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Rapid diagnosis of COVID-19 in the first year of the pandemic: A systematic review

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ABSTRACT

Background: COVID-19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a global health threat and remains a challenge for modern medicine. Rapid and accurate diagnosis of COVID-19 is vital for proper disease and outbreak management. Our review aimed to analyze scientific articles published in the literature addressing the rapid tests available for COVID-19 diagnosis at the first year of the pandemic. **Methods:** A systematic review was performed from October 22 to 27, 2020, searching data published in PubMed and Google Scholar databases, using subject headings or keywords related to point of care and rapid test diagnostic for SARS-CoV-2 and COVID-19. **Results:** The first survey identified 403 articles, but only 23 met the defined criteria for the systematic analysis. The sensitivity and specificity parameters were assessed in 19 studies, and the data suggested that there was lower sensitivity in the period 1 to 7 days after the emergence of symptoms (~38%) higher sensitivity at 8 to 14 days (~90%), and the highest at 15 to 39 days (~98%). Accuracy was reported in six studies, reporting values above 50%. Only three studies reported a possible cross-reaction. **Conclusions:** Our findings indicate that the rapid tests used in the first year of the pandemic were tested with a small number of samples and not adequately validated. And the studies that described them were conducted with little scientific rigor.

1. Introduction

The Coronavirus Disease 2019 (COVID-19) outbreak, caused by the new Severe Acute Respiratory Syndrome Coronavirus type 2 (SARS-CoV-2), has rapidly progressed worldwide after its origin in Wuhan, Hubei Province, China [1,2]. Since the World Health Organization (WHO) declared COVID-19 as a pandemic [3], the number of cases has increased exponentially, with more than 102 million cases reported up to February 2021, with a death toll exceeding 2.2 million [4].

The rapid and accurate diagnosis of COVID-19 is vital for proper disease and outbreak management, allowing immediate and precise public health surveillance, prevention and control measures [5]. At present, diagnostic tests for COVID-19 rely on two main categories: 1) methods in which the clinical specimen is examined directly for the presence of virus particles, virus antigen, or viral nucleic acids; and 2) serological tests that detect anti-SARS-CoV-2 antibodies.

Real-time reverse transcriptase polymerase chain reaction (RT-PCR), a molecular test of virus nucleic acid found in respiratory samples [6], is

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currently used as the reference standard for diagnosing COVID-19. However, this method has limitations that include possible false-negative results if the amount of viral genome is insufficient or if the correct time window of viral replication is missed [7]. Hence, patients suspected of having COVID-19 may receive an incorrect diagnosis, leading to greater disease spread or inappropriate treatment in the case of false positives and greater spread of the disease mistaken clinical conduct [8,9].

Serological tests, called immunoassays, are rapid and simple techniques based on the qualitative or quantitative detection of anti-SARS-CoV-2 immunoglobulins. There are several types of serological tests available, including ELISA (enzyme-linked immunosorbent assay), chemiluminescence immunoassay (CLIA) fluorescence, and qualitative immunochromatography [10-14]. Serological tests for antibodies against SARS-CoV-2 are an important tool for epidemiological studies, to determinate the immunization status of a population [15-19] and identify asymptomatic patients [15-17].

Combined RT-PCR and serological tests increase both the sensitivity and accuracy of COVID-19 diagnosis [7]. Thus, the complementary use of IgM-IgG antibody tests may guarantee a correct diagnosis, allow early intervention, and play a critical role in fighting outbreaks [20,21]. Due to their advantages, many serological tests for COVID-19 have become available in a short period, including some marketed for use as rapid, point-of-care (POC) tests. These tests provide results within minutes of administering the test and may extend testing to communities and populations that cannot readily access care [22]. In addition, POCs contribute to the rapid screening of sick patients and containment of the spread of Sars-CoV-2 [23].

However, the pace of development of these rapid diagnostic tests (RDT) exceeded that of rigorous evaluation, and many questions about their accuracy remain [24]. It is not clear whether the scientific evidence supports the continued use of these tests for COVID-19. Therefore, in this review, we searched for studies related to POC tests and RDT for SARS-CoV-2 infection.

2. Methods

For this systematic review, we searched the PubMed and Google Scholar databases from November/2019 to November/2020, with no restrictions on language. These databases are large enough to find all relevant articles on the topic of the article. We used subject headings/sub-headings (when applicable) or keywords for the concepts of point-of-care diagnostics or rapid test diagnostic for COVID-19 or SARS-CoV-2. The inclusion criteria were randomized trials, cohort, or case-control studies reporting experimental data on POC tests or RDT for SARS-CoV-2 infection. The WHO and UNICEF mention that rapid diagnostic test (RDT) provides results in 15–30 min. Because of this, we considered POC and RDT quick tests providing results within 30 min after administration [25,26]. We excluded review articles, editorials, case reports, modeling studies, duplicate articles, preprints, and studies that did not perform POC or RDT for COVID-19. Three investigators independently screened the titles and keywords, and two independently screened the full-text papers. A third reviewer resolved any disagreements between the reviewers at the full-text stage. The authors used PICO to describe all the components related to the identified problem and to structure the research question. Then, the bibliographic search for evidence was carried out by the selection of the search terms identification of terms (descriptors) related to each component of the PICO strategy. The descriptors are classified as controlled: known as “medical subject headings” or “subject descriptors”, which are used for the indexation of articles in the databases. The most known vocabularies of controlled descriptors are: MeSH (MEDLINE/PubMed), DeCS (BIREME) and Emtree (EMBASE). Not controlled: represent the textual words and their synonyms, orthographic variations, acronyms and correlates. Moreover, the PICO strategy also used Boolean operators which was represented by the connector terms AND, OR and NOT. These terms

allow for combinations of descriptors that will be used in the search, with AND for a restrictive combination, OR for an additive combination and NOT for an excluding combination. Therefore, the combination of components of the PICO strategy for the finalization of the search strategy: after the selection of the search terms and use of Boolean operators for each of the four components of the PICO strategy, these must be inter-related in the following final strategy: (P) AND (I) AND (C) AND (O). Such final strategy must be inserted in the search box existent in the databases, so that evidence is located by means of a bibliographic search [27].

3. Results

Fig. 1 shows the selection process of the studies. Initially, 257,120, we identified possible results through the database searches. Then, after screening the titles and keywords, we selected 403 studies for inclusion in the whole selection process. Twenty-three studies met the inclusion criteria. Tables 1 and 2 summarize the studies by test method.

We identified 14 studies evaluating the levels of IgG, and IgM antibodies [5,28-39], one assessing the levels of IgG, IgM and IgA antibodies [7], one the levels of IgG and IgA antibodies [40], one the levels of IgG antibodies only [41], and one the levels of IgM antibodies [42]. Also, we identified four rapid tests directed to the detection of antigenic structures (44–47) and two for the detection of RNA [42,47].

Among the included studies, 18 reported the sensitivity of the test [5,7,27-38,42,44-48], and 14 reported the specificity [5,28,29,33-37,41,43-47]. Fig. 2 shows a graphical representation of the sensitivity and specificity of the analyzed tests. Only five studies provided information about the accuracy of the test [36,42,45-47]. Four studies [32,39-41] performed tests to detect antibodies but did not reveal any of these parameters. Cross-reaction was assessed in three studies [7,29,43], and different diseases were considered, such as mycoplasma, influenza,

