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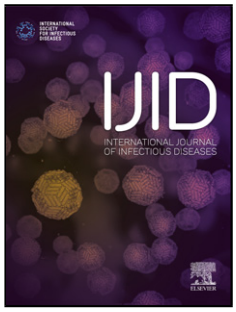

Diagnostic performance of commercially available COVID-19 serology tests in Brazil

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Running Title: Performance of serological tests for COVID-19 in Brazil

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Diagnostic performance of commercially available COVID-19 serology tests in Brazil

Highlights

- The performance of SARS-CoV-2 serological tests until the 14th day of symptoms is markedly low, and its use is not recommended at this stage;
- The performance of SARS-CoV-2 serological tests increases with the duration of symptoms and the clinical severity of the disease;
- In general, IgM and IgA antibodies were not earlier or more sensitive markers for the SARS-CoV-2 diagnosis, reaching their highest positivity rate after 14 days of the onset of symptoms;
- LFIA tests are more specific than ELISA tests for SARs-Cov-2 diagnostic;
- Infectious diseases prevalent in tropical regions, such as HIV, leishmaniasis, arbovirus and malaria may be related to false-positive results in the SARS-CoV-2 tests.

Abstract

Timely and accurate laboratory testing is essential to manage the global COVID-19 pandemic. Reverse transcription-polymerase chain reaction remains the gold-standard for SARS-CoV-2 diagnosis, but several practical issues limit the test use. Immunoassays have been indicated as an alternative for individual and mass testing. Objectives: To access the performance of twelve serological tests for COVID-19 diagnosis. Methods: We conducted a blind evaluation of six lateral flow immune assays (LFIAs) and six enzyme-linked immunosorbent assays (ELISA) commercially available in Brazil to detect anti-SARS-CoV-2 antibodies. Results: Considering patients with seven or more days of symptoms, the sensitivity ranged from 59.5% to 83.1% for LFIAs and from 50.7% to 92.6% for ELISAs. For both methods, the sensitivity increased with

clinical severity and days of symptoms. The agreement between LFIA performed in digital blood and serum was moderate. Specificity was, in general, higher for LFIAs than for ELISAs. Infectious diseases prevalent in the tropics, such as HIV, leishmaniasis, arboviruses and malaria, represent conditions with the potential to cause false positive results, which significantly compromises their specificity. Conclusion: The performance of immunoassays was only moderate, affected by the duration and clinical severity of the disease. Absence of discriminatory power between IgM/IgA and IgG has also been demonstrated, which prevents the use of acute phase antibodies for decisions on social isolation.

Keywords: antibody, COVID-19, performance, SARS-CoV-2, rapid test, serology, diagnosis, accuracy

INTRODUCTION

A novel corona virus (SARS-CoV-2) disease (COVID-19) was first identified in Wuhan City, Hubei Province, China, in December 2019, followed by an outbreak across the world. On March 12, World Health Organization (WHO) declared COVID-19 a global pandemic (1) and, four months later, more than 12 million cases and 550,000 deaths have already been reported worldwide. In July 2020, Brazil exceeded the mark of 1,800,000 cases notified (2), at the same time exceeding the total of 70,000 deaths confirmed by the disease, becoming the second country in the Americas with more cases, after the United States, and the epicenter of the pandemic in Latin America (3). Preventing transmission to control the spread of SARS-CoV-2, from symptomatic and asymptomatic individuals (4), is the main objective of any containment strategy. The approach of testing, tracking and tracing has become a central tool to achieve this

objective (5). However, the response to the coronavirus disease pandemic has been hampered by a lack of aggressive testing for the infection in several regions of world. To date, assays based on the reverse-transcription polymerase chain reaction (RT-PCR) in respiratory samples are the gold standard for COVID-19 diagnosis (6). This diagnostic strategy has been limited by significant logistics and capacity constraints, ranging from the short time of high viral excretion in respiratory secretions, availability of well-equipped laboratories, trained personnel, reagents, swabs used for the collection of nasopharyngeal specimens and personal protective equipment for health care providers collecting samples. Thus, RT-PCR is particularly challenging in resource-limited settings. Additionally, RT-PCR execution is relatively time consuming and highly dependent on the pre-analytical phase.

In this context, numerous immunological tests, based on antigens or antibodies detection and including point-of-care or conventional platforms, have recently become available and approved for use worldwide. The tests developed to detect SARS-CoV-2 antibodies are typically based on lateral flow immune assays (LFIAs), enzyme-linked immunosorbent assays (ELISAs) or chemiluminescent immunoassays (CLIAs). Unlike tests based on viral detection, whose diagnostic window is short and related to the period of viral excretion, serological tests would have the advantage of being longer-lasting markers of infection, which has classically been used as a tool in assessing the population dissemination of infections.

Currently, the available tests predominantly target antibodies to the main surface proteins of the novel coronavirus (7). In theory, the serological strategy, and a point-of-care approach based on rapid tests, would have the potential to significantly improve the current testing capacity for COVID-19. Serology is easier to perform, requiring less technical expertise and equipment and has a much lower unitary cost than RT-PCR

assays. The samples are blood collected in tubes or taken from digital pulp, which pose a lower potential risk to the health care staff. Serology can be performed in a basic clinical laboratory and community settings, thereby reaching a wider application. These potential advantages have been sufficient to encourage government from several countries, especially those with limited resources, and employers in the private sector, to acquire and use serological tests on a large scale during the COVID-19 pandemic, both as a diagnostic tool and as a marker of previous infection and guarantee of immunity. However, the serodiagnostic power of the specific IgM, IgA and IgG antibodies against SARS-CoV-2 remain largely uncertain, such as the relationship between the presence of antibodies and presence of immunity against re-infection (8). Although serologic tests contribute little to urgent decisions on social withdrawal and quarantine, from a public health perspective, serological analysis could be useful to estimate epidemiological variables, such as the attack rate and case fatality rate, which are necessary to assess the virus community transmission and its burden (9). Regardless of the intended use, the first stage of any decision on the implementation of serological tests in the context of the COVID-19 pandemic is the careful analysis of their performances over the various stages of the infection, in different specimens and their specificity, challenged in the face of other clinical conditions. Here, we aimed to describe the accuracy of serological assays for COVID-19 registered in Brazil up to May 2020, the comparative performance of rapid tests performed in digital whole blood and serum and between patients with severe and mild clinical manifestations, in addition to the positivity of the different antibodies among patients with less than seven days, between 7 and 14 days and more than 14 days after the onset of symptoms**.**

MATERIALS AND METHODS

Study Design

The panel-based study comprised 289 serum samples from 173 symptomatic patients with confirmed SARS-CoV-2 infection and 116 negative controls. All the cases (SARS-CoV-2 positive) were confirmed by RT-PCR testing of nasopharyngeal or oropharyngeal swabs and had their clinical condition and demography data compiled. The RT-PCR tests used for case confirmation were performed according to the protocols proposed by Center for Diseases Control/USA (10) or Charité Hospital/Germany (11), both accepted by World Health Organization (12). The negative control sera were all obtained before January 2020, the milestone of the introduction of the new coronavirus in Brazil, from patients with serological markers for other infectious or no infectious diseases. Only one sample per individual was included in this panel.

Source of samples and ethical approval

Sera from confirmed cases of SARS-CoV-2 infection were provided by the Minas Gerais State Department of Health, which is responsible for collecting and storing a biorepository since the beginning of the COVID-19 pandemic. Additionally, the performance of LFIAs performed in blood and serum was compared in a group of 32 patients who consented to be double tested. The SARS-CoV-2 RT-PCR confirmed cases included hospital patients and outpatients. For each case, information about the presence of acute respiratory distress syndrome (ARDS) presence, according to the definition adopted in Brazil ($\geq$ 30 breaths/min or an oxygen saturation $\leq$93% at rest), was registered. Negative control sera were collected before the emergence of the novel coronavirus in previous studies and were kindly provided by their legal guardian, with authorization from the Ethical Board of Instituto René Rachou, according to the

Brazilian legislation for research with humans. Only serum samples were available for the control group, for this reason, no control was submitted to SARS-CoV-2 RT-PCR. Due to the small volume of serum available from patients with malaria, samples from ten patients infected by *Plasmodium vivax* and nine patients with malaria by *Plasmodium falciparum* were pooled and included in the control. The main demographic and clinical characteristics of cases and the serological or parasitological markers that defined the diagnosis of the controls are available as supplementary material.

Ethics Approval

Ethics approval was obtained from the institutional review board of Instituto René Rachou, Fundação Oswaldo Cruz, CAAE: 30960120.0.0000.5091, approval numbers 4.001.133 and 4.128.388.

Sample calculation

The minimum sample required for this validation was estimated through one-sample proportion test, using Statistics/Data Analysis software (Stata), version 11.0. As premises were considered a power of 80% and significance of 5% to reproduce the sensitivity and specificity of tests with an expected binomial exact 95% confidence limits, based on the lowest performance rates reported by the manufacturers to ANVISA up to May 18, 2020 (86% for sensitivity and 98% for specificity). Based on that, it was defined as the minimum of 149 cases with seven or more days of symptoms and 116 controls. Additionally, the minimum number of 20 tests performed on digital blood and serum from SARS-CoV-2 confirmed patients was estimated as sufficient to identify a minimum difference of 20% between the sensitivity in the two clinical specimens. Two of the manufacturers involved in this validation provided fewer tests than requested, and

the evaluation was carried out with a proportional sub-group of case samples and negative controls, chosen randomly.

Research and selection of tests registered in Brazil

A search for diagnostics for COVID-19 was carried out with records in force at the Brazilian National Health Surveillance Agency (ANVISA) through the Agency's website (https://consultas.anvisa.gov.br/#/saude/). The search strategy was based on the terms “COVID 19”, “SARS”, “nCOV”, “COV” and “coronavirus” and was carried out on May 18, 2020. Sixty-seven serological tests registered in Brazil to diagnose SARS-CoV-2 were identified: 55 lateral flow immunoassays (LFIAs), six ELISA assays, four chemiluminescence and two immunofluorescence tests. Five manufacturers did not present any commercial contact information, thirty-eight manufacturers did not respond to contact, and other three refused to participate. All the companies responsible for producing the tests identified using the commercial contact available were invited to participate in this validation. By the end of June, nine companies had sent kits for validation and nine others were committed to donating the tests, if not yet received. Thus, twelve registered and commercially available serological tests for SARS-CoV-2 diagnosis were included in this analysis and their main characteristics are shown in Table 1. There were six LFIA and six ELISA tests.

Among the LFIA tests, only one exhibits a total antibody detection line, while in the other five the cassette display two test lines (M and G lines) and a quality control line (C line). All the kits used capture reaction to detect SARS-CoV-2 antibodies and were based on the colloidal gold-labeled immunochromatography principle and one-step method with results obtained within 10-30 minutes, using whole blood, serum or plasma samples. Briefly, the sample was absorbed by capillary action and mixed with the

SARS-CoV-2 antigen-dye conjugate. The conjugate binds to the antibodies present in the sample and, after adding the buffer, the antibody-conjugate complex migrates chromatographically across the membrane and finds the test region, in which the anti-human IgG and anti-IgM antibodies are immobilized, forming a colored line. The presence of this line indicates a positive result and its absence indicates a negative result. Among the ELISA tests, three were based on IgG detection, one on IgM, one on IgA and one on IgA and IgM indistinctly.

Samples preparation and tests execution

The serum samples were randomly coded and kept frozen at -70ºC until needed, and then was thawed for ten minutes at room temperature and homogenized before testing. The tests were carried out following each manufacturer's instructions strictly. To avoid comparison between tests, all the samples were submitted to a test before moving on to the next test. The reproducibility of the LFIA kits was assessed using the interpretation of the results by three independent observers using the Kappa index and the final result defined was that indicated by at least two of the three readers. For one of the LFIA kits, the result was obtained using a micro reader provided by the manufacturer. For LFIA blood-serum comparison, approximately 10% of the SARS-CoV-2 confirmed patients represented in this panel was consecutively recruited to donate capillary blood for testing, until reaching the minimum of 20 tests performed of each brand test.

Data Analysis

The performance parameters of interest were sensitivity, specificity and accuracy, defined as follows: i. sensitivity (S): proportion of positive tests among diseased individuals; ii. specificity (E): proportion of negative tests among non-diseased

individuals; iii. accuracy: the sum of true positives and true negatives among the total number of tests performed. Exact binomial confidence limits of 95% (95%CI) were calculated for each performance parameter individually by test and sensitivity was also stratified by time since symptom onset. The Kappa index was interpreted following the criteria of Landis and Koch (1977)(13) and interpreted as follows: < 0, no agreement; 0–0.2, slight agreement; 0.2–0.4, fair agreement, 0.4–0.6, moderate agreement; 0.6–0.8, substantial agreement; 0.8–1, almost perfect agreement. McNemar's test was used to determine the statistical differences between tests (all diagnostic tests were applied to a same set of samples). χ^2 test at a significance level of 0.05 was used to determine the statistical differences. Analyses were performed using SPSS version 23 and MedCalc statistical software version 19.4.

RESULTS

The patients whose serum samples were tested in this study were diagnosed with COVID-19 between April 21 and June 10, 2020, in Minas Gerais, Brazil. The age ranged from 22 to 96 years (median 47.5 years), and 52.6% were female. Regarding the length of symptoms, 25 patients had up to 6 days of symptoms (15%), 74 patients had between 7 and 14 days from the onset of symptoms (43%) and 74 patients had 15 days or more from the onset of symptoms. Among this latter group, 19 (26%) patients had between 31 and 60 days since the onset of symptoms and 13 (17.5%) had more than 2 months since the onset of symptoms. Fifty-nine percent of patients met the criteria for ARDS. Negative control serum was collected from the adult patients with a serological or parasitological marker for the following diseases: dengue, Zika, Chagas disease, syphilis, toxoplasmosis, viral hepatitis, malaria, visceral leishmaniasis, cytomegalovirus, Epstein Barr virus infection and HIV infection. Besides these, the

control panel also comprised 26 sera (22%) from patients under investigation for acute febrile illness or metabolic disease, without confirmation of an infectious condition. Tables 2 and 3 summarize the sensitivity by serological test and immunoglobulin class detected. For LFIA tests, the sensitivity for IgM ranged from 13.3% to 72.3% and that for IgG, ranged from 51.4 to 65.9%. For all except one LFIA tests, the sensitivity of the IgG detection alone was numerically higher than that observed for IgM band and for all of them, the highest detection rates were observed by combining the IgM and IgG results, ranging from 52.6% to 75.1% (Table 1). Among the ELISAs, a test based on the IgA/IgM detection exhibited the highest sensitivity (90.2%, 95%CI 84.9-93.8%). For IgG, the sensitivity for ELISAs ranged from 58.7% to 76.8% (Table 3). Considering only patients with seven or more days of symptoms, the sensitivity ranged from 59.5% to 83.1% for LFIA and from 50.7% to 92.6% for ELISAs (Table 4). As expected, the sensitivity for patients with less than seven days of symptoms was in general poor, up to 40% for all except one ELISA test. The sensitivity tends to increase with the number of days from the day of symptoms onset. However, there was substantial overlap between the sensitivity 95% confidence interval for the groups of confirmed cases with 7 to 14 days and more than 14 days of symptoms (Table 5).

For all LFIA tests and three of six ELISAs, the sensitivity was higher among patients presenting with ARDS than among those presenting with mild symptoms (Table 6). Except for one LFIA, which presents an exceptional low specificity (81%, 95%CI 72.9%-87.1%), the specificity of all other LFIAs was in general high, varying between 97.4% and 100% (Table 4). Overall, sensitivity for ELISA assays was higher than for LFIA. Excluding one IgG based ELISA test, which presented a sensitivity rate of 58.7%, the rates varied from 66.9% to 92.6%. By contrast, the specificity for ELISA tests was in general lower than that for LFIA, except for the same test presenting the

lowest sensitivity referred above, which exhibited the highest specificity among the ELISA tests (95.8%). Agreement between the results of LFIA performed in digital blood and serum varied markedly among different commercial kits, from perfect to only slight agreement (Table 7). Among the 116 control sera, only 21 did not show reactivity to any of the 12 tests evaluated, 53 were positive in one test, 27 in two tests, 10 reacted positively in three tests and five controls showed positive reaction in four different tests. The patients of control group whose sample reacted falsely in more than three different SARS-Cov-2 serological tests had serum markers to HIV, dengue, zica, Chagas disease, syphilis, toxoplasmosis or parasitological confirmation of visceral leishmaniasis or malaria.

DISCUSSION

This is the first study to assess comparatively the clinical performance of the serological tests available to diagnose SARS-CoV-2 in Brazil. Although some systematic reviews have already been published on the subject (14) (15), none has included data from Brazil, the current epicenter of the COVID-19 pandemic in Latin America. Local accuracy data based on real scenarios are essential considering the marked regional differences in the performance of the tests. In the case of SARS-CoV-2 serological tests, this information is especially relevant to the current reality of Brazil, a developing country that faces serious budgetary constraints and that has been performing sub-optimally in relation to its mass testing capacity. By contrast, several successful strategies implemented worldwide, such as aggressive testing and isolation, have promoted transmission control (16) (17). In this sense, the inverse association between testing capacity and mortality from COVID-19 has been consolidated as evidence of the impact of the isolation of those infected and the tracing and quarantining of their

contacts (3). The role of diagnostic testing and its impact on the community transmission are dependent on the types of tests available and on the logistical arrangements. RT-PCR based assays performed on respiratory specimens remain the gold standard for COVID-19 diagnosis. However, it is a time-consuming method limited by several practical issues, including relatively invasive sampling and the need for specialized operators and certified laboratories, making its use particularly challenging in resource-limited settings. Additionally, the test offers a narrow window of diagnostic opportunity, typically between the 4th and 6th day of symptom onset (18), coinciding with the peak viral load in the upper respiratory tract (19), further restricting its possibility of mass use. In this context, serologic immunoassays have been proposed as an alternative diagnostic tool for use during the acute and symptomatic phases. To date, many commercial companies have developed serological assays to detect SARS-CoV-2. These assays are mainly directed against two immunogenic targets: S protein, which is the most exposed viral protein, or its receptor-binding domain (RBD), or N protein, which is abundantly expressed (20). Despite the large number of tests approved for commercialization after a quick evaluation process in many countries, some sanitary authorities have recommended caution and conditioned authorization to validation of test performance on a national scale (21, 22). In general, when there is a second confirmatory test, sensitivity is the most desired parameter for screening. In Covid-19 pandemic scenario, both sensitivity and specificity are important parameters for screening. Contrary to this rationale, our results confirm that up to the 6^{th} day of symptoms, serological tests should not be used because of their extremely low sensitivity. This aspect limits the serology application in the stage where the greatest viral excretion is expected and, consequently, with the greatest risk of disease transmission. However, later serology testing has also been considered to diagnose

symptomatic cases not detected by RT-PCR or those who did not have access to this test. False negative RT-PCR would be expected, to an extent difficult to estimate (19), related to flaws in the swab collection process, conditions of storage/transport of the sample, variations in the viral load and excretion and the time of infection when the collection was performed. For this use, as a complementary test, several days after the onset of symptoms, it is important to know the serological tests performance along the disease's phases. Although the sensitivities of all serological tests tend to increase with the number of days of symptoms and with the clinical severity, based on the results presented here, the highest sensitivity rate observed after14 days of symptoms reaches only a moderate level, just over 90% for a few tests in the best scenario. Thus, in regions with SARS-CoV-2 prevalence below 10%, a reality in many regions of the world, the positive predictive value (PPV) of these tests remain below 80%, that is, these tests will produce around 20% of false-positive results if they have a very high specificity. Additionally, for tests with specificities lower than 95%, this moderate sensitivity will generate an even lower PPV, even with a disease prevalence above 20%. However, considering that a set of clinical manifestations could be used as disease suspicion criteria increasing the pre-test probability, in theory, immunoassays could play a complementary function to RT-PCR, enhancing COVID-19 detection sensitivity and accuracy (21), at rates that need to be established. An important observation presented here is a test performance in general lower than that described by others (24, 25). The main difference between this and those studies lies on the studied population. Here, approximately 40% of the cases did not meet the Covid-19 severity criterion adopted in Brazil while in most of the first studies only hospitalized patients were tested. Furthermore, the test's performance in the two groups stratified according to the clinical criterium was significantly different, reinforcing the link between clinical

severity and positivity in serological tests. Other factors as sample size issues and genetic specificities could also justify that difference. On the other hand, as a common result among validation studies, immunoassays still will produce delayed information, if the critical period of viral transmission is considered (26). For a more accurate performance, serological tests should be used after two weeks after symptoms onset in a context of high probability. Unlike PCR-based tests, serology cannot be used to confirm the presence of the SARS-CoV-2 virus, making its use limited for clinical decision-making or as a reinforcement in the recommendation for social isolation. There is even less evidence to support serological testing of asymptomatic individuals, as proposed for the screening of contacts of COVID-19 confirmed cases or in the supposed assessment of protective immunity. The antibody presence and circulating titers may exhibit behavior different from that observed in symptomatic infection. Assuming a similar performance of serological tests after a silent infection, and considering the performance reported here and the still low SARS-CoV-2 prevalence in general, except in few hotspots, we can expect many more false-positive than true positive results.

In relation to the choice between LFIA and ELISA tests, in addition to performance, logistics issues and total cost involved should be considered. To assist in this decision, cost-effectiveness analysis needs to be conducted and should guide more accurate decisions applied to different scenarios where pre-test probability or disease prevalence are estimated.

The inadequacy of the use of IgA and IgM antibodies as markers of contagiousness need to be highlighted. Our results revealed that both increase directly with the number of days of symptoms, reaching the highest rate among samples from patients with more

than 14 days of symptoms, a period in which the SARS-CoV-2 infectivity is considered low (27).

Specificity was homogeneously high for all LFIA tests, except for one test, assembled and packaged in Brazil but imported from a manufacturer based in the United States, where the kit had its FDA (Food and Drug Administration) license revoked in June 2020, in addition to more than 70 other tests, because of a poor performance detected by independent analyses (28).

Although low specificity was not a problem for most LFIAs, data for ELISAs differ significantly from that described in the package inserts of the tests and in relation to other accuracy studies carried out in China and in countries in the northern hemisphere. Infectious diseases prevalent in the tropics, such as leishmaniasis, arboviruses and malaria, were for the first time described as cause of false-positivity in tests for SARS-CoV-2, which raises concern about its use in these regions. On the other hand, there were few samples from patients with acute respiratory symptoms enrolled in the control group, which would be the real control for this validation. Even so, other studies contemplating samples from acute respiratory patients, including other endemic coronavirus confirmed cases, have revealed similar specificity rates (14, 29, 30). Additionally, another group of patients possibly prone to cross-reactivity are those with chronic autoimmune diseases, as indicated by some previous observations with SARS-CoV, an association still not confirmed for SARS-CoV-2 (31).

High specificity, in turn, is one of the most important properties required for a test to be used in epidemiological surveys. Estimation of the extent of the population that has already been infected in the community is essential to understand the spread of the epidemic and the main characteristics of the virus, its attack rate, its lethality and the impact of the various prevention and control interventions. These parameters would also

be useful to monitor the resumption of social and economic activities. A limiting factor, however, is the lack of knowledge of the longevity of these antibodies, an issue that will require studies with longer observation periods. In this panel, only 19 patients were between 31 and 60 days and another 13 had more than 60 days since the onset of symptoms. This small sample of evaluated patients does not allow us to confirm the lack of differences in the positivity rates of the tests over time.

Another limiting factor for the use of LFIA as a point-of-care test is the heterogeneity observed between the results of tests performed using fingerstick whole blood and serum, with the kappa varying from 0.2 to 1.0. This observation cautions the possibility of lower performance using blood, which is variable among kits, and an undeniable commitment to the more striking potential advantages of LFIA: agility and decentralization in mass testing. Few studies until now have addressed this issue because most have presented the results of tests performed solely on serum. In addition, the sample sizes evaluated were generally small and the results conflicting (30, 32).

Finally, the most controversial point regarding the use of immunoassays use is the lack of robust evidence of a correlation between circulating antibodies and acquired immunity (33), that is, whether the antibodies detected are protective for a significant period of time. Thus far, no definitive study has been conducted on the titers of neutralizing antibodies necessary for protection from SARS-CoV-2. However, there are several reports of new detection of the virus through molecular tests among individuals who again present signs and symptoms of the disease (34), and reports of the occurrence of new outbreaks in regions where the infection would have already reached high levels of exposure of the population. This casts doubt on the ability of viral exposure to produce protective immunity, and, consequently, on the role of immunoassays to

determine the immunity of health care workers and support the resumption of social activities and the re-start of economic sectors.

The accumulated experience with SARS-CoV, another coronavirus with strong genetic similarity to the current SARS-CoV-2, and involved in an outbreak in 2002, provides some insights on immunity. Specifically, the presence of antibodies has been extended for at least three years, being more intense among patients with the most severe forms, with progressive and significant reduction in the neutralizing antibody over time (35). Thus, at this point, any use of serology as a marker of immunity and criteria to allow or prevent the resumption of social life is only speculation and should not be recommended.

