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Rapid Serological Test for COVID-19, One-Step-COVID-2019: Accuracy and Implications for Pandemic Control

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Abstract: Background: Accurate and rapid testing for COVID-19 is critical for effective disease management and control. The One-Step-COVID-2019-Test was developed as a rapid serological test to detect antibodies against SARS-CoV-2. Objective: To estimate the accuracy of the rapid serological test for COVID-19 using One-Step-COVID-2019. Methods: We conducted a population-based serological survey with a stratified sampling of 593 adults between October and December 2020, prior to mass vaccination and during a period of limited availability of rapid tests. Participants provided 7.5 mL of serum, which was tