunoassay (LFIA) test, a rapid IgM-IgG combined antibody test, showed the lowest turn-around time (15 min); however, its specificity was as high as 90.63%. While there are some reports about the accuracy of these rapid immunoassay kits, there are no publications to refer to the obtained results.

The sensitivity of serological methods mainly depends on the number of days post-infection; it can reach 100% after 15 - 39 days of disease onset. The sensitivity of serological methods was greater than 90% in most of the assays. However, a cross-reaction with SARS and MERS-COV was reported in an *in vitro* immunohistochemistry sandwich ELISA quantum dots (QD) Immunochromatographic technology assay (12). A combination of IgG and IgM antibody tests against SARS-CoV-2 showed an increased positivity rate compared to single IgG or IgM tests or molecular methods (10). In another study, the combination of serological tests (total antibody) and molecular methods (RT-PCR) showed a 12% higher sensitivity rate, suggesting ELISA + RT-PCR a better diagnostic indicator, even for the early diagnosis of COVID-19 infection (within seven days after disease onset). These results show that serological assays could play a role as a supplement to RNA detection methods to improve diagnostic sensitivity and specificity (11).

### 3.2.2. Molecular Methods

Target specific detection, amplification, or sequencing of genetic materials of SARS-CoV-2 in clinical samples could be done by different molecular methods. These methods can detect and/or amplify the target sequences using degenerative primers, multiple primer sets, or a single set of primers. At the time of writing this article, more than 202 molecular assays had been developed for the detection of SARS-CoV-2, 168 of which were commercialized (<https://www.finddx.org/covid-19/pipeline/>) and 60 products were CE-IVD-certified. The validated products included Caspr Biotech Phantom 1.0 Dx (near-point-of-care [POC]), Coyote Bioscience 2019-nCoV Prep Free QPCR Assay (near-POC), Fluxergy LLC Fluxergy Sample-to-Answer SARS-CoV-2 RT-PCR, General Biologicals Corporation GB SARS-CoV-2 Real-Time RT-PCR, Integrated Nano-Technologies Palladium, and STILLA COVID-19 Multiplex Crystal Digital PCR detection kit. Other molecular methods that their applications for SARS-CoV-2 were studied include the electrical detection of SARS-CoV-2 nucleocapsid protein using nanosensors and aptamer, NGS-based target capture for SARS-CoV-2 detection and screening, loop-mediated isothermal amplification (LAMP), Colorimetric and Isothermal Detection Kit, and CRISPR (clustered regularly interspaced short palin-

dromic repeats)-Cas systems. As the RT-PCR methods, the WHO (<https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance>) and the US Centers for Disease Control and Prevention (<https://www.cdc.gov/coronavirus/2019-ncov/lab/index.html>) developed RT-PCR Diagnostic Panels for the universal detection of SARS-like betacoronaviruses and the specific detection of SARS-CoV-2. Other reference laboratories also proposed alternative RT-PCR assays that generally target conserved gene loci of the virus in RNA extracts of clinical samples. These assays initially do a screening and therefore need to complete two or three sets of reactions to confirm the results. The integration of these tests into a multiplex real-time PCR panel has improved their application for screening of suspected patients during the pandemic in most countries.

In a comparative study on metagenomic-NGS (mNGS), RT-PCR, and CRISPR, in which mNGS was used as the reference method, PCR- and CRISPR-nCoV both presented a specificity of 100%. Besides, RT-PCR could detect the virus in 90.4% of the positive cases, which was explained due to the lower titer of the virus in some of the studied samples. However, CRISPR showed greater sensitivity (100%). In this study, higher sensitivity and lower turn-around-time of the CRISPR system (RT-PCR took about 1.5 hours, mNGS approximately 20 hours, and CRISPR around 40 min) and its usefulness in under-resourced settings where thermal cyclers are not accessible suggested the superiority of this technology over other methods for the detection of SARS-CoV-2 in clinical samples (44). In a recent review of the detection of human coronavirus infections, 17 diagnostic methods were summarized as cleared Chinese kits for the laboratory diagnosis of SARS-CoV-2 infection. The methods used by these products were included fluorescence RT-PCR, NGS, immune colloidal gold technique, isothermal amplification and microarray, magnetic particle chemiluminescence, and chemiluminescence immunoassay (54). However, the sensitivity and specificity of the cleared diagnostic kits were not presented clearly.

The PCR and real-time PCR methods are among the common test methods developed by different companies and national or international health authorities. All of these methods target ORF1AB, spike (S), nucleocapsid (N), envelope (E), and RNA-dependent RNA polymerase (RdRP) genes. The assays were tested on RNA extracts of saliva, nasal, and pharyngeal swabs, bronchoalveolar lavage fluid, sputum, and stool samples. The detection limits of the molecular assays reviewed in our study ranged between 0.109 copies/ $\mu$ L and  $10^5$  copy/ $\mu$ L and their sensitivity was between 45% to 97.6%. The highest sensitivity was reported for the RT-LAMP (iLACO) method used for the amplification of the ORF1AB gene when compared to the RT-PCR method

(97.6%). Among the targeted genes, ORF1AB showed the highest specificity and the N gene showed the highest sensitivity for the detection of SARS-CoV-2 RNA in clinical samples using different molecular methods. The analysis of sequence diversity among different loci of the SARS-CoV-2 genome in strains recovered from different countries showed a nucleotide substitution rate of  $8 \times 10^4$  substitutions per site per year (Figure 2). These substitutions were also depicted at gene loci that were mainly targeted for the amplification and diagnosis of the infection in RNA extracts of clinical samples using molecular methods reviewed in this article. This diversity was not limited to different geographic locations but was reported among isolates in each country (Figure 2).

In a study of isothermal amplification methods, researchers combined isothermal amplification with CRISPR-Cas12 DETECTR technology to develop a rapid detection method for SARS-CoV-2 (42). In this assay, RNA extracts of nasopharyngeal or oropharyngeal swab samples were used for the detection of N and E genes as targets. The assay was comparable in accuracy to the qRT-PCR method approved by the WHO and US CDC. Low cost, minimum requirement of laboratory equipment, and short turn-around-time (30 - 45 min) were proposed as its advantages. In a study by Song et al., a closed-tube Penn-RAMP, a two-stage isothermal dsDNA amplification method, was developed for the detection of SARS-CoV-2. The assay utilized both recombinase polymerase amplification (RPA) and LAMP techniques in a single tube. This RNA-based point-of-care (POC) technology combines both a paper-based POC diagnostic device and LAMP, which can be integrated with a smartphone application.

### 3.2.3. Proteomic Assays

Among the other proposed detection methods for SARS-CoV-2, shotgun proteomics were proposed in an in silico study for screening of this virus in proteolytically digested body fluids (55). In this method, digested viral peptides are screened by liquid chromatography-mass spectrometry (LCMS systems). However, no in vitro test on human clinical samples was done for the validation of the proposed method. The application of the fluorescence immunochromatographic assay that uses microbial antigens for diagnosis was proposed in a study of 239 participants suspected to COVID-19 infection. The assay could detect the N protein in either swab or urine samples of patients in the early phase (3 - 5 days after clinical onset) of the disease occurrence (19). In this study, the existence of the N antigen in nasopharyngeal swabs was detected in all participants that had positive RT-PCR results (ORF1ab and N). Although the performance of the assay on the nasopharyngeal swab samples showed a sensitivity of 68% compared to RT-PCR, it

showed a capacity for the detection of the virus in ~74% of urine samples among patients with positive nasopharyngeal antigen tests.

## 4. Discussion

In this review, due to the numerous biases in studies and the inconsistency of studies in terms of gold standard methods, it was not possible to perform meta-analyses on their results. Thus, it is necessary to use qualitative analyses to obtain some answers about the diagnostic accuracy of tests. The researchers of this study admit that because of the bias in the studies, it is not possible to draw definite conclusions from them, but based on the analysis of the effect of each type of bias on the results, the effect of compensatory measures used by each study in this field, and comparing the results of different studies, it can be seen which of the results of the study is closer to reality. We also use logical inferences in qualitative analysis to judge the results of studies.

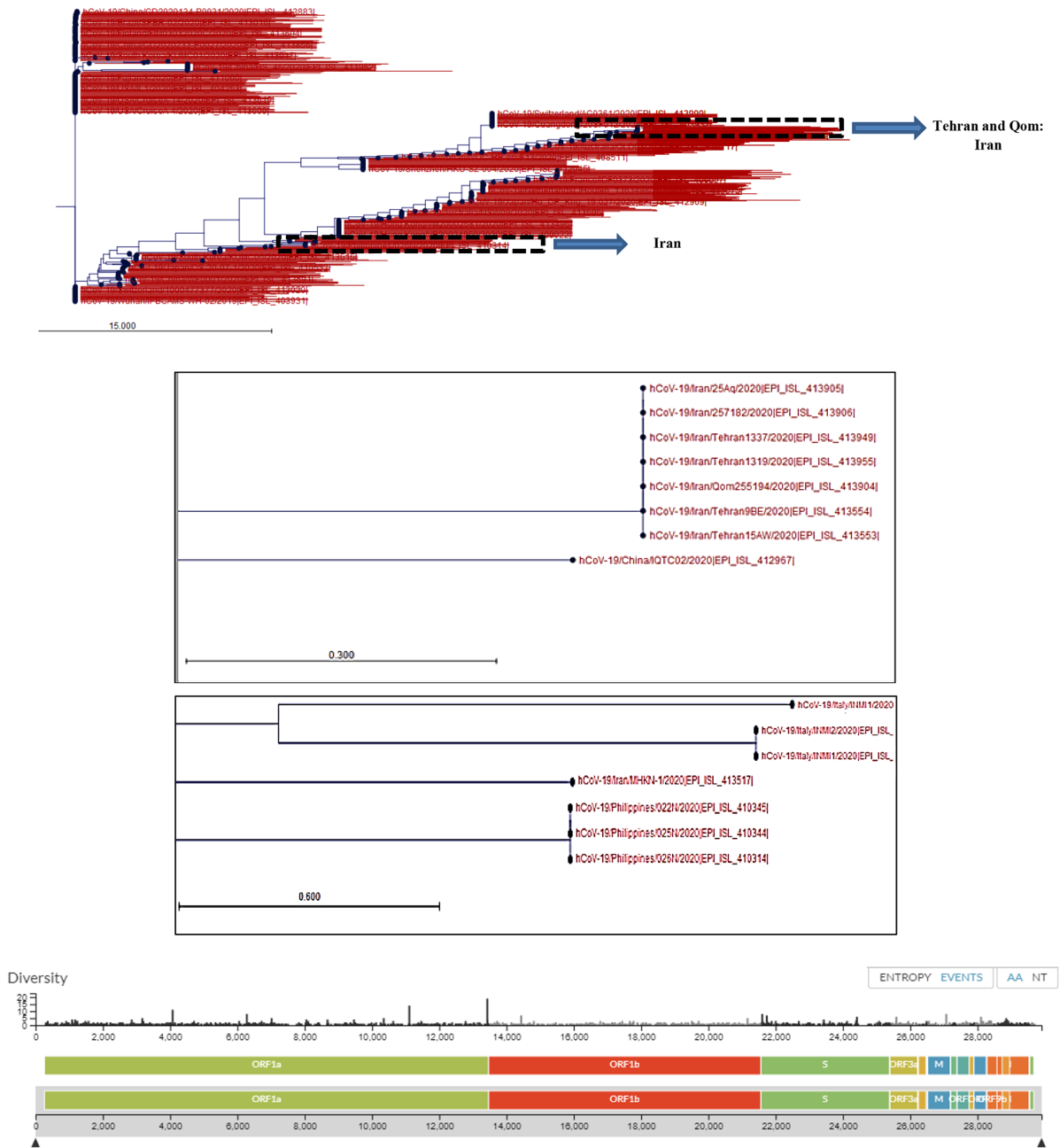
To analyze the validity of the results of the studies used in this review, the following factors were considered:

### 4.1. The Sampling Method and Correct Diagnostic Study Protocol

In most of the studies, infected and healthy samples were selected separately; that is, patient samples were selected from approved patients, and healthy samples were selected from individuals who did not have any specific viral disease. Known as spectrum bias, this bias is one of the most important factors that disrupt the results of sensitivity and specificity estimates. In studies that have this type of bias, both indicators are usually shown to be greater than the actual value. Studies by Xiang et al. (9), Zhao et al. (14), Li et al. (12), Yu et al. (30), Zhu et al. (31), Cleemput et al. (34), Broughton et al. (42), and Jiang et al. (48) had this type of bias. Studies by Liu et al. (17) and Cai et al. (18) partially encountered this problem although they tried to compensate to some extent by adding people with other diseases. Studies by Diao et al. (19), Jiang et al. (36), and Yang et al. (47) did not have this type of bias, but for reasons that we will discuss below, their results were not conclusive.

### 4.2. How to Choose the Gold Standard and the Final Confirmation of Virus Infection?

In most of the studies, the final confirmation of contamination was based on the two guidelines by WHO and a guideline from China; given that the final confirmation in these two guidelines was based on NAAT and RT-PCR, respectively, the sensitivity and specificity of tests that perform the same function as NAAT cannot be judged correctly. In other words, the sensitivity and specificity of



**Figure 2.** Phylogenetic tree of SARS-CoV-2 strains recovered from different countries around the world. Phylogenetic tree was drawn using CLC Genomic Workbench 11.0.1 based on the released sequences of SARS-CoV-2 from different countries, including Iran. The sequence of Wuhan-Hu-1/2019 was used as the reference. The Iranian strains were clustered at two distinct locations, which were originally related to one strain from China (seven Iranian strains, Upper dashed box) and one strain isolated from Philippine (Lower dashed box). Nucleotide diversity of SARS-CoV-2 isolates at nucleotide levels was provided from GISAID-Global Initiative on Sharing All Influenza Data-database based on the shared data updated on 2020-03-20.

these cases were falsely higher than the actual value. This problem can be seen even in studies that have not had spectrum bias.

#### 4.3. Indirect Conclusions From the Data

Since the direct conclusions are erroneous for the above reasons, we tried to obtain an estimate of the cor-

rectness of the reported indicators based on indirect conclusions from the data. The findings by Liu et al. (17) and Pan et al. (20) are significant in this regard. In the studies by Liu et al. (17), out of 238 patients who were clinically highly suspicious, 85 cases were reported to be RT-PCR-negative. In this study, the ratio of positive antibodies in both approved and unapproved groups and their clinical status were similar. In the Pan et al. (20) study, there were 95 RT-PCR-confirmed samples and 39 RT-PCR-negative samples, which were clinically confirmed. In this study, the ratio of positive antibodies in both approved and unapproved groups was similar, and the results were similar to the findings in the Liu et al. study (about 80% of their antibody tests were positive). These results indicate that the antibody and RT-PCR tests are epidemiologically independent of each other.

The study's authors conclude that patients who are highly suspicious in terms of clinical symptoms can be considered definitive. Logically, this conclusion is correct because when an independent test (antibody test) in both PCR-positive and PCR-negative independent populations, is positive by 80%, it can be proven by using a mathematical argument that all people in the PCR-negative population are sick. Otherwise, the antibody test will be 80% false positive, and since this is not possible, the only assumption is that both groups are sick. Based on the above arguments, it can be concluded that:

Clinically highly suspicious cases are similar to those in which RT-PCR is positive, and both groups can be considered definite patients (the author has done the same).

A 64% sensitivity for RT-PCR is close to reality.

Because antibody tests and RT-PCR are epidemiologically independent of each other, they can complement each other.