In summary, our observations revealed marked differences among the serological tests registered in Brazil. Generally, the sensitivity was only moderate, with insufficient performance for use before seven days of symptoms, as expected for a method based on the search for antibodies. The sensitivity rates reach around 80% to 90% for LFIA and ELISA after 14 days from the onset of symptoms, respectively, confirming that immunoassays are not suitable tools for screening SARS-CoV-2 virus infection in the general population, except for regions presenting high prevalence rates, over 20%. Specificity was better for LFIA than ELISA. These results also confirm the inadequacy of using immunoassays as a reference test in the validation of point-of-care tests. The increase in positivity with the time of symptoms even for the acute phase antibodies, IgM and IgA, confirm that their detection cannot be used as an indication of infectivity and evidence to support the quarantine recommendation. Studies addressing the local performance of immunoassay tests should contribute to the rational use of this diagnostic tool in the COVID-19 pandemic context. The lack of information about the antigen used prevents a deeper discussion about the reasons for the performance

differences among tests. This information, if was required by the health regulatory agencies, could contribute to the understanding of the role of various antigens and accelerate the development of new tests.

Of the three uses recommended so far, as a diagnostic method for acute cases, as a marker of immunity to allow the resumption of social life and as an instrument for measuring viral dissemination in epidemiological studies, only the last one seems to be justified. Seroprevalence can play an important role in the understanding of COVID-19 spread, however, to estimate the extent of the population that has already been infected in the community, the estimated prevalence rates need to be adjusted by test sensitivity and specificity. As an epidemiological tool, seroprevalence could still be helpful to assess the impact of different collective interventions on different demographics retrospectively. As a diagnostic tool for symptomatic patients, serology represents delayed information, greatly limiting its role in decision making. The inaccuracy of detecting IgM or IgA, as markers of active infectivity, was also confirmed by our results. Further data need to be gathered correlating antibody detection and protective immunity, besides the duration of protection.

CONCLUSION

Many questions remain unanswered regarding the value of serological testing in COVID-19 diagnosis and monitoring. These findings confirm the little usefulness of immunoassays for individual diagnosis up to seven days of symptoms and the relationship between sensitivity and time. Although attractive due to their lower cost and ease of execution, serological tests have the main disadvantage of a late positivity during the disease course. The sub-optimal performance of available serological tests for COVID-19 and the serum-blood reproducibility inconsistencies for LFIAs raises questions about

the usefulness of using such methods for medical decision making. Additionally, the detection of SARS-CoV-2 antibodies does not guarantee the protection against COVID-19 infection, because there is no confirmation that anti-SARS-CoV-2 antibodies are neutralizing antibodies. More studies addressing the cross-reactivity of SARS-CoV-2 antigens with other infectious diseases should be carried out. Serology assays may be a tool to study the seroepidemiology of COVID-19, and clinically validated serologic tests with good performance will provide a more accurate picture of the overall spread of COVID-19.

Author Contributions: Conceptualization: GC, DMA, MLF, CSS; methodology: GC and DMA; tests execution: DMA, MJP, LLA and MLF; formal analysis: GC, MLF, DMA, LLA; writing, original draft preparation: GC; writing, review and editing: GC, MF, CS, MP, JS, VF, LA, AR, DA; supervision: AR; project administration: CSS, VF, JWS, VF, MLF. All authors have read and agreed to the published version of the manuscript.

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Declaration of interests

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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References

1. World Health Organization (WHO). Coronavirus disease (COVID-2019) situation reports. 2020. Available at <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports>. Assessed on July 13, 2020.

2. BRASIL. Ministério da Saúde. Available at <https://coronavirus.saude.gov.br>. Assessed on July 13, 2020.

3. Max Roser HR, Esteban Ortiz-Ospina and Joe Hasell Coronavirus Pandemic (COVID-19). Published online at OurWorldInData.org. 2020. Retrieved from: https://ourworldindataorg/coronavirus> online resource. Assessed on July 13, 2020.

4. Lee S, Meyler P, Mozel M, Tauh T, Merchant R. Asymptomatic carriage and transmission of SARS-CoV-2: What do we know? Can J Anaesth. 2020;67(10):1424-1430. doi:10.1007/s12630-020-01729-x

5. World Health Organization (WHO). Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19). World Health Organisation, 2020. Assessed on July 13, 2020.

6. Tang YW, Schmitz JE, Persing DH, Stratton CW. Laboratory Diagnosis of COVID-19: Current Issues and Challenges. J Clin Microbiol. 2020;58(6).

7. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020;395(10224):565-74.

8. Wölfel Rea. Virological assessment of hospitalized patients with COVID-2019 Nature. 2020:pp. 1-10.

9. Lee CY, Lin RTP, Renia L, Ng LFP. Serological Approaches for COVID-19: Epidemiologic Perspective on Surveillance and Control. Front Immunol. 2020;11:879. doi:10.3389/fimmu.2020.00879.

10. Centers for Disease Control and Prevention. A CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. Available online: https://www.fda.gov/media/134922/download

11. Corman V, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu D, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance. 2020;25, 2000045.

12. World Health Organization. (2020). Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases: interim guidance, 2 March 2020. World Health Organization. https://apps.who.int/iris/handle/10665/331329

13. Landis JR KG. An application of hierarchical kappa-type statistics in the assessment of majority agreement among multiple observers. Biometrics 1977;33:363–74.

14. Deeks JJ, Dinnes J, Takwoingi Y, Davenport C, Spijker R, Taylor-Phillips S, et al. Antibody tests for identification of current and past infection with SARS-CoV-2. Cochrane Database Syst Rev. 2020;6:CD013652.

15. Lisboa Bastos M, Tavaziva G, Abidi SK, Campbell JR, Haraoui LP, Johnston JC, et al. Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. BMJ. 2020;370:m2516.

16. Lee VJ, Chiew CJ, Khong WX. Interrupting transmission of COVID-19: lessons from containment efforts in Singapore. J Travel Med. 2020;27(3).

17. Song JY, Yun JG, Noh JY, Cheong HJ, Kim WJ. Covid-19 in South Korea - Challenges of Subclinical Manifestations. N Engl J Med. 2020;382(19):1858-9.

18. To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis. 2020;20(5):565-74.

19. Kucirka LM, Lauer SA, Laeyendecker O, Boon D, Lessler J. Variation in False-Negative Rate of Reverse Transcriptase Polymerase Chain Reaction-Based SARS-CoV-2 Tests by Time Since Exposure. Ann Intern Med. 2020; May 13. doi: 10.7326/M20-1495.

20. Lee CY, Lin RTP, Renia L, Ng LFP. Serological Approaches for COVID-19: Epidemiologic Perspective on Surveillance and Control. Front Immunol. 2020;11:879.

21. Food and Drug Administration (FDA). Regulatory information. 2020. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/policy-coronavirus-disease-2019-tests-during-public-health-emergency-revised. Assessed on July 17, 2020.