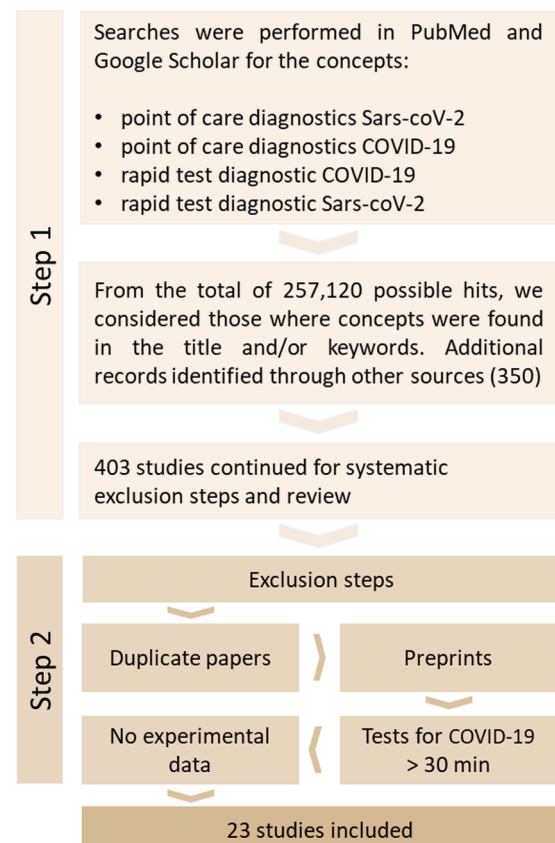


Fig. 1. Study selection.

Table 1
Serological POC tests and RDT for detection of antibodies against SARS-CoV-2.

