Rapid Serological Tests for SARS-COV-2: Diagnostic Performance of Four Commercial Assays

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Research Article

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Abstract

Background. This study aimed to assess the diagnostic performance of lateral flow immunochromatographic assays (LFA) of four different manufacturers to identify SARS-CoV-2 antibodies (IgM, IgG or total), comparing them with the nucleic acid amplification test (NAAT) or clinical defined (definite or probable SARS-CoV-2 infection respectively).

Methods. 119 serum samples were randomly selected by convenience and distributed in the groups: (1) Group with SARS-CoV-2 infection [n=82; RT-qPCR positive (*definite, n=*70), and probable (n=12)]; (2) other diseases [n= 27; other viruses identified (n=8), SARS of other etiologies (n=19)]; (3) healthy control group (n=10). LFA essays of four manufacturers were compared: MedTest Coronavírus (COVID-19) IgG/IgM (MedLevensohn, Brazil); COVID-19 IgG/IgM ECO Test (Ecodiagnóstica, Brazil); Camtech COVID-19 IgM/IgG Rapid Test Kit (Camtech Diagnostics Pte Ltd, Singapore); and one Step COVID-19 Test for total antibodies (Guangzhou Wondfo Biotech Co, China).

Results. The four tests studied showed high diagnostic performance characteristics for the diagnoses of definite or probable SARS-CoV-2 infection. The best measures were for the Wondfo test: sensitivity (86.59%; 95%CI, 77.26-93.11%); specificity (100%; 90.51-100%); DOR (257; 60-1008); LR+ (33.43; 4.82-231.85); LR– (0.13; 0.08 - 0.23); accuracy (90.76%; 84.06- 95.29%); Matthews Correlation coefficient (MCC) 0.82. Although considering only the probable SARS-CoV-2 infection (PCR-) cases, all the kits studied showed limited values.

Conclusion. Our data demonstrate the excellent performance of LFA for the diagnoses of definite or probable SARS-CoV-2 infection. There was substantial heterogeneity in sensitivities of IgM and IgG antibodies among the manufacturers. LFA tests cannot replace molecular diagnostics, but should be used as additional screening tool.

Introduction

The World Health Organization (WHO) declared the severe acute respiratory syndrome-related *coronavirus (*SARS-CoV-2*)* outbreak as a world pandemic on March 12th 2020. In Brazil, the first case was described at the end of February 2020. After that, the disease extended to all regions, presenting a heterogeneous epidemiological pattern, with high morbidity and mortality and a devastating effect on the public health system of the country. Currently (September 11, 2020), six months after the pandemic was declared by the WHO, there were 900,000 deaths in the entire world. In Brazil, 4,197,000 cases were notified, with 128,000 deaths associated.

The SARS-CoV-2 pandemic presents important diagnostic challenges [1]. An accurate diagnosis is fundamental. Moreover, hospitals would benefit from rapid detection of this virus infection in individuals who are admitted to hospitals with respiratory symptoms and suspicion of SARS-CoV-2 infection [2].

The diagnosis of SARS-CoV-2 infection involves collecting the correct specimen from the patient at the right time [3].

The molecular diagnosis is considered the gold-standard. It is made by searching viral RNA in respiratory samples: combined swab (nasopharynx and oropharynx), nasopharyngeal aspirate, bronchoalveolar lavage or sputum.

The large variety of available serological tests requires proper validation before deciding on the development of assays for specific applications [3]. Since the beginning of the pandemic, medical companies and research institutes have been working on the development and approval of tests to detect current viral infection and immunity to SARS-CoV-2 [4].

Serology tests are comparatively easier to perform, but their utility may be limited by the performance and the fact that antibodies appear later during the disease course [5]. Serology tests to detect the presence of antibodies to SARS-CoV-2 aim to identify previous SARS-CoV-2 infection, and may help to confirm the presence of current infection [1]. SARS-CoV-2 serology testing relies on targeted antibodies binding to SARS-CoV-2 specific antigens. Its greater technical ease, speed, possibility of application in existing laboratory networks and less need for technical expertise resulted in a greater search for such tests. Consequently, there has been commercialization of several diagnostic kits in the country. Among the methodologies for serological diagnosis, there was a great availability of rapid immunochromatographic tests, which are mostly being commercialized without an appropriate validation process. These tests require less technical expertise and equipment compared to nucleic acid detection. The samples are blood that can be collected in tubes, which poses less potential risk to the staff handling the samples. The tests can be performed in a basic clinical laboratory and in smaller community settings, reaching, therefore, a wider public. However, their utility may be limited by the performance issues of rapid tests in general, and the fact that antibodies appear later during the disease course [5].Therefore, numerous lateral flow immunochromatographic assays (LFAs) to detect the presence of IgM, IgG or total antibody (Atb) against SARS-CoV-2 have been introduced onto the market, and some countries have stocked up on such rapid tests.

This study aimed to assess the diagnostic performance of LFAs of four different manufactures and compare them with the nucleic acid amplification test (NAAT, definite) or the clinical defined test (probable) in individuals with SARS-CoV-2 infection. Tests were selected based on their availability and approval by the National Health Surveillance Agency (ANVISA) in Brazil at the time of the study.

Material And Methods

The samples consisted of serum specimens sent to the Virology Laboratory, Hospital de Clínicas, Universidade Federal do Parana (HC-UFPR), Brazil, a tertiary care academic center for diagnostic purposes. Immunological and RT-qPCR for SARS-CoV-2 assays were performed in the HC-UFPR virology laboratory, which is certified by the Health Secretary of Paraná, Brazil.

This study was approved under a waiver of informed consent by the HC-UFPR institutional review board, Brazil.

Patients admitted to the HC-UFPR between March 1st and August 7th 2020 were considered eligible if they had respiratory symptoms that were suspicious of COVID-19. The performances of the four different LFAs were evaluated in serum samples obtained on corresponding dates that respiratory samples were collected for the performance of NAAT. A total of 119 serum or plasma samples were randomly selected for convenience, and distributed in the following groups (Figure 1):

Group with SARS-CoV-2 (COVID-19, n=82)

Definite **SARS-CoV-2** infection, RT-qPCR positive (n=70)

Serum samples from COVID-19 patients, who tested positive for SARS-CoV-2 on RT-qPCR from nasopharyngeal samples. Participants admitted to the hospital COVID-19 unit or intensive care unit (ICU), n=60 (86%); outpatients (n=10; 14%); male 38 (54%); median (IQR) of age was 50 (38; 58.5) years old; time after symptoms onset 17 (12; 23) days. Two participants (2.9%) were asymptomatic.