22. United Kingdom (UK). Department of Health & Social Care. Coronavirus (COVID-19) scaling up our testing programmes. Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/878121/coronavirus-covid-19-testing-strategy.pdf. Assesses on July 17, 2020.

23. Liu R, Liu X, Yuan L, Han H, Shereen MA, Zhen J, et al. Analysis of adjunctive serological detection to nucleic acid test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection diagnosis. Int Immunopharmacol. 2020;86:106746.

24. Nicol T, Lefeuvre C, Serri O, Pivert A, Joubaud F, Dubee V, et al. Assessment of SARS-CoV-2 serological tests for the diagnosis of COVID-19 through the evaluation of

three immunoassays: Two automated immunoassays (Euroimmun and Abbott) and one rapid lateral flow immunoassay (NG Biotech). J Clin Virol. 2020;129:104511.

25. Khomer WS, Ruhl C, Ciesek S, Rabenau HF. Clinical performance of different SARS-CoV-2 IgG antibody tests. J Med Virol. 2020; 1-5.

26. Xiao AT, Tong YX, Gao C, Zhu L, Zhang YJ, Zhang S. Dynamic profile of RT-PCR findings from 301 COVID-19 patients in Wuhan, China: A descriptive study. J Clin Virol. 2020;127:104346.

27. Bullard J, Dust K, Funk D, Strong JE, Alexander D, Garnett L, et al. Predicting infectious SARS-CoV-2 from diagnostic samples. Clin Infect Dis. 2020.

28. Food and Drug Administration (FDA News). FDA Nixes Chembio's EUA for COVID-19 Antibody Test. Available at: <https://www.fdanews.com/articles/197693-fda-nixes-chembios-eua-for-covid-19-antibody-test>. Assessed on July17, 2020.

29. Premkumar L, Segovia-Chumbez B, Jadi R, Martinez DR, Raut R, Markmann A, et al. The receptor binding domain of the viral spike protein is an immunodominant and highly specific target of antibodies in SARS-CoV-2 patients. Sci Immunol. 2020;5(48).

30. Whitman JD, Hiatt J, Mowery CT, Shy BR, Yu R, Yamamoto TN, et al. Test performance evaluation of SARS-CoV-2 serological assays. medRxiv. 2020.

31. Wang Y, Sun S, Shen H, Jiang L, Zhang M, Xiao D, et al. Cross-reaction of SARS-CoV antigen with autoantibodies in autoimmune diseases. Cell Mol Immunol. 2004;1(4):304-7.

32. Li Z, Yi Y, Luo X, Xiong N, Liu Y, Li S, et al. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. J Med Virol. 2020.

33. GeurtsvanKessel CH, Okba NMA, Igloi Z, Bogers S, Embregts CWE, Laksono BM, et al. An evaluation of COVID-19 serological assays informs future diagnostics and exposure assessment. Nat Commun. 2020;11(1):3436.

34. Kang H, Wang Y, Tong Z, Liu X. Retest positive for SARS-CoV-2 RNA of "recovered" patients with COVID-19: Persistence, sampling issues, or re-infection? J Med Virol. 2020.

35. Manners C, Larios Bautista E, Sidoti H, Lopez OJ. Protective Adaptive Immunity Against Severe Acute Respiratory Syndrome Coronaviruses 2 (SARS-CoV-2) and Implications for Vaccines. Cureus. 2020;12(6):e8399

Table 1. The list of tests evaluated and their main characteristics

Test	Registration number	Manufacturer - country	Product batch	Storage temperature (°C)	Method	Sample volume	Specimen	Biomarker (antibody)
One Step COVID-2019 Test	80537410048	Guangzhou Wondfo Biotech CO., LTD. - China	W195004112 W19500458	2 to 30°C	Lateral flow immune assay	10µL	Blood, serum, plasma	Total IgG/IgM
TR DPP® COVID-19 IGM/IGG - Bio-Manguinhos	80142170039	Fundação Oswaldo Cruz - Brazil	204EXVD01Z	2 to 30°C	Lateral flow immune assay	10µL	Blood, serum, plasma	IgM and IgG
COVID-19 IgG/IgM ECO Teste	80954880132	Eco Diagnostica Ltda - Brazil	202005043	2 to 30°C	Lateral flow immune assay	20µL (blood), 10µL (serum or plasma	Blood, serum, plasma	IgM and IgG
COVID-19 IgG/IgM	80258020106	Qingdao Hightop Biotech CO., LTD. - China	COV1252004C	4 to 30°C	Lateral flow immune assay	20µL (blood) 10µL (serum or plasma	Blood, serum, plasma	IgM and IgG
Imuno-Rápido COVID-19 IgG/IgM	10310030208	Wama Produtos Para Laboratorio LTDA - Brazil	20E017	2 to 30°C	Lateral flow immune assay	20µL (blood), 10µL (serum or plasma)	Blood, serum, plasma	IgM and IgG
COVID-19 IgG IgM Gold Analisa Diagnóstica LTDA	80022230214	Gold Analisa Diagnóstica LTDA	200653	2 to 30°C	Lateral flow immune assay	20µL (blood), 10µL (serum or plasma)	Blood, serum, plasma	IgM and IgG

COVID-19 ELISA IgM+IgA	80263710088	Vircell Microbiologists - Spain	20ECOVMA107 20ECOVMA108	2 to 8°C	ELISA	5µL	Serum or plasma	IgM/IgA
COVID-19 ELISA IgG	80263710087	Vircell Microbiologists - Spain	20ECOVG107	2 to 8°C	ELISA	5µL	Serum or plasma	IgG
Allserum EIA COVID19 IgM	80047580200	Mbiolog Diagnosticos Ltda. - Brazil	4A20 and 5A20A	2 to 8°C	ELISA	10µL	Serum or plasma	IgM
Allserum EIA COVID19 IgG	80047580201	Mbiolog Diagnosticos Ltda. - Brazil	1B20	2 to 8°C	ELISA	10µL	Serum or plasma	IgG
Anti-SARS-CoV-2 ELISA (IgA)	10338930226	Euroimmun AG - Germany	E200507BE	2 to 8°C	ELISA	10µL	Serum or plasma	IgA
Anti-SARS-CoV-2 ELISA (IgA)	10338930227	Euroimmun AG - Germany	E200507AE	2 to 8°C	ELISA	10µL	Serum or plasma	IgA