In a study by Pan et al., 70 people were examined as patients other than COVID-19, and the results of antibody testing in four of them showed a false positive. This finding confirms that a 100% specificity is incorrect for the ELISA test and it is due to the effect of spectrum bias. Based on the above findings, it can be said that the sensitivity of the tests whose gold standard was RT-PCR was much lower than the reported value (considering that the sensitivity of RT-PCR is 64%). A study by Jiang et al. (36), which examined the sensitivity and specificity of RT-PCR based on the WHO guidelines and could be considered conclusive in terms of the sampling method, reported a 78.2% sensitivity for RT-PCR. However, considering that the NAAT method is the gold standard in the WHO guideline, and because of its similarity to RT-PCR in targeting sites, this study may have undergone an overestimation of RT-PCR sensitivity and specificity.

Another point in the findings of the above studies by

Liu et al. (13) and Pan et al. is that the method chosen by these two studies to find definitive patients minimized bias incorporation, while the result of Ren et al. study was affected by this bias.

Based on the foregoing discussion, it can be said that the sensitivity results cannot be cited in any of the reviewed studies, except for Liu et al. (17) and Pan et al. (20) studies. The results of specificity in any of the studies, even two studies by Liu et al. (17) and Pan et al. (20) cannot be cited due to limitations in the selection of the healthy group.

## 5. Conclusions

The RT-PCR test alone cannot act as a gold standard method. Since the results of antibody tests and other methods are independent of RT-PCR, they can be used as supplementary diagnostic tests. Although clinical findings are invaluable for the direct diagnosis of the infection, in many cases, they can be considered more valuable than serological tests. Based on the findings from the analysis of the study results, it is recommended that future COVID-19 diagnostic studies use combined gold standards with full follow-up, because trusting on a test as the gold standard leads to bias in measurements. Clinical prediction rule studies can be helpful to find an effective diagnostic pathway.

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## Footnotes

**Authors' Contribution:** Armin Shirvani, Leila Azimi, and Masoud Alebouyeh collected data, analyzed them, and wrote the draft. Armin Shirvani wrote the discussion section. Roxana Mansour Ghanaie, Fatemeh Fallah, Sedigheh Rafiei Tabatabaei, Maryam Rajabnejad, Shahnaz Armin, Seyed Alireza Fahimzad, Fariba Shirvani, Ahmad Reza Shamshiri, Saeid Maham, and Abdollah Karimi designed the review, read the draft of the manuscript, and provided comments. Abdollah Karimi and Masoud Alebouyeh supervised the study.

**Conflict of Interests:** Nothing to declare.

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**Table 1.** Review of Studies on the Newly Developed Methods for Laboratory Diagnosis of SARS-CoV-2 in Clinical Samples