Probable **SARS-CoV-2** infection (n= 12)

Serum samples from participants who tested RT-qPCR negative for SARS-CoV-2 on nasopharyngeal samples, but fulfilled the World Health Organization (WHO) clinical diagnostic case definitions for SARS-CoV-2 [6] (Suppl table 1). All participants admitted in the hospital COVID-19 unit or intensive care unit (ICU); male 6 (50%); median (IQR) of age was 61.5(47.5, 74.5) years old; time after symptoms onset 11 (7.5; 19) days (*definite* vs. probable, p=0.046 and 0.118, respectively).

Group with other diseases (RT-qPCR for SARS-CoV-2 negative, n= 27)

Other viruses were identified in patients (n=8); RT-qPCR positive on a nasopharyngeal swab on a respiratory panel RT-qPCR test for other virus infections, Rhinovirus (n=6) and Coronavirus 229e/NL63 (1case); Epstain-Barr virus and Cytomegalovirus (EBV/CMV, n=1).

Severe acute respiratory syndromes (SARS), RT-qPCR for SARS-CoV-2 on respiratory samples were either negative and did not fulfill the WHO case definitions for SARS-CoV-2 [6] or other etiologies were identified (n= 19); the etiologies were: asthma, chronic obstructive pulmonary disease, endocarditis, pneumonia (2 cases), respiratory insufficiency, heart failure, hypoxia, pulmonary thromboembolism, stroke, cystic fibrosis (2 cases), viral infection, tuberculosis (2 cases), and bronchiectasis.

Median (IQR) of age was 54 (41, 76) years old; male 13 (48%) (*definite* vs. probable vs. other diseases, p=0.078 and 0.851, respectively); time after symptoms onset 4 (3; 8.5) days.

Group without disease (Healthy control group, n=10)

Ten serum samples from blood donors were collected in 2015 (HIV, HCV, HBV, HTLV I/II, syphilis, and Chagas disease negative). This group was not tested for SARS-CoV-2 by RT-qPCR as the samples were taken before the emergence of the virus in China [6].

Lateral flow immunochromatographic assays (LFAs)

All essays were based on the colloidal gold-labeled immunochromatography (GICA) principle and the one-step method with results obtained within 15 minutes, using serum or plasma samples. The kits use capture reaction to detect SARS-CoV-2 IgM/IgG or total antibody in the samples.

IgG, IgM, and Total antibodies for SARS-CoV-2.

The MedTest Coronavírus (COVID-19) IgG/IgM (MedLevensohn, Brazil), the COVID-19 IgG/IgM ECO Test (Ecodiagnóstica, Brazil), and the Camtech COVID-19 IgM/IgG Rapid Test Kit (Camtech Diagnostics Pte Ltd, Singapore) detect IgM and IgG. According to the manufacturer, the diagnostic characteristics of the tests are as follows: Eco test IgG (sensitivity, 95%; specificity, 99%; PPV 95%; NPV 100%); IgM (sensitivity, 90%; specificity,94%; PPV 89%; NPV 94%); MedTest IgG (sensitivity, 95.74%; specificity, 99.3%; PPV 97%; NPV 99%); IgM (sensitivity, 86.8%; specificity, 98.6%; PPV 94%; NPV 97%). The One Step COVID-19 Test (Guangzhou Wondfo Biotech Co.,China) kit detects antibodies of both IgM and IgG isotypes, without distinction, reactive towards SARS-CoV-2 antigens not specified by the manufacturer or in the literature [7]. According to the manufacturer, that test has 86.4% (95% confidence interval (CI) = 82.4–89.6%) sensitivity and 99.6% (95% CI = 97.6–99.9%) specificity.

Samples were tested in parallel in the four assays. The tests were performed at room temperature according to the manufacturer's instructions. For all tests, the recommended sample volume of 10µl serum was added to the specimen well on the individual test cassettes followed by the supplied buffer. The result was visually read after 10 minutes, by two researchers; in case of doubt, a third one checked it. For combination of the IgM and the IgG kit, the test card or cassette has two test lines (M and G lines) and a quality control line (C line). The M line was fixed with a monoclonal anti-human IgM antibody for detecting SARS-CoV-2 antibody; the G line was fixed with a reagent for detecting SARS-CoV-2 antibody; the C line was fixed with a quality control antibody. Any visible band for IgG, IgM or unspecified immunoglobulin was an indicative for a positive result. The test card of the Wondfo kit has only one test line (T line) and a quality control line (C line). This kit does not differentiate IgM or IgG, thus, results were interpreted as positive or negative for SARS-CoV-2 antibody.

RT-qPCR for SARS-CoV-2

The RT-qPCR for SARS-CoV-2 was composed of dual positive results from a single NAAT targeting two different SARS-CoV-2 genes. Samples were collected with a rayon swab, and transported immediately to the virology laboratory in a viral transport medium (VTM). Samples were taken from the oral cavity and subsequently from the nasal cavity using a nasopharyngeal rayon swab. We performed RT-qPCR using the XGEN-Master COVID-19 (XGEN) for qualitative detection of nucleic acid in RT-qPCR format-reverse transcription, followed by amplification of a conserved region of the ORF1ab and N genes for SARS-Cov-2 [8], using specific primers and a fluorescence-labeled probe in respiratory samples. Specificity: 100% for SARS-CoV-2 (ORF1ab gene), 10 copies/reaction, with probability ≥ 95%. Sensitivity – SARS-CoV-2 (ORF1ab gene): 10 copies/reaction, with probability ≥ 95%. Sensitivity – SARS-CoV-2 (N gene): 50 copies/reaction, with probability ≥ 95%.

Statistical Analyses

The results were presented as the median (interquartile, IQR), number (n) and percentage, as appropriate. Categorical variables were compared between groups using the Fisher's exact test and continuous variables were compared using the Mann–Whitney or Kruskal–Wallis test for non-parametric data, as appropriate. We performed the comparison of concordance and discordance proportions of the different kits with the McNemar test for paired nominal data. Results were considered significant at the 5% alpha level.

Clinical Performance characteristics of the tests

We evaluated, for each kit, the clinical performance of the LFA for SARS-CoV-2 IgM/IgG or total antibody (index test) in *predicting the* SARS-CoV-2 infection*. The* RT-qPCR for SARS-CoV-2 was the reference method. We analyzed separately the diagnostic performance for the detection of IgM and IgG antibodies in each test. For the calculation of SARS-CoV-2 IgG clinical performance, we included samples from patients who presented symptoms onset ≥ 8 days. If an IgG result was positive before the eighth day, we considered it true positive (n= 75). For IgM, we included all the samples collected.