Table 2. Sensitivity by lateral flow test and immunoglobulin class detected

Test	IgM			IgG			IgM or IgG		
	n	Positive	Sensitivity (95% CI)	n	Positive	Sensitivity (95% CI)	n	Positive	Sensitivity (95% CI)
One Step COVID- 2019 Test (Guangzhou Wondfo Biotech)	-	-	-	-	-	-	173	124	71.7 (64.3-78.2)
TR DPP® COVID-19 IGM/IGG Bio-Manguinhos (Fundação Oswaldo Cruz)	173	91	52.6 (45.2-59.9)	173	109	63 (55.6-69.8)	173	119	68.8 (61.5-75.2)
COVID-19 ECO IGM/IGG (Eco Diagnostica)	173	125	72.3 (65.2-78.4)	173	114	65.9 (58.6-72.6)	173	130	75.1 (68.2-80.9)
COVID-19 IgG/IgM (Qingdao Hightop Biotech)	173	39	22.5 (16.9-29.3)	173	89	51.4 (44.0-58.7)	173	91	52.6 (45.2-59.9)
Imuno-Rápido COVID-19 IgG/IgM (Wama Produtos Para Laboratorio)	173	70	40.5 (33.5-47.9)	173	108	62.4 (54.9-69.3)	173	119	68.8 (61.6-75.2)
COVID-19 IgG IgM (Gold Analisa Diagnóstica)	173	23	13.3 (9.1-19.2)	173	98	56.6 (49.2-63.8)	173	100	57.8 (50.4-64.9)

Table 3. Sensitivity by ELISA test and immunoglobulin class detected

Test	Antibody detected	n	Positive	Sensitivity (95% CI)
Covid-19 ELISA IgA+gM (Vircell Microbiologists)	IgA/IgM	173	156	90.2 (84.9-93.8)
Covid-19 ELISA IgG (Vircell Microbiologists)	IgG	173	133	76.8 (70.0-82.5)
Anti-SARS-CoV-2 ELISA IgA Euroimmun	IgA	109	83	76.1 (67.1-83.1)
Anti-SARS-CoV-2 ELISA IgG Euroimmun	IgG	109	64	58.7 (49.3-67.5)
Allserum EIA COVID-19 IgM (Mbiolog)	IgM	166	76	45.8 (38.4-53.4)
Allserum EIA COVID-19 IgG (Mbiolog)	IgG	166	100	60.2 (52.6-67.3)

Table 4. Sensitivity after seven days post-symptoms onset, specificity and global accuracy by test

Test	Method	Antibody detected	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy (95% CI)
One Step COVID- 2019 Test (Guangzhou Wondfo Biotech)	Lateral flow assay	Total IgM/IgG	79.7 (72.3-85.9)	100 (96.8-100)	88.6 (84.2-92.2)
TR DPP® COVID-19 IGM/IGG Bio-Manguinhos (Fundação Oswaldo Cruz)	Lateral flow assay	IgG + IgM	73.6 (65.9-80.0)	81.0 (72.9-87.1)	76.9 (71.5-81.6)
COVID-19 ECO IGM/IGG (Eco Diagnostica)	Lateral flow assay	IgG + IgM	83.1 (76.2-88.3)	99.1 (95.2-99.8)	90.1 (85.9-93.1)
COVID-19 IgG/IgM (Qingdao Hightop Biotech)	Lateral flow assay	IgG + IgM	59.5 (51.4-67.1)	100 (96.8-100)	77.3 (71.9-81.9)
Imuno-Rápido COVID-19 IgG/IgM (Wama Produtos Para Laboratorio	Lateral flow assay	IgG + IgM	75.0 (67.5-81.3)	97.4 (92.6-99.1)	84.8 (79.9-88.7)
COVID-19 IgG IgM (Gold Analisa Diagnóstica)	Lateral flow assay	IgG + IgM	64.9 (56.9-72.1)	98.3 (93.9-99.6)	79.5 (74.2-84.2)
Covid-19 ELISA IgA/IgM (Vircell Microbiologists)	ELISA	IgA	92.6 (87.2-95.8)	23.3 (16.5-31.8)	62.1 (56.1-67.70
Covid-19 ELISA IgG (Vircell Microbiologists)	ELISA	IgG	83.8 (82.7-92.9)	53.4 (47.4-59.3)	70.4 (64.6-75.6)
Anti-SARS-CoV-2 ELISA IgA (Euroimmun)	ELISA	IgA	82.9 (74.0-89.2)	82.2 (72.7-89.8)	82.6 (76.2-87.6)
Anti-SARS-CoV-2 ELISA IgG (Euroimmun)	ELISA	IgG	67.0 (56.9-75.7)	95.8 (88.5-98.5)	79.6 (72.8-85.0)
Allserum EIA COVID-19 IgM (Mbiolog)	ELISA	IgM	50.7 (42.6-58.8)	70.4 (61.2-78.2)	59.2 (53.0-65.2)
Allserum EIA COVID-19 IgG (Mbiolog)	ELISA	IgG	66.9 (58.8-74.1)	98.1 (93.4-99.5)	80.4 (75.0-84.8)

Table 5.: Sensitivity by serology test according to onset of symptoms timing

	<7 days Positive/total	**7-14 days Positive/total**	**>14 days Positive/total**	**Total Positive/total**
	% (95% CI)	**% (95% CI)**	**% (95% CI)**	**% (95% CI)**
One Step COVID- 2019 Test (Guangzhou Wondfo Biotech)				
IgM + IgG	6/25	56/74	62/74	124/173
	24.0 (11.5-43.4)	75.7 (64.8-84.0)	83.7 (73.7–90.4)	71.7 (64.3–78.2)
TR DPP® COVID-19 IGM/IGG Bio-Manguinhos (Fundação Oswaldo Cruz)				
IgM	7/25	38/74	46/74	91/173
	28 (14.3–47.6)	51.4 (40.2–62.4)	62.2 (50.8–72.4)	52.6 (45.2–59.9)
IgG	6/25	45/74	58/74	109/173
	24 (11.5–43.4)	60.8(49.4–71.1)	78.4 (67.7–86.2)	63.0 (55.6–69.8)
IgM + IgG	10/25	48/74	61/74	119/173
	40 (21.9–61.3)	64.9 (53.5–74.9)	82.4 (72.2–89.4)	68.8(61.5–75.2)
COVID-19 ECO IGM/IGG Teste (Eco Diagnostica)				
IgM	7/25	57/74	61/74	125/173
	28 (14.3–47.6)	77 (63.0–82.6)	82.4 (72.2–89.4)	72.3 (65.2–78.4)
IgG	5/25	44/74	65/74	114/173
	20 (8.9–39.1)	59.5 (48.1–69.9)	87.8 (78.4–93.4)	65.9 (58.6–72.6)
IgM + IgG	7/25	57/74	66/74	130/173
	28 (14.3–47.6)	77 (66.2–85.1)	89.2 (80.1–94.4)	75.1 (68.2–80.9)
COVID-19 IgG/IgM (Qingdao Hightop Biotech)				
IgM	2/25	16/74	21/74	39/173
	8 (2.2–24.9)	21.6 (13.7–32.2)	28.4 (19.4–39.5)	22.5 (16.9–29.3)
IgG	2/25	36/74	51/74	89/173
	8 (2.2–24.9)	48.6 (37.6–59.8)	68.9 (57.6–78.3)	51.4 (44.0–58.7)
IgM + IgG	3/25	37/74	51/74	91/173
	12 (4.2–29.9)	50 (38.9–61.1)	68.9 (57.6–78.3)	52.6 (45.2–59.9)
Imuno-Rápido COVID-19 IgG/IgM (Wama Produtos Para Laboratorio)				