No.	Tests	Type of Sample	Limit of Detection	Sensitivity	Specificity	Reference method	Turn Around	Kit	References
<b>Serological Assays</b>									
1	Enzyme-linked immunosorbent assay (ELISA) IgG and IgM antibodies	Serum-COVID-19 vs. Serum of healthy people		55/63, 87.3%	100%	RT-PCR, WHO guidelines (NAAT)	ND	IgG/IgM antibody ELISA kits were manufactured by Zhu Hai Liv Zon Diagnostics Inc.	Xiang et al. (9)
2	COVID IgM/IgG antibodies kit	Blood of confirmed and not-confirmed cases	A Cut-off value of 0.88 and 1.02 for IgM and IgG, respectively	Positive rate increased when a combination of IgM and IgG tests was used		Nucleic acid test	ND	Diagreat company	Jia et al. (10)
3	Chemiluminescence	Serum		86.9%	99.2%	Total Ab compared to RNA only or IgM only tests	ND		Xia et al. (11)
	ELISA			94.8%	100%				
	Colloidal gold			96.2%	95.2%				
4	Immunohistochemistry sandwich ELISA quantum dots (QD) immunochromatographic technology (synthetic peptides corresponding to different areas of Nucleoprotein (N))	Blood of immunized mouse with synthetic peptides and lung biopsy of a confirmed patient	100 ng/mL. 10 ng/mL	Not presented	Minimal cross-reaction with SARS and MERS-CoV	ND	ND	In-house	Li et al. (12)
5	ELISA assay for IgM and IgG antibodies against N protein of SARS-CoV-2	Serum of confirmed or suspected patients	ND	83%	100%	RT-PCR assays and clinical findings	ND	Enzyme-Linked Immunosorbent Assays kit (Lizhu, Zhuhai, China)	Liu et al. (13)
6	Double-antigens sandwich immunoassay (Ab-ELISA); (receptor-binding domain (RBD) of the spike protein)	Plasma		89.6% and 100, for days 8-14 and 15-39 after the onset, respectively	99.10%	RT-PCR	ND	Enzyme linked immunosorbent assay (ELISA) kits by Beijing Wantai Biological Pharmacy	Zhao et al. (14)
	IgM			73.3% and 94.3%	98.60%				
	IgG			54.1% and 79.8%	99.00%				
7	Point-of-care lateral flow immunoassay (LFIA) test- A rapid IgM-IgG combined antibody test	Blood, serum, and plasma		88.66%	90.63%	RT-PCR and clinical findings	15 minutes	Enterprise Co., Ltd., China	Jiang et al. (15)
8	In-house and commercial ELISAs (Si-specific IgG and IgA antibodies)	Serum		ND	100%	Plaque-reduction neutralization test	ND	In-house and commercial kits (EUROIMMUN Medizinische Labordiagnostika AG)	Okba et al. (16)
9	ELISA (IgM and IgG antibodies against recombinant nucleocapsid and recombinant spike proteins)	Serum		80.4 - 82.2%	100%	RT-PCR		Lizhu, Zhuhai, China and Hotgen, Beijing, China	Liu et al. (17)
10	Colloidal gold-immunochromatographic assay (GICA)	Serum-COVID-19 vs. Serum of healthy people		75/91, 82.4%	100%	RT-PCR		IgG/IgM antibody GICA kits (lot number 2001010220), manufactured by Zhu Hai Liv Zon Diagnostics Inc, China	Xiang et al. (9)
11	Peptide-based luminescent immunoassay (magnetic chemiluminescence enzyme immunoassay, MCLIA) to detect IgG and IgM (synthesized peptides from the orf1a/b, spike (S), and nucleocapsid (N))	Serum of confirmed cases with RT-PCR	Mean chemiluminescence (CL) values for IgG and IgM as $0.152 \pm 0.109$ and $0.151 \pm 0.107$ , respectively	71.4%, 57.2%, and 81.5% for IgG, IgM, and total antibody, respectively	100%	RT-PCR	ND		Cai et al. (18)