The following clinical performance measures were calculated: sensitivity; specificity; accuracy (efficiency); positive and negative predictive values (PPV, NPV); Youden index [9]; positive and negative clinical utility index (CUI+, CUI−). The CUI values were classified as follows: excellent, ≥0.81; good, ≥0.64; fair, ≥0.49; poor, ≤0.49; and very poor, ≤0.36 [10, 11]. We calculated the positive and negative likelihood ratio (LR+, LR−) and diagnostic odds ratio (DOR), in which a LR+ value ≥10.0 indicates that a positive test almost confirmed the disease, a value of ~6.0 indicates that the disease was present, and a value of ~1.0 indicates that the test was not able to show whether the disease was present or not. A LR+ value ≤0.1 indicates that the disease was practically absent [12, 13]. The higher the DOR value the better the test is. The Matthews correlation coefficient (MCC) is a value between −1 and +1. A coefficient of +1 represents a perfect prediction, 0, no better than random prediction, and −1 indicates total disagreement between prediction and observation.

As the Wondfo test detects total antibodies, in order to compare its results with other tests, we evaluated the clinical performance results of all kits considering any positive results (IgM or IgG), in a subsequent analysis, we evaluated them separately (IgM and IgG).

Positive rates and levels of agreement between the kits were assessed using Cohen's Kappa coefficients of agreement, which may be interpreted as follows: values ≤ 0 as indicating no agreement (i.e., purely random), 0.01–0.20 as none to slight, 0.21–0.40 as fair, 0.41– 0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1.00 as almost perfect agreement [14].

Results

The groups with definite and probable SARS-CoV-2 infection were comparable for time of symptoms starting, and for age, they were comparable with the group with other disease, although different for age on PCR- and PCR+ groups.

Clinical performance of LFAs

Overall, the analysis of the tests showed a good sensitivity; One Step Wondfo and MedTest presented the best results. In addition to the high sensitivity, they were the most accurate tests (table 1). Medtest and Wondfo presented higher sensitivity, specificity, and accuracy, with less extended 95% confidence intervals (Figure. 2).

IgG, IgM or total antibodies

For the diagnoses of definite or probable SARS-CoV-2 infection, the four tests studied showed high diagnostic performance characteristics. The higher sensitivity was for Camtech, the accuracy was similar for Med test, Camtech and Wondfo. Diagnostic specificity was 100% for the MedTest and Wondfo. LR+ was higher than 30 for the MedTest and Wondfo, indicating a positive test - almost confirming the SARS-CoV-2 infection, although it was limited for the EcoTest and Camtech. The highest DOR was seen in the Wondfo test.

For the PCR+ cases, the sensitivity and accuracy were higher for all the brands we studied.

Although for the probable SARS-CoV-2 infection (RT-qPCR negative), all the brands studied showed a limited value for total Atb, IgM and IgG, but a high specificity was seen for IgM and IgG.

The Wondfo and MedTest showed the best clinical diagnostic performance characteristics with a high LR (+), which indicates that a positive test almost confirmed the disease, and a high Youden´s index, DOR, accuracy and MCC (Table 1).

IgM

For the diagnoses of definite or probable SARS-CoV-2 infection, the IgM displayed a higher sensitivity and accuracy for the MedTest and Camtech. We observed a high LR+ for the MedTest only; for the other manufacturers, it was very low. The highest DOR, accuracy, Youden´s index and MCC were seen in the MedTest. All the brands showed high diagnostic specificity for IgM detection (Table 2).

IgG

The detection of the IgG sensitivity for SARS-CoV-2 infection was low, although it was specific; the MedTest and Cantech showed similar diagnostic performance characteristics. For definite SARS-CoV-2, the IgG had a very high sensitivity. The accuracy, Youden´s index and MCC were similar for the three kits (Table 3).

Asymptomatic participants

Two participants (2.9%) were asymptomatic and were positive for SARS-CoV-2 RT-qPCR from nasopharingeal samples; Wondfo was positive in both patients; however, only one of them tested positive for IgM in the MedTest and Camtech. The IgG was not positive in all assays performed. For the other participant tested positive for both IgM and IgG in all assays, but the Camtech test was positive only for IgG.

Analytical specificity (cross-reaction)

To calculate the analytical specificity of the LFAs, we considered 15 serum samples from the not SARS-CoV-2 group, in which other etiologies were identified. Eight samples contained other viruses, and seven samples from cases of clinical and laboratory investigation were compatible with bacterial infection (pneumonia, 3 samples: one community and two nosocomial pneumonia; pleural tuberculosis (Tb), 2 samples; sepsis associated with cystic fibrosis, 2 samples).

For the Wondfo and MedTest IgM and IgG and the Camtech IgG, no cross reaction was identified, the analytical specificity was 100%. However for EcoTest, the analytical specificity for IgG or IgM was 80% each. For IgM, 1 sample cross-reacted with Rhinovirus, and 2 samples with Tb. Concerning IgG, 1 sample cross-reacted with Rhinovirus, and 2 samples with sepsis).

For Camtech there was a cross-reaction of IgM with 4 samples (2 samples with Rhinovirus, one community-acquired pneumonia, and one pleural Tb). The analytical specificity was 73.30%.

Analytical interference

Analytical interference is defined as the effect of a substance present in the sample that alters the correct value of the result. There was hemolysis in one sample in the group with definite SARS-CoV-2 infection. This sample tested positive for IgM, IgG and total antibodies by all the four kits in the group with RT-qPCR positive.

Agreement between LFA kits

We observed a difference between the agreement and discordance proportion between the Wondfo and Camtech testes and between the Camtech and the MedTest (p= 0.0005 and 0.0001, respectively); all the other comparisons were not significant. Considering the clinical performance characteristics, Wondfo and MedTest worked better than Camtech. The agreement between LFA kits is shown in table 4.

Discussion

In this study, we report the performance characteristics of the LFA for IgG, IgM and total antibody anti SARS-CoV-2 from three different manufacturers for IgG and IgM and one for total antibody anti-SARS-CoV-2. We used sera from hospitalized adults patients with definite (RT-qPCR proved) and probable (clinical, epidemiologic, and radiologic criteria, with RT-qPCR negative) SARS-CoV-2 infection

Overall, the LFA to identify SARS-CoV-2 antibodies (IgM, IgG or total) in the definite and probable cases showed high sensitivity, specificity, and PPV. This indicates that a positive result is often seen in those with SARS-CoV-2 infection, and a negative result is often seen in those without the infection. The very high PPV that indicates a false positive is rare, which suggests this can be a potential confirmatory test. A high CUI+ suggested LFA are excellent for case finding, and a CUI- is good for screening. The overall value of this single test for combined screening and case finding is good. The clinical performance characteristics presented higher values considering only the definite

SARS-CoV-2 cases determined by positive RT-qPCR. However, for probable cases, the clinical performance characteristics showed limited values. Overall, the manufacturers, DOR and MCC of IgM were higher than IgG, and the diagnostic sensitivity and specificity were similar for IgG and IgM.