AUTHOR / METHODS USED	SARS-CoV-2 Test	SENSITIVITY			SPECIFICITY / ACCURACY	N
		Day after symptom onset				
		01 – 07	08 – 14	15 – 39		
Yang <i>et al.</i> / Immunochromatography for the qualitative detection of the antibody IgM / IgG to novel Coronavirus (COVID-19) in human whole blood / serum / plasma (USA)	IgM	ND	82%	ND	94% / 92%	
	IgG	ND	ND	94%	97% / 96%	
Abreu <i>et al.</i> / The LFI, SGTi-flex COVID-19 IgM / IgG (Sugentech, Republic of Korea), which is a nanoparticle-based immunochromatographic test kit for the qualitative determination of IgM and IgG COVID-19 antibodies in human whole blood (finger pricked or venous), serum or plasma.	IgM/IgG	ND	75%	ND	80,30% / ND	164 = 27 positives samples and 137 negatives (rt-PCR)
Spicuzza <i>et al.</i> / LFA 2019 IgG / IgM Antibody Rapid Test Kit (Beijing Diagreat Biotechnologies Co., Ltd)	IgM/IgG	ND	ND	83%	93% / ND	37 (30 patients admitted to the Infectious Disease Covid 19 or Pneumology Units and 7 healthy controls)
Cassaniti <i>et al.</i> / VivaDiag COVID-19 IgM/IgG Rapid Test lateral flow immunoassay (LFIA) with serum or whole blood sample	IgM/IgG	18.4%	ND	ND	91.7% / ND	50 patients confirmed positive for SARS-CoV-2 by rRT-PCR
Li <i>et al.</i> / Lateral flow qualitative immunoassay (LFQI) for the rapid determination of the presence or absence of both anti-SARS-CoV-2-IgM and anti-SARS-CoV-2-IgG in human serum, and plasma), manufactured by Jiangsu Medomics Medical Technologies. Specimens (whole blood)	SARS-CoV-2 IgG-IgM combined antibody	88.66%	ND	ND	90.63% / ND	A total of 525 cases were tested: 397 (positive) clinically confirmed (including rRT-PCR test) SARS-CoV-2-infected patients and 128 non-SARS-CoV-2-infected patients (128 negative).
Lee <i>et al.</i> / SARS-CoV-2 was detected in a throat swab sample by rRT-PCR.	IgG		100.0%		98% / 98.6%	1 case of COVID-19 confirmed by rRT-PCR
Lyu <i>et al.</i> / LFA (COVI040, GenBody Inc., Cheonan, Republic of Korea) to detect IgM or IgG and ELISA for IgG (RAI010R, BioVendor Laboratories) against SARS-CoV-2	IgM/IgG	ND	ND	ND	ND / ND	400 all samples from 2015 - 2017
Capello <i>et al.</i> / VivaDiag™ COVID-19 IgM / IgG Rapid Test is an in vitro diagnostic test for the qualitative determination of COVID-19 IgM and IgG antibodies	IgM/IgG	ND	ND	ND	ND	30 (56% men, 44% women; general age 60.9 + 2.71 years; x ± SEM)
Pan <i>et al.</i> / Blood samples were collected, and blood serum, plasma or whole blood were subjected to ICG assay in accordance with the manufacturer's protocol (Zhuhai Livzon Diagnostisic Inc.).	IgM	11.1%	78.6%	74.2%		A total of 86 samples from 67 cases of real-time rRT-PCR confirmed SARS-CoV-2 positive Antibody detection in nucleic acid-negative "clinically diagnosed" patients
	IgG	3.6%	57.1%	96.8%	ND	
	IgM	22.2%	33.3%	57.1%	ND	
	IgG	44.4%	66.7%	71.4%	ND	
Zhong <i>et al.</i> / ELISA methods for serum samples to detect IgM and IgG antibodies	rN-based IgM		97.9%		99.7% / ND	47 COVID-19 patients ho were nucleic acid-positive (Table S1) and 300 healthy controls were analyzed
	rN-based IgG		97.9%		99.7% / ND	
	rS-based IgM		89.1%		97.0% / ND	
	rS-based IgG		95.7%		85.7% / ND	
Chemiluminescence methods for serum samples to detect IgM and IgG antibodies	IgM		97.7%		95.2% / ND	
	IgG		95.6%		96.6% / ND	
	rN-based IgM	68.2%	ND	ND	ND	
	rN-based IgG	70.1%	ND	ND	ND	
Liu <i>et al.</i> / Two Enzyme-Linked Immunosorbent Assay (ELISA) kits based on recombinant SARS-CoV-2 nucleocapsid protein (rN) and spike protein (rS) were used for detecting IgM and IgG antibodies.	rN-based IgM and- or IgG	80.4%	ND	ND	ND	214 patients diagnosed with COVID-19. All laboratory confirmed positive for SARS-CoV-2 by rRT-PCR
	rS-based IgM	77.1%	ND	ND	ND	
	rS-based IgG	74.3%	ND	ND	ND	
	rS-based IgM and- or IgG	82.2%	ND	ND	ND	
To <i>et al.</i> / Antibody levels against the SARS-CoV-2 internal nucleoprotein (NP) and surface spike protein receptor binding domain (RBD) were measured using EIA (EIA of IgG and IgM against internal viral NP and RBD).	IgM and IgG (EIA)		ND		ND	108 serum specimens were obtained from 23 patients confirmed positive for SARS-CoV-2 by rRT-PCR
Zhang <i>et al.</i> / anti-SARSr-CoV IgG and IgM ELISA kits (MaxiSorp Nunc-immuno).	IgM	Day 0	Day 5	Day 15	ND	16 patients were viral nucleotide detection positive
	IgG	ND	50.0%	81.0%	ND	
Okba <i>et al.</i> / Serologic assays for detection of SARS-CoV-2 neutralizing, spike protein-specific, and nucleocapsid-specific antibodies. Performed anti-SARS-CoV-2 S1 IgG and IgA ELISAs by using β-versions of 2 commercial kits (EUROIMMUN Medizinische Labordiagnostika AG	IgG and IgA (ELISA)		ND		ND	Serum samples (n = 10) collected from 3 patients + serum samples (n = 31) All serum samples from patients with rRT-PCR-confirmed SARS-CoV-2
Jin <i>et al.</i> / SARS-CoV-2 IgM and IgG chemiluminescence immunoassay (CLIA). All serum antibody tests were performed by iFlash3000 fully automatic chemiluminescence immunoassay analyzer from Shenzhen YHLO Biotech Co., Ltd (China).	IgM	48.1%	ND	ND	100.0% / ND	27 patients with laboratory confirmed infection and at least one viral serological test (Laboratory confirmation of the virus was based on the result of real time rRT-PCR).
	IgG	88.9%	ND	ND	90.9% / ND	