12	Fluorescence immunochromatographic assay (Nucleocapsid protein)	Nasopharyngeal swab sample and urine		68%	100%	RT-PCR	within 10 minutes		Diao et al. (19)
13	Colloidal gold-based immunochromatographic strip (IgG and IgM)	Serum, plasma, and whole blood		68.6% in total		RT-PCR and clinical findings	15 min	Zhuhai Livzon Diagnostic Inc.	Pan et al. (20)
				92.9% in the intermediate stage (8-14 days of onset)					
				96.8% in the late stage ( $\geq$ 15 days of onset)					
<b>B</b>	<b>Molecular Assays</b>								
14	qRT-PCR (ORFlab and nucleocapsid protein (N))	Throat swab samples, sputum samples, and alveolar lavage fluid samples of suspected cases	50 copies/ $\mu$ L to 105 copies/ $\mu$ L	42/81 (51.9%)		RT-PCR		2019-nCoV nucleic acid detection kit according to the manufacturer's protocol (Shanghai ZJ Bio-Tech Co Ltd)	Xiang et al. (9)
15	qRT-PCR (N and RdRP)			N has 7-43 fold greater sensitivity than RdRP; ~10 fold increased sensitivity		RT-PCR		PCLMDIM nCoV one step RT-PCR Kit from PCL Inc, Korea, Cat # PCLMD2019001-50	Kim et al. (21)
16	Automated RT-qPCR (N, E, and RdRP)		21, 141, and 350 copies/reaction for N, E, and RdRP, respectively	N and RdRP showed the highest analytical sensitivity		RT-PCR			Barra et al. (22)
17	Droplet digital PCR (ddPCR) (ORFlab and N)	Pharyngeal swab samples from suspected patients	0.109 copies/ $\mu$ L and 0.021 copies/ $\mu$ L for ORFlab and N primers/probe sets	500 times higher than qRT-PCR		RT-PCR		QX200 Droplet Digital PCR System using supermix for probe (Bio-Rad)	Suo et al. (23)
18	qRT-PCR vs. CT scan	Throat and nasal swabs		Between 45 and 60% vs. 97%		RT-PCR			Al-Tawfiq et al. (24)
19	qRT-PCR assay (RdRp/Orf1 and N region)		15 copies/reaction; ORFlab (China), 2019-nCoV-N2, N3 (USA), and NIID-2019-nCoV-N (Japan) sets	ORFlab: ORFlab (China) set might be most sensitive than other sets; N: 2019-nCoV-N2, N3 (USA), and NIID-2019-nCoV_N (Japan) sets may be recommended for the sensitive qRT-PCR assay of N region		RT-PCR			Jung et al. (25)
20	qRT-PCR	Nasopharyngeal swabs or sputum	10 copies/reaction	RealStar SARS-CoV-2 kit seemed to be more sensitive		RT-PCR	1:50- 2:15 hrs	QuantiTect Virus + Rox Vial kit (QIAGEN); SuperScript III One-step RT-PCR System with Platinum TaqDNA Polymerase (Invitrogen); RealStar SARS-CoV-2 RT-PCR kit 1.0 (Altona)	Konrad et al. (26)
21	qRT-PCR ((RdRp)helicase (Hel), spike (S), and nucleocapsid (N) genes)	Respiratory and non-respiratory samples	11.2 RNA copies/reaction	COVID-19 -RdRp/Hel showed higher sensitivity vs. RdRp -P2 assay	More specific vs. RdRp-P2 assay	RT-PCR			Chan et al. (27)
22	Multiplex-PCR-based method and multiplex-PCR-based metagenomic method		1.15 copies	ND	ND	RT-PCR			Li et al. (28)
23	Digital PCR (dPCR) (ORF1ab, N and E genes)	Pharyngeal swab	2 copies/reaction	Higher than RT-qPCR	100%	RT-qPCR			Dong et al. (29)