In the present study, the Wondfo test presented the best clinical performance characteristic, followed by the MedTest. Our findings are in accordance with previous studies, in which the Wondfo test was one of the two best-performing LFA of the ten evaluated, with pooled estimates of sensitivity (84.8%; 95% confidence interval (CI) = 81.4–87.8%) and specificity (99.0%; 95% CI = 97.8–99.7%) [15, 16]. However, the Wondfo test has the advantage of not differentiating between IgM and IgG. For IgM and IgG, the best results of clinical performance characteristic were with the MedTest. The Youden´s index, accuracy and MCC of IgG were similar for the three manufacturers.

Test performance characteristics as provided by manufacturers were similar with those observed in our study. In a meta-analysis of diagnostic test accuracy of commercial assays registered in Brazil, all cases that were confirmed by RT-qPCR, considering the diagnostic characteristics reported by the manufacturers, the following results were found: for the detection of IgM antibodies (eight tests; 951 samples), the pooled diagnostic accuracy measures (95%CI) included sensitivity (82%; 76–87), specificity (97%; 96–98), DOR (168; 92–305), LR+ (31.3; 19.7–49.7), LR− (0.19; 0.14–0.25), and summary receiver operating characteristic (SROC, 0.98; 0.96–0.99); for the detection of IgG antibodies (eight tests; 1503 samples), the pooled diagnostic accuracy measures included sensitivity (97%; 90–99), specificity (98%; 97–99), DOR (1994; 385–10334), LR+ (56.6; 30.6–104.7), LR− (0.03; 0.01–0.11), and SROC (0.99; 0.98–1.00) [17].

In the present study, the median time after the symptoms appeared was 11 and 17 days in the groups with definite and probable SARS-CoV-2. We observed that the positivity of immunological tests for SARS-CoV-2 diagnosis are time dependent. Patients with at least 8 days of symptoms presented a higher sensitivity [18, 19]. Antibody-mediated immunity in SARS-CoV-2 specific IgM and IgG are detectable in the serum between 7 and 14 days after the onset of the symptoms, respectively. The SARS-CoV-2 Virus RNA peak occurs at 3-5 days after exposure; virus RNA inversely correlated with neutralizing antibody titers. In the acute phase of the disease, nucleic acid detection of SARS-CoV-2 in respiratory samples was greater for antibody detection in the diagnosis of COVID-19 [18, 20, 21, 22, 23, 24]. After that period, tests that detect the presence of specific antibodies are recommended [25, 26]. Antibody tests could play a useful role in the detection of previous SARS-CoV-2 infection if applied 15 or more days after the onset of symptoms. The persistence of antibody rises is currently unknown, as there is very little data beyond 35 days post-symptoms onset [1] as well as about the extension of protection of neutralizing antibodies against subsequent infection with the virus [27]. The sensitivity of LFA was 11.1% from the 1st to the 7th day after the onset of the symptoms; 92.9% from the 8th to the 14th days and 96.8% from day 15 after the onset of the symptoms [28].

Our study will add to the previous ones as SARS-CoV-2 infection cases were based on RT-qPCR and clinical radiologic criteria, i.e. definite and probable SARS-CoV-2 cases, respectively [6]; 15% of the samples included in the

SARS-CoV-2 group were probable infection. Although these cases fulfill the clinical and radiological criteria for SARS-CoV-2 infection, some cases could be miss enrolled. Both hospitalized and out participants were included. In this study, two participants were asymptomatic with a positive result of RT-qPCR from nasal samples, the LFT were positive on both serum samples. In spite of these results, more studies are necessary to define the diagnostic performance characteristics on asymptomatic cases. It would be of great importance to establish the value of LFT on population studies to define the behavior of the community or the population by the end of the quarantine, opening of schools, etc.

Confusion matrices (or error matrix) are routinely used to evaluate binary classification problems as clinical performance characteristics of a diagnostic test; however, a unified statistical rate that is able to correctly represent the quality of a binary prediction is not available [29]. Therefore, to overcome this, we used in our study the association of several performance metrics, as the Youden's Index, likelihood ratios, DOR, the clinical utility index, and the MCC.

LFA tests are simple and can provide a rapid confirmation of SARS-CoV-2 while being limited due to higher rates of false-negative results when collected in the early-phase of symptoms onset [17]. Antibody testing may therefore be relevant in the following settings: diagnosis of patients who seek medical attention later than seven days after the onset of the symptoms; contact tracing; determining potential immunity and risk of infection; and sero-epidemiological studies to understand the extent of SARS-CoV-2 spread [30]. Serological testing can detect past cases of SARS-CoV-2 for which reverse transcription-PCR (RT-q

PCR) testing was not performed or nasopharyngeal swab sampling resulted in false negatives. Serological tests require exceptional sensitivity and specificity in order to have an adequate positive predictive value, especially when seroprevalence is low [31]. To date, most SARS-CoV-2 serological tests on the market have inadequate performance characteristics to be used widely in population or in clinical testing [32].

There are some study limitations to consider. The main limitation is he small number of samples and the lack of blinding of the index test and reference standard. Our study included a comparison of four different LFA, but more tests are available on the market. We included the majority patients admitted in the hospital, which represents clinically more severe patients. Only 14% were outpatients, of these, only two were asymptomatic. Therefore, it is unclear if the results could be expanded to asymptomatic or oligosymptomatic persons or even used on serological community surveys. Other authors have already questioned the utility of these tests for seroprevalence surveys for public health management purposes [1]. Only adults were included; reports on the dynamics and detection of SARS-CoV-2 antibodies in children are lacking and require urgent attention. In the majority of the studies, sensitivity has mainly been evaluated in hospitalized patients; thus, it is unclear whether these tests are able to detect lower antibody levels usually seen in mild and asymptomatic COVID-19 patients [1].