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Table 1 (continued)

AUTHOR / METHODS USED	SARS-CoV-2 Test	SENSITIVITY			SPECIFICITY / ACCURACY	N
		Day after symptom onset				
		01 – 07	08 – 14	15 – 39		
Zhao <i>et al.</i> / Test for total antibodies, IgM and IgG against SARS-CoV-2 in plasma samples using enzyme linked immunosorbent assay (ELISA) kits supplied Beijing Wantai Biological Pharmacy Enterprise Co., Ltda.	Total antibodies	ND	89.6%	100.0%	99.1% / ND	173 cases of COVID-19 confirmed by rRT-PCR
	IgM	ND	73.3%	94.3%	98.6% / ND	
	IgG	ND	54.1%	78.9%	99.0% / ND	
	RNA by rRT-PCR	ND	66.7%	45.5%	ND	
Guo <i>et al.</i> / Western blot and ELISA assays to evaluate the potential cross-reactivities between SARS-CoV-2 and other human coronavirus.	rRT-PCR CONFIRMED CASES	IgM	75.6%	ND	ND	208 blood samples from 2 cohorts: 39 patients confirmed positive for SARS-CoV-2; 101 inpatients; 135 plasma samples collected in 2018; 150 plasma samples from healthy adults in 2018-2019 (control); plasma samples positive for human CoV-229E, -NL63, -OC43, -NLU1 and SARS-CoV
	rRT-PCR NO CONFIRMED CASES	IgM	93.1%	ND	ND	
	IgM	85.4%	ND	ND	ND	
	IgA	92.7%	ND	ND	ND	
	IgG	ND	77.9%	ND	ND	
	rRT-PCR		ND		ND	
	rRT-PCR		98.6%		ND	
	+ Igs					

Table 2

POC tests and RDT for direct detection of SARS-CoV-2.

AUTHOR / METHODS USED	SARS-CoV-2 Test	SENSITIVITY			SPECIFICITY	N
		Days after symptom onset				
		01 – 07	08 – 14	15 – 39		
Huang <i>et al.</i> / (RT-LAMP) to achieve the detection of SARS-CoV-2 in nasopharyngeal secretions in 30 minutes (China)	Direct Test RNA	100%	ND	ND	100% / ND	16 clinic samples; with 8 positives (rt-PCR) and 8 negatives
Wang <i>et al.</i> / SARS-CoV-2 nucleic acid was detected using rRT-PCR (kit provided by Shanghai Zhijiang Biotechnology Co., Shanghai, China; detection instrument provided by Hongshi Biotechnology Co., Shanghai, China).	RNA by rRT-PCR	ND	ND	ND	ND	86 patients: 72 with IgM-positive sera to different diseases (Influenza A and B, <i>Mycoplasma pneumoniae</i> , <i>Legionella pneumophila</i> , HIV infection, hypertensive and diabetes mellitus) without symptoms of COVID-19, 14 COVID-19 positive patients. and 36 RF-IgM-positive serum samples
Wang <i>et al.</i> / GICA and ELISA were used for SARS-CoV-2 IgM detection (kit provided by Beijing Hotgen Biotechnology Co., Beijing, China, for GICA for ELISA). Optical density in ELISA plates using a Microplate Reader (PHOMO, Autobio Diagnostics Co., Zhengzhou, China).	Direct Test Structural antigens	67%	ND	ND	96% / ND	-
Mohanty <i>et al.</i> / STANDARD Q COVID-19 do SD Biosensor LFIA for the qualitative detection of SARS-CoV-2 specific antigens present in the human nasopharynx (South Korea)	Direct Test Structural antigens	ND	75%	ND	100% / 80.5%	251 = 122 men (48.6%) and 129 women (51.4%), aged 16-75 (average: 40.2 years)
Diao <i>et al.</i> / Detection of NP antigen with double antibody sandwich fluorescence immunochromatographic analysis technology	Direct Test Structural antigens	93,90%	ND	ND	100% / 96,1%	127 = 82 rt-PCR positives and 45 negatives. 53.5% were male and the median age was 38 years
Porte <i>et al.</i> / Fluorescence antigen rapid test kit Bioeasy 2019-Novel (2019-nCoV) with anti-SARS-CoV-2 IgY antibodies (fluorescence immunochromatographic assay) (Bioeasy Biotechnology Co., Shenzhen, China)	Direct Test Structural antigens	30%	ND	ND	100% / 50%	148 nasopharyngeal samples (RT-Qpcr: 42 negative and 106 positive). Median age of the study population was 57.5 (range: 0-94) with a sex ratio of 0.8 (64 men and 84 women)
Scohy <i>et al.</i> / COVID-19 Ag Respi-Strip is an immunochromatographic test with reagent tape designed to detect the SARS-CoV-2 antigen in nasopharyngeal secretions in 15 minutes (Coris BioConcept, Gembloux, Belgium)	Direct Test Structural antigens					