IgM	5/25	32/74	33/74	70/173
	20 (8.7–39.1)	43.2 (32.5–54.5)	44.6 (33.8–55.9)	40.5 (33.5–47.9)
IgG	6/25	40/74	62/74	108/173
	24 (11.5–43.4)	54.1 (42.9–64.9)	83.8 (73.8–90.5)	62.4 (54.9–69.3)
IgM + IgG	8/25	47/74	64/74	119/173
	32 (17.2–51.6)	63.5 (52.1–73.6)	86.5 (76.9–92.5)	68.8 (61.6–75.2)
COVID-19 IgG IgM (Gold Analisa Diagnóstica)				
IgM	0/25	14/74	9/74	23/173
	0 (0-13.3)	18.9 (11.6-29.3)	12.2 (6.6–21.6)	13.3 (9.1–19.2)
IgG	4/25	39/74	55/74	98/173
	16.0 (6.4–34.6)	52.7 (42.9–64.9)	74.3 (62.9–83.8)	56.6 (49.2–63.8)
IgM + IgG	4/25	41/74	55/74	100/173
	16.0 (6.4–34.7)	55.4 (44.1–66.2)	74.3 (63.3–82.9)	57.8 (50.4–64.9)
Covid-19 ELISA IgA/IgM (Vircell Microbiologists)				
IgA + IgM	19/25	66/74	71/74	156/173
	76.0 (56.6–88.5)	89.2 (80.1–94.4)	95.9 (88.7–98.6)	90.2(84.9–93.8)
Covid-19 ELISA IgG (Vircell Microbiologists)				
IgG	9/25	55/74	69/74	133/173
	36.0 (20.3–55.5)	74.3 (63.3–82.9)	93.2 (85.1–97.1)	76.8 (70.0–82.5)
Anti-SARS-CoV-2 ELISA IgA (Euroimmun)				
IgA	5/15	36/47	42/47	83/109
	33.3 (15.1–58.3)	76.6 (62.8–86.4)	89.4 (77.4–95.4)	76.1 (67.3–83.1)
Anti-SARS-CoV-2 ELISA IgG (Euroimmun)				
IgG	1/15	22/47	41/47	64/109
	6.7 (1.2–29.8)	46.8 (33.3–60.8)	87.2 (74.8–93.9)	58.7 (49.3–67.5)
Allserum EIA COVID-19 IgM (Mbiolog)				
IgM	4/24	36/71	36/71	76/166
	16.7 (6.7–35.9)	50.7 (39.4–62.0)	50.7 (39.4–62.0)	45.8 (38.4–53.4)
Allserum EIA COVID-19 IgG (Mbiolog)				

IgG	5/24	39/71	56/71	100/166
	20.8 (9.2-40.4)	54.9 (43.4–65.9)	78.9 (68.1–86.8)	60.2 (52.6–67.3)

Table 6. Sensitivity by serology test according to clinical severity

	ARDS present	**ARDS absent**	**P value**
Lateral flow assay			
One Step COVID- 2019 Test (Guangzhou Wondfo Biotech)	78.6 (69.5-86.1)	61.4 (49.0-72.3)	0.02
TR DPP® COVID-19 IGM/IGG Bio-Manguinhos (Fundação Oswaldo Cruz)	75.7 (66.6-82.9)	58.6 (46.9-69.4)	0.03
COVID-19 ECO IGM/IGG (Eco Diagnostica)	82.5 (73.8-89.3)	64.3 (52.6-74.5)	0.01
COVID-19 IgG/IgM (Qingdao Hightop Biotech)	63.1 (53.5-71.8)	37.1 (26.7-48.8)	0.00
Imuno-Rápido COVID-19 IgG/IgM (Wama Produtos Para Laboratorio)	77.7 (68.7-84.7)	55.7 (44.1-66.7)	0.00
COVID-19 IgG IgM (Gold Analisa Diagnóstica)	66.0 (56.4-74.4)	45.7 (34.6-57.3)	0.01
ELISA			
Covid-19 ELISA IgA/IgM (Vircell Microbiologists)	90.3 (83.1-94.6)	90.0 (80.7-95.1)	0.95
Covid-19 ELISA IgG (Vircell Microbiologists)	83.5 (75.2-89.4)	67.1 (55.5-76.9)	0.02
Anti-SARS-CoV-2 ELISA IgA (Euroimmun)	81.8 (69.6-89.9)	70.4 (57.2-80.9)	0.24
Anti-SARS-CoV-2 ELISA IgG (Euroimmun)	67.3 (54.1-78.2)	50.0 (37.1-62.9)	0.10
Allserum EIA COVID-19 IgM (MBiolog)	55,1(44,7-65,2)	32,4(21,5-44,8)	0.00
Allserum EIA COVID-19 IgG (Mbiolog)	68,4(58,2-77,4)	48,5(36,2-61,0)	0.01

ARDS: Respiratory acute distress syndrome

Table 7. Agreement between results of the tests performed on serum and capillary blood among SARS-Cov-2 confirmed cases

Results of tests performed in digital blood		Results of test performed in serum		Total	Kappa
		Negative	Positive		
One Step COVID- 2019 Test	Negative	2	3	5	0.50
	Positive	0	15	15	
Total		2	18	20	
TR DPP® COVID-19 IGM/IGG Bio-Manguinhos	Negative	1	1	2	0.24
	Positive	3	16	19	
Total		4	17	21	
COVID-19 ECO IGM/IGG Teste	Negative	2	0	2	1.0
	Positive	0	26	26	
Total		2	26	28	