Diagnosis of suspected cases is confirmed by nucleic acid amplification tests (NATs) with real-time PCR, using respiratory samples. NATs are the reference standard because of their high specificity, although sensitivity may

depend on the timing of the disease onset, sampling location, and severity of the illness [33]. However, NAT as a reference standard remains suboptimal as some cases of actual infections were missed due to false-negative RT-qPCR results [1]. In some patients, NATs were only positive in sputum and negative in nasopharynx, whereas the patients in this study were only tested from nasopharyngeal swabs. There is a proportion of 2/3 of patients with strongly suggestive clinical manifestations, but the molecular test does not detect the viral target. This may be due to the inadequate period of sample collection or limitation of the molecular test [25]. The best period to have the test results is between the 3rd and 4th days as they are time-dependent [25]. Other limitation of the RT-qPCR for the diagnosis of SARS-CoV-2 is the incorrect collection, transport, and processing of the samples [34], and the day of the samples collection [35, 36]. Besides this, it requires certified laboratories, expensive equipment, and trained technicians [17]. False-negative cases of SARS-CoV-2 are being increasingly reported. Laboratory diagnosis through RT-qPCR testing alone lacks adequate sensitivity to be recommended as the only valid criterion to confirm SARS-CoV-2 infection diagnosis. The association of the test with clinical, epidemiologic and radiologic criteria are recommended to increase the accuracy of diagnosis [37].

In conclusion, our data demonstrate excellent performance of the antibodies assays studied. We observed substantial heterogeneity in sensitivities of IgM and IgG antibodies between manufacturers. The high specificity of LFAs may contribute to rapidly confirm the presence of SARS-CoV-2 infection and accelerate decision-making in emergency rooms and routing to appropriate hospital wards. Nonetheless, these LFA tests cannot replace molecular diagnostics in acute-care settings, but should only be used as an additional screening tool when the improvement of hospital logistics is expected and their limitations are carefully considered.

List Of Abbreviations

WHO- World Health Organization

SARS-CoV-2- severe acute respiratory syndrome-related *coronavirus*

LFA- lateral flow immunochromatographic assay

Atb- antibody

NAAT- nucleic acid amplification test

HC-UFPR- Hospital de Clínicas, Universidade Federal do Parana

GICA- *Gold Immunochromatography* Assay

EBV- Epstain-Barr virus and

CMV- cytomegalovirus

Tb- tuberculosis

CSF- cerebrospinal fluid

CNS- central nervous system

PPV- positive predictive value

NPV- negative predictive value

TP- true positive

TN- true negative

FP- false positive

FN- false negative

CUI+ - clinical utility index positive

CUI- - clinical utility index negative

LR+ positive likelihood ratio

LR- negative likelihood ratio

DOR- diagnostic odds ratio

K- Kappa statistic

WBC- white blood cells

RBC- red blood cells

TP- total proteins

GL- glucoses

LA- lactic acid

Standards for Reporting of Diagnostic Accuracy Studies (STARD)

Declarations

This study was approved under a waiver of informed consent by the HC-UFPR institutional review board, Brazil.

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Ethical approval: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the HC-UFPR institutional Review Board, Brazil.

Informed consent: Informed consent was waived the Institutional Review Board.

Data statement: The data is available to access under request to the corresponding author after approval of the IRB.

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Tables

Table 1. Clinical performance characteristics of LFA in serum to detect SARS-CoV-2 antibodies

IgM and/or IgG	MedTest		EcoTest		Camtech		Wondfo	
PCR+ PCR-	**Value**	**95% CI**	**Value**	**95% CI**	**Value**	**95% CI**	**Value**	**95% CI**
TP	69		67		76		71	
Sensitivity (%)	84.15	74.42-91.28	81.71	71.63-89.38	92.68	84.75-97.27	86.59	77.26-93.11
Specificity (%)	100.00	90.51-100.00	78.38	61.79-90.17	78.38	61.79-90.17	100.00	90.51-100.00
LR(+)	32.50	4.68 -225.50	3.78	2.03-7.04	4.29	2.31-7.94	33.43	4.82-231.85
LR(-)	0.16	0.10 -0.26	0.23	0.14-0.38	0.09	0.04-0.21	0.13	0.08 - 0.23
DOR	203	46.80-867	16.43	14.5-18.53	47.67	57.75-37.81	257.15	60.25- 1008
Youden's Index	0.842	0.649-0.913	0.601	0.334-0.796	0.711	0.465-0.874	0.866	0.678-0.931
CUI+	0.841	0.767-0.916	0.730	0.635-0.825	0.839	0.765-0.912	0.866	0.797-0.935
CUI-	0.740	0.657-0.823	0.517	0.400-0.634	0.649	0.537-0.762	0.771	0.690-0.851
PPV(%)	100.00		89.33	81.81-93.98	90.48	83.68-94.62	100.00	
NPV (%)	74.00	63.35-82.41	65.91	54.28-75.90	82.86	68.71-91.41	77.08	66.00-85.36
Accuracy (%)	89.08	82.04-94.05	80.67	72.42-87.34	88.24	81.05-93.42	90.76	84.06-95.29
MCC	0.79		0.57		0.72		0.82	
PCR+								
TP	68		65		69		69	
Sensitivity (%)	97.14	90.06-99.65	92.86	84.11-97.64	98.57	92.30-99.96	98.57	92.30-99.96
Specificity (%)	100.00	90.51-100.00	78.38	61.79-90.17	78.38	61.79-90.17	100.00	90.51-100.0
LR(+)	37.37	5.40 -258.87	4.29	2.32-7.96	4.56	2.47- 8.43	37.92	5.48-262.56
LR(-)	0.03	0.01-0.11	0.09	0.04-0.22	0.02	0.00- 0.13	0.01	0.00-0.10
DOR	1245	540-2353	47.66	58-36.18	228	0-64.85	3792	0-2625
Youden's	0.9714	0.8057-	0.7124	0.459-	0.7695	0.541-	0.986	0.828-