legionella, pneumophila, HIV, and chronic diseases. Only two studies evaluated cross-reaction for other coronaviruses [7,29].

4. Discussion

Serological tests are an essential tool for diagnosing of COVID-19; however, there is uncertainty about the accuracy of the point-of-care (POC) and rapid diagnostic tests (RDT), that are currently available. We, therefore, aimed to analyze the scientific literature published in this

area. Here, we overview of the rapid POC tests used to diagnose SARS-CoV-2 infection from 2,739 samples.

We identified 23 studies reporting on the use of POC tests and RDT for the diagnosis of COVID-19. We divided these studies into serological POC tests and RDT for detecting IgM and/or IgG antibodies against SARS-CoV-2 (Table 1), and POC tests and RDT for direct detection SARS-CoV-2 (Table 2). The reviewed studies used different quick diagnosis methods such as Lateral flow immunoassay (LFIA), chemiluminescence, enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay

Performance of SARS-CoV-2 rapid diagnostic tests

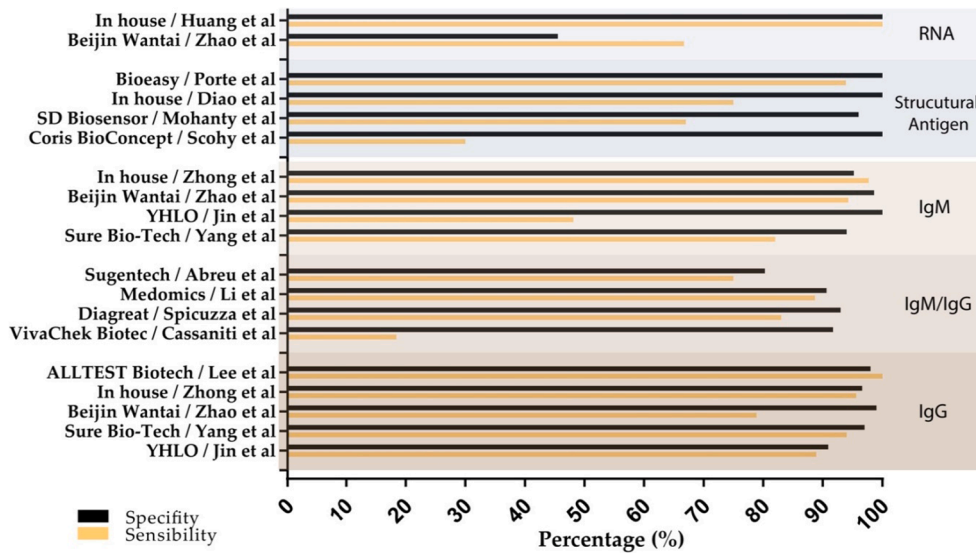


Fig. 2. Sensitivity and specificity of tests.