24	RT-LAMP (iLACO)	ND	10 copies of ORF1ab/reaction	97.6% (42/43) vs. RT-PCR		RT-PCR			Yu et al. (30)
25	RT-LAMP coupled with nanoparticles-based biosensor (NBS) assay (RT-LAMP-NBS) (orf1a/b and N)	Oropharynx swab	12 copies/reactions	100%	100%	RT-PCR		1 h	Zhu et al. (31)
26	Nanopore target sequencing (NTS) of 11 related gene fragments of orf1ab	Nasopharyngeal swab	10 copies/mL	More sensitive than qRT-PCR		RT-PCR	6 - 10 h		Wang et al. (32)
27	Bait capture platform for culture- and amplification-free next-generation sequencing of SARS-CoV-2	In silico	ND	ND	ND	ND	ND	ND	Nasir et al. (33)
28	Genome Detective Coronavirus Typing Tool			100%	100%	A reference dataset of previously published coronavirus whole-genome sequences			Cleemput et al. (34)
29	SENsitive Splint based one-step isothermal RNA detection (SENSR) (RdRp)	RNA from clinical samples	0.1 aM, 6 molecules per 100 $\mu$ L reaction	ND	ND	ND	30 min		Woo et al. (35)
30	Combination of RT-PCR and CT, Two targets: RdRp+, E or N+	Pharyngeal and nasal swabs and stool		91.90%	66.8% RT-PCR is superior to CT in diagnosing mild infections	RT-PCR, WHO guideline	ND	ND	Jiang et al. (36)
31	Two multiplex real-time RT-PCR assays targeting ORF1b and N gene regions	Sputum and cell culture supernatant	$\geq$ 10 copies of chimeric plasmid	N gene assay is about 10 times more sensitive than the ORF-1b gene assay		RT-PCR		In-house method	Chu et al. (37)
32	RT-PCR (E and RdRp)	Respiratory samples	5.2 and 3.8 copies per reaction at 95% detection probability, respectively		100%	RT-PCR		World Health Organization	Corman et al. (38)
33	Real-time RT-PCR (ORF1ab, nucleocapsid protein-NP)	Nasal, pharyngeal swabs, bronchoalveolar lavage fluid and sputum	Not presented	Not presented		RT-PCR		Shanghai Huirui Biotechnology Co., Ltd.	Liu et al. (39)
34	Real-time reverse-transcription PCR (rtPCR); RdRp, S, E, N	Pharyngeal swab	1 - 10 virus particles	High	Not presented	RT-PCR	less than 4 hours		Won et al. (40)
35	CRISPR-Cas12 (RdRp, ORF1b and ORF1ab genes)	Saliva	10 copies/ $\mu$ L			ND			Lucia et al. (41)
36	CRISPR-based DETECTR Lateral Flow Assay; CRISPR-Cas12 based (E and N genes + human RNase P gene as a control)	Respiratory swab	10 copy/ $\mu$ L reaction (in vitro); Decreased to 500 and 1,500 copies/ $\mu$ L in raw samples carried in transport media and PBS	90%	100%	RT-PCR	30 min (RNA)- 45 min (Sample to results)		Broughton et al. (42)
37	CRISPR-Cas13 detection system (Orf1ab)		10 copies/ $\mu$ L	Not presented	Not presented	ND			Metsky et al. (43)
38	CRISPR/Cas13-mediated enzymatic signal amplification (Orf1ab)	Respiratory samples	1 - 7.5 copies	100%	100%	Metagenomic next-generation sequencing (m-NGS)	40 min		Hou et al. (44)
39	All-in-One-dual (AIOD)-CRISPR (CRISPR-Cas2a)	In vitro	1.2 copies	ND	ND	RT-PCR	40 min		Ding et al. (45)
40	LAMP (ORF1a-A, GeneNA)	Respiratory specimens (swabs)	4.8 copies/ $\mu$ L	In agreement with the real-time detection		RT-PCR			Zhang et al. (46)

41	RT-LAMP (ORFlab gene, E gene and N gene)	Throat and nasal swabs	1000 copies/mL (5 copies)	Similar to RT-PCR. The ORFlab gene was very specific and the N gene was very sensitive	99%	RT-PCR		In-house RT-LAMP vs RT-PCR (Jiangsu Bioperfectus Technologies Co., Ltd.)	Yang et al. (47)
42	Single-step reverse transcriptase loop-mediated isothermal amplification (RT-LAMP)	Spiked molecular grade water and sputum samples	500 copies/mL	91.4%	99.5%	qRT-PCR	< 30 min		Jiang et al. (48)
43	RT-LAMP (Nsp3, S, N)	In vitro	100 copies	ND	100%	ND	30 min		Park et al. (49)
44	RT-ERA: isothermal exponential amplification methods via reverse transcription plus subsequent enzymatic recombinase amplification (ERA); (N-gene); nfo affinity probe plus LF strips	In vitro	0.32 aM (i.e. $0.32 \times 10^{-18}$ M) of RNA or 0.2 copy/ $\mu$ L	ND	ND	ND	30 min		Xia et al. (50)
45	closed-tube Penn-RAMP, a two-stage; isothermal dsDNA amplification method	ND		Greater than LAMP or RT-PCR alone		ND	> 1 h		Yang et al. (51)
46	Double-quencher probes (YCH assay) (N1 and N2)	In vitro	> 10 copies of DNA positive control	Greater than US CDC and CDC) in the USA and the National Institute of Infectious Diseases (NIID) in Japan		RT-PCR			Hirotsu et al. (52)

<sup>a</sup> Not reported or detected; RT-PCR: Reference real-time PCR method reported by the WHO or other international organizations as described in each article.