Index		0.9965		0.878		0.901		0.999
CUI+	0.971	0.937-1.000	0.827	0.745-0.908	0.883	0.817-0.949	0.986	0.961-1.000
CUI-	0.949	0.905-0.992	0.669	0.558-0.780	0.748	0.658-0.857	0.974	0.942-1.000
PPV(%)	100.00		89.04	81.43-93.77	89.61	82.35-94.10	100.00	
NPV (%)	94.87	82.52-98.64	85.29	71.02-93.21	96.67	80.44-99.51	97.37	84.09-99.62
Accuracy (%)	98.13	93.41-99.77	87.85	80.12-93.37	91.59	84.63-96.08	99.07	94.90-99.98
MCC	0.96		0.73		0.81		0.98	
PCR-								
TP	01		02		07		02	
Sensitivity (%)	8.33	0.21-38.48	16.67	2.09-48.41	58.33	27.67-84.83	16.67	2.09-48.41
Specificity (%)	100.00	90.51-100.00	78.38	61.79-90.17	78.38	61.79-90.17	100.00	90.51-100.00
LR(+)	5.57	0.55-56.78	0.77	0.19-3.14	2.70	1.24-5.87	8.36	0.95-73.88
LR(-)	0.92	0.77-1.09	1.06	0.78-1.44	0.53	0.27 -1.06	0.83	0.65-1.07
DOR	6.054	0.714-52.02	0.726	0.244-2.180	5.094	4.59-5.538	10.072	1.461-69.05
Youden's Index	0.083	-0.093-0.385	-0.050	-0.36-0.386	0.367	-0.105-0.75	0.168	-0.074; 0.484
CUI+	0.083	0-0.552	0.033	0.0-0.313	0.272	0.0-0.598	0.167	0.0-0.614
CUI-	0.771	0.690-0.841	0.583	0.467-0.699	0.669	0.558- 0.78	0.787	0.709-0.866
PPV(%)	100.00		20.00	5.77-50.49	46.67	28.67-65.57	100.00	
NPV (%)	77.08	73.93-79.96	74.36	68.14-79.72	85.29	74.41-92.04	78.72	74.18-82.65
Accuracy (%)	77.55	63.38-88.23	63.27	48.29-76.58	73.47	58.92-85.05	79.59	65.66-89.76
MCC	0.25		-0.05		0.34		0.36	

True positive (TP); positive predictive value (PPV); negative predictive value (NPV); positive likelihood ratio (LRP); negative likelihood ratio (LRN); diagnostic odds ratio (DOR, the higher the DOR value the better the test is.); clinical utility index positive (CUIP); clinical utility index negative (CUIN); Matthews Correlation coefficient (MCC)

Table 2. Clinical performance characteristics of LFA in serum to detect SARS-CoV-2 IgM antibodies

IgM	MedTest		EcoTest			Camtech
PCR+/PCR-	**Value**	**95% CI**	**Value**	**95% CI**	**Value**	**95% CI**
TP	68		48		72	
Sensitivity (%)	82.93	73.02- 90.34	58.54	47.12- 69.32	87.80	78.71- 93.99
Specificity (%)	100.00	90.51- 100.00	83.78	67.99- 93.81	81.08	64.84- 92.04
LR (+)	32.04	4.62 - 222.32	3.61	1.70- 7.68	4.64	2.37- 9.09
LR (-)	0.17	0.11- 0.28	0.49	0.37- 0.66	0.15	0.08- 0.27
DOR	188.47	42-794	7.367	4.595-11.636	30.933	29.625-33.667
Youden's Index	0.829	0.635; 0.903	0.423	0.151; 0.631	0.689	0.436; 0.860
CUI+	0.829	0.752; 0.907	0.520	0.392; 0.648	0.800	0.718; 0.882
CUI-	0.725	0.641; 0.810	0.400	0.294; 0.505	0.608	0.496; 0.720
PPV (%)	100.00	-	88.89	79.00- 94.45	91.14	84.01- 95.27
NPV (%)	72.55	62.12- 80.98	47.69	40.47-55.02	75.00	62.18- 84.55
Accuracy (%)	88.24	81.05- 93.42	66.39	57.15-74.78	85.71	78.12-91.45
MCC	0.78	-	0.39	-	0.68	-
PCR+						
TP	67		46		66	
Sensitivity (%)	95.71	87.98- 99.11	65.71	53.40- 76.65	94.29	86.01-98.42
Specificity (%)	100.00	90.51-100.00	83.78	67.99- 93.81	81.08	64.84-92.04
LR+	36.83	5.32- 255.17	4.05	1.91- 8.59	4.98	2.55- 9.73
LR-	0.04	0.01- 0.13	0.41	0.29- 0.58	0.07	0.03- 0.18
DOR	921	532-1962	9.87	6.59-14.81	71.14	85-54.05
Youden's Index	0.9571	0.785- 0.991	0.495	0.214- 0.705	0.754	0.509- 0.905
CUI+	0.957	0.915- 0.999	0.581	0.452- 0.710	0.852	0.777- 0.928
CUI-	0.925	0.874- 0.976	0.472	0.363- 0.582	0.715	0.612- 0.819
PPV (%)	100.00	-	88.46	78.33-94.21	90.41	82.84-94.85
NPV (%)	92.50	80.30-97.39	56.36	47.55-64.79	88.24	74.09-95.16
Accuracy (%)	97.20	92.02-99.42	71.96	62.45-80.22	89.72	82.35-94.76
MCC	0.94	-	0.47	-	0.77	-
PCR-						
TP	01		02		06	
Sensitivity (%)	8.33	0.21-38.48	16.67	2.09-48.41	50.00	21.09-78.91

Specificity (%)	100.00	90.51- 100.00	83.78	67.99-93.81	81.08	64.84-92.04
LR+	5.57	0.55- 56.78	1.03	0.24-4.43	2.64	1.10-6.34
LR-	0.92	0.77-1.09	0.99	0.74-1.33	0.62	0.34- 1.11
DOR	6.054	0.714-52.09	1.04	0.324-3.331	4.258	3.325-5.712
Youden's Index	0.083	-0.093- 0.385	0.005	-0.299 - 0.422	0.311	-0.141- 0.710
CUI+	0.083	0.0- 0.552	0.042	0.0-0.350	0.231	0.0- 0.57
CUI-	0.771	0.690- 0.851	0.633	0.526- 0.741	0.676	0.568- 0.783
PPV (%)	100.00	-	25.00	7.17- 58.98	46.15	26.33- 67.27
NPV (%)	77.08	73.93- 79.96	75.61	69.88- 80.56	83.33	73.55- 89.99
Accuracy (%)	77.55	63.38- 88.23	67.35	52.46- 80.05	73.47	58.92- 85.05
MCC	0.25	-	0.005	-	0.30	-

True positive (TP); positive predictive value (PPV); negative predictive value (NPV); positive likelihood ratio (LRP); negative likelihood ratio (LRN); diagnostic odds ratio (DOR, the higher the DOR value the better the test is.); clinical utility index positive (CUIP); clinical utility index negative (CUIN); Matthews Correlation coefficient (MCC)

Table 3. Clinical performance characteristics of LFA in serum to detect SARS-CoV-2 IgG antibodies