(EIA), RT-LAMP, rRT-PCR, colloidal gold immunochromatography assay (GICA), and fluorescence assays.

Despite the methodology applied, diagnostic tests should be requested at different stages of infection considering both its sensitivity and specificity [10-14,48] and its accuracy and chance of cross-reactions [35,36]. However, four serological studies included in this review [31,38-40] did not display any of the abovementioned parameters.

4.1. Specificity and sensitivity

The analytical specificity of a test is its ability to detect the intended target while not being affected by interfering substances under well-controlled laboratory conditions. The analytical sensitivity often describes the lowest amount of analyte that can be accurately measured through an assay. Adequate analytical specificity and sensitivity will, in the end, lead to optimal clinical performance.

Evidence on the diagnostic sensitivity and specificity for COVID-19 POC tests and RDTs was found, respectively, in 18 and 14 studies (Tables 1 and 2). Therefore, the sensitivity and specificity parameters together were assessed in 19 studies. Pooled sensitivities and specificities are shown in Fig. 2.

In respect of SARS-CoV-2 contagion test criteria, Zhao et al. [33] reported that in the period from 1 to 7 days after the advent of symptoms, the RT-PCR method has a sensitivity of 67%, while between 8 and 14 days, the sensitivity decreases to 54%, and from 15 to 39 days, it drops to 45%.

In comparison, IgM and IgG levels have a sensitivity of 29% and 19%, respectively, within 7 days of symptoms; 73% and 54%, from 8 to 14 days; and 94% and 89%, from 15 and 39 days. Thus, RT-PCR should be used as a diagnostic tool from 1 to 7 days of symptoms to obtain the best results [49,50], while serological assays for combined or isolated antibodies (IgG and IgM) present higher sensitivity after 15 days of symptoms [51].

From the 18 studies reporting POC/RDT sensitivity, only seven studies (two reporting direct detection and five identifying antibodies) reported good sensitivity, ranging from 90% to 100% (Fig. 2). This observation shows that although the POC/RDT has the advantage of producing results quickly, the sensitivity values vary depending on the type of test, the brand, and the lot; and for most tests found, the values are low.

Regarding the different methods, RT-LAMP exhibited 100%

sensitivity from 1 to 7 days, and ELISA showed a sensitivity increase for both IgG and IgM after 15 days of contagion [34]. The chemiluminescence technique seems to be more sensitive from day 8 to 14 of contagion, with a 97.7% sensitivity for IgM and 95.6% for IgG [34]. The enzyme-linked fluorescent assay (ELFA) showed a sensitivity of 18.4% on the first day of symptoms [34]; these data agree with Rong et al. [14], who suggested that this methodology is not capable of detecting antibodies early.

As for specificity, most of the studies presented values higher than 90% (Fig. 2).

4.2. Accuracy and Cross-reaction

Accuracy was reported in six studies, describing values above 50%, frighteningly low, and reinforces the lack of reliable information. Indeed, there is some uncertainty about the accuracy of COVID-19 POC tests. Most of them are produced in China and do not have Food and Drug Administration (FDA) approval. In addition, these tests have frequently been found to be inaccurate and nonspecific, with the occurrence of cross-reactivity with other viruses, such as influenza [52]. Only three studies described cross-reactions. They considered various diseases [7,28,42] such as mycoplasma, influenza, legionella, pneumophila, HIV and chronic diseases. Of these, only two evaluated cross-reaction for other coronaviruses [7,28].

Many tests can cross-react with other microorganisms, resulting in false-positive results [53]. A false-positive test may disrupt individuals' lives and contribute to a false impression about the disease with the consequent quarantine requirements [55,43,14,56,57]. Both false-positive and false-negative results can have devastating consequences in respect of this epidemic. A false-negative result will lead to undetected cases that propagate the epidemic.