IgG	MedTest		EcoTest		Camtech	
PCR+/PCR-	**Value**	**95% CI**	**Value**	**95% CI**	**Value**	**95% CI**
TP	59		63		59	
Sensitivity (%)	78.67	67.68- 87.29	84.00	73.72- 91.45	78.67	67.68- 87.29
Specificity (%)	100.00	90.51- 100.00	89.19	74.58- 96.97	97.30	85.84- 99.93
LR+	30.39	4.37-211.13	7.77	3.06- 19.71	29.11	4.20- 201.92
LR-	0.21	0.14- 0.33	0.18	0.11- 0.30	0.22	0.14- 0.34
DOR	144	31.21-639	43.17	27.82-65.70	132.32	30- 594
Youden's Index	0.787	0.582- 0.873	0.732	0.483- 0.884	0.7597	0.535- 0.872
CUI+	0.787	0.696, 0.877	0.790	0.701-0.879	0.774	0.680-0.867
CUI-	0.698	0.611, 0.785	0.654	0.553-0.755	0.674	0.582-0.765
PPV (%)	100.00	-	94.03	86.13- 97.56	98.33	89.48- 99.76
NPV (%)	69.81	59.96-78.12	73.33	61.80- 82.38	69.23	59.22- 77.71
Accuracy (%)	85.71	77.84- 91.61	85.71	77.84- 91.61	84.82	76.81- 90.90
MCC	0.74	-	0.70	-	0.72	-
PCR+						
TP	58		62		57	-
Sensitivity (%)	87.88	77.51- 94.62	93.94	85.20- 98.32	86.36	75.69- 93.57
Specificity (%)	100.00	90.51- 100.00	89.19	74.58- 96.97	97.30	85.84- 99.93
LR+	33.84	4.88- 234.75	8.69	3.44- 21.97	31.95	4.61- 221.41
LR-	0.12	0.06- 0.23	0.07	0.03- 0.18	0.14	0.08- 0.26
DOR	282	81.33-1020	124	115-122	228	7.62-850
Youden's Index	0.8788	0.680- 0.946	0.8313	0.598- 0.953	0.837	0.615- 0.935
CUI+	0.879	0.806-0.952	0.882	0.812-0.953	0.849	0.768-0.930
CUI-	0.822	0.749-0.896	0.795	0.709-0.882	0.778	0.696-0.860
PPV (%)	100.00	-	93.94	85.98-97.51	98.28	89.16- 99.75
NPV (%)	82.22	70.72- 89.85	89.19	76.02- 95.55	80.00	68.50- 88.04
Accuracy (%)	92.23	85.27- 96.59	92.23	85.27- 96.59	90.29	82.87- 95.25
MCC	0.85	-	0.83	-	0.81	-
PCR-						
TP	01		01		02	
Sensitivity (%)	11.11	0.28- 48.25	11.11	0.28- 48.25	22.22	2.81-60.01

Specificity (%)	100.00	90.51- 100.00	89.19	74.58- 96.97	97.30	85.84- 99.93
LR+	7.09	0.71- 71.11	1.03	0.13- 8.12	8.22	0.83- 80.97
LR-	0.89	0.71- 1.12	1.00	0.77- 1.29	0.80	0.56- 1.14
DOR	7.966	1-63.49	1.03	0.169-6.295	10.275	1.482-71.026
Youden's Index	0.111	-0.092- 0.483	0.003	-0.251- 0.452	0.195	-0.114- 0.599
CUI+	0.111	0.00- 0.645	0.022	0.00- 0.351	0.148	0.00- 0.635
CUI-	0.822	0.749- 0.896	0.718	0.622- 0.814	0.815	0.737- 0.892
PPV (%)	100.00	-	20.00	3.07- 66.38	66.67	16.88- 95.17
NPV (%)	82.22	78.59- 85.35	80.49	76.14- 84.21	83.72	78.32- 87.98
Accuracy (%)	82.61	68.58- 92.18	73.91	58.87- 85.73	82.61	68.58- 92.18
MCC	0.30	-	0.003	-	0.31	-

True positive (TP); positive predictive value (PPV); negative predictive value (NPV); positive likelihood ratio (LRP); negative likelihood ratio (LRN); diagnostic odds ratio (DOR, the higher the DOR value the better the test is.); clinical utility index positive (CUIP); clinical utility index negative (CUIN); Matthews Correlation coefficient (MCC)

Table 4. Cohen's *Kappa* coefficients and tests agreement for IgM, IgG and Total antibodies

	***Kappa* (95% CI)**	**agreement**	**p**
MedTest vs. Wondfo	0.965 (0.918; 1.013)	perfect	0.500
EcoTest vs. MedTest	0.754 (0.634; 0.875)	good	0.180
Camtech vs MedTest	0.730 (0.602; 0.858)	good	0.0001
Wonfo vs. EcoTest	0.752 (0.630; 0.874)	good	0.424
Camtech vs EcoTest	0.605 (0.451; 0.758)	good	0.078
Wonfo vs. Camtech	0.778 (0.659; 0.897)	good	0.0005

Figures

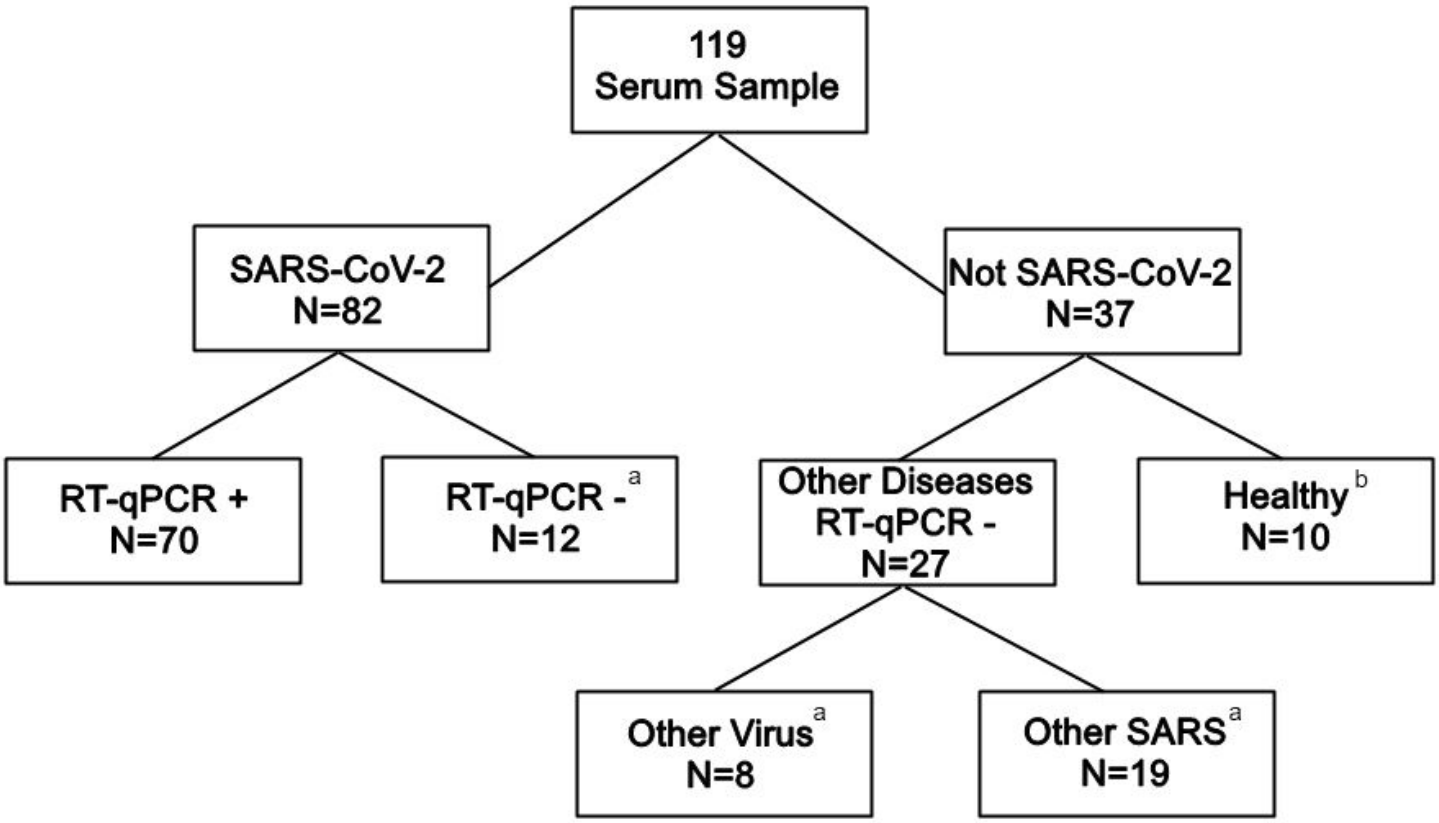

Figure 1

Standards for Reporting of Diagnostic Accuracy Studies (STARD) diagram of participants flow through the validation of lateral flow immunochromatographic assays (LFA) for identification of SARS-CoV-2 antibodies in serum samples; reference standard RT-qPCR for SARS-CoV-2 on nasopharyngeal swabs; and clinical, epidemiology, and radiologic criteria [6]. a. Participants who tested RT-qPCR negative for SARS-CoV-2 on nasopharyngeal samples. b. Participants were not tested for SARS-CoV-2 by RT-qPCR as the samples were taken before the emergence of the virus in China.

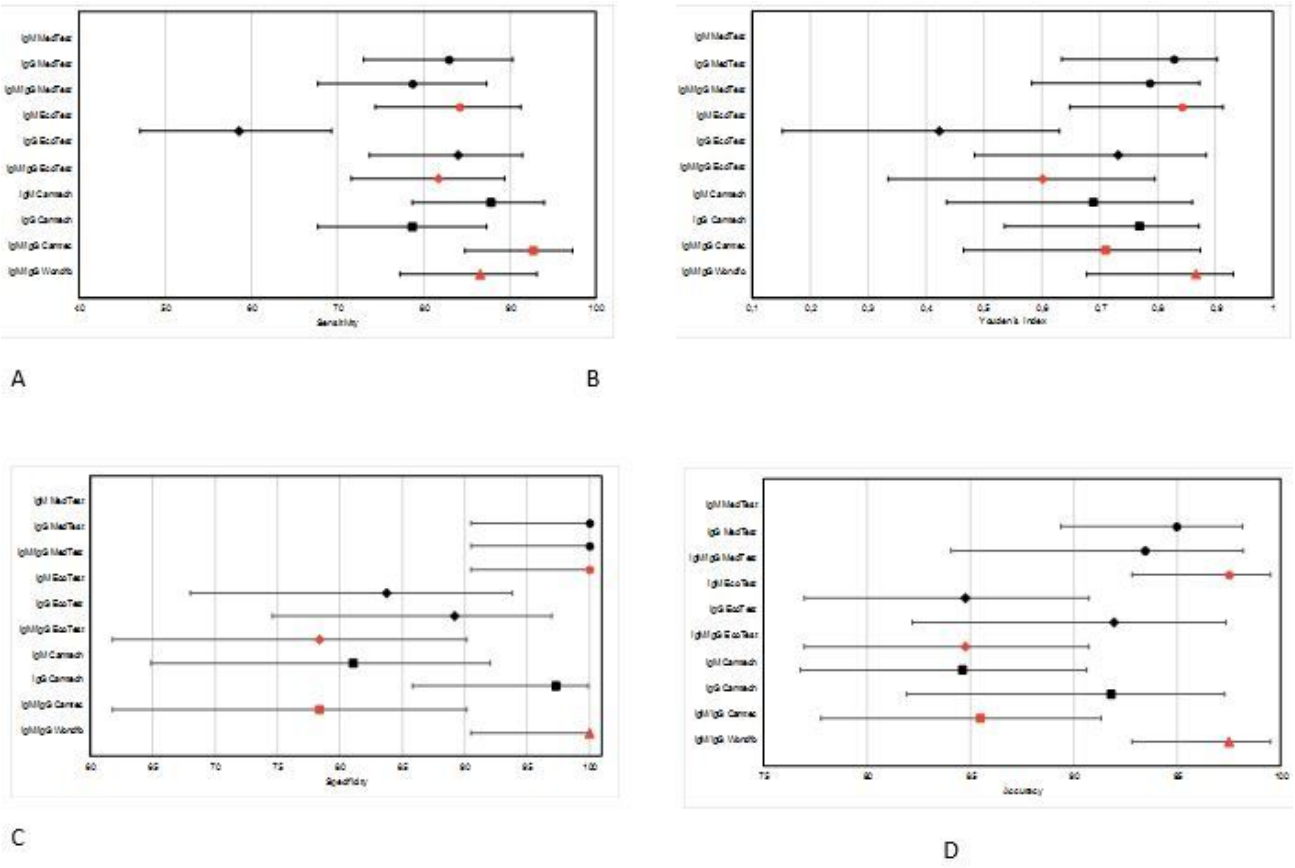

Figure 2

Comparison of lateral flow immunochromatographic assays (LFA) diagnostic performance for IgM, IgG, or total antibodies to diagnose SARS-CoV-2 infection.

Supplementary Files

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- Suppl.Table1.docx