5. Study limitations

In general, the limitations of the studies identified in this review were: low sample numbers (varying between 16 and 525 individuals); a lack of validation; and diverse clinical parameters [19,48]. Thus, we found significant variability and little scientific rigor in the data, and there is a clear need for more studies about this type of COVID-19 test to determine their reliability and safety for use in clinical practice.

5. Final Considerations

Block serial evaluations for symptom days seem to be urgently needed to confirm the sensitivity and specificity of rapid point-of-care tests, being subsequently validated by techniques such as immunochromatography, ELISA, chemiluminescence, and fluorescence [55,57]. Overall, the studies suggest that direct diagnostic tests are more sensitive and specific during the earlier phase of symptom onset (with best results between 1 and 3 days), and serological tests that detect IgM and IgG antibodies are more accurate after 15 days of contagion, with titers increasing according to symptoms evolution [35,43,56,55].

Moreover, due to the urgent need imposed by the COVID-19 pandemic, validation of POC tests/RDT should not be delayed, as they are a cornerstone for the extensive use of rapid tests. Nevertheless, questions on the quality of rapid COVID-19 tests remain, as there is a real lack of indisputable and conclusive evidence. Therefore, increasing the number of samples tested and comparing them with molecular techniques should be the key strategy for a better diagnosis, validation of rapid tests, and, consequently, technical decision-making by health-care workers. However, for excellent diagnostic reliability, a larger number of samples should be evaluated to validate rapid tests such as POC and RDTs.

To develop more sensitive and specific tests, companies must adopt criteria in selecting antigens used in their fabrication. These antigens must be specific and immunogenic and can be selected by bioinformatics tools. Similarly, the production of glycosylated antigens, such as the Spike protein, must be done in eukaryotic cells to preserve their antigenic characteristics.

For the development of direct test tests that detect viral components, it is fundamental to use monoclonal or polyclonal antibodies that have been tested for cross-reactivity to other pathogens. Especially in the case of tests for COVID-19, these antibodies and antigens should be tested for reactivity to antigens and antibodies of alphacoronavirus, as they are ubiquitous in the population and can generate false-positive results in not validated tests. Finally, this information should be present in the test inserts. The fact that they are not shown raises suspicions that these criteria were not used to manufacture the tests.

Regarding the ability of these tests to recognize new virus variants, there are already some works showing differences in the binding of monoclonal antibodies due to the existence of variants. However, even kits that use monoclonal antibodies to detect antigens will probably detect new variants because they accumulate mutations in various parts of the genome and not necessarily in the same target proteins of the test.

And if the kit uses polyclonal antibodies, the mutations present in some variants should not prevent binding to these antibodies either. In these kits, however, the use of polyclonal antibodies can impair the accuracy of the test. It occurs because the antibodies will bind to antigens that present epitopes of related viruses, such as Alpha coronavirus 229E and NL63, Beta coronavirus OC43, and HKU1, which share many epitopes in the protein spike with sarscov2.

In conclusion, our findings corroborate the need for further studies to determine the best rapid testing method. These studies are pivotal to establishing rapid tests as an essential surveillance tool against the SARS-CoV-2 epidemic. We also found that the citizen tests in this article were released to the market without being adequately validated and did not show information about cross-reactivity with other pathogens, especially against other coronaviruses. All of these tests and the technological advances employed in them, and the pandemic lessons could be very useful in developing new tests for diagnosing other diseases and pathogens.

Author contributions

All authors have read and agreed to the published version of the manuscript. LP was responsible for the conception and design of the manuscript; AL, BM, BP, RL, DR, MG and PC analyzed and interpreted the data and completed the draft; MR, JS and LJ carried out a critical review of intellectual content; and HDMC and NM contributed with the

final draft of the manuscript and with resources for the research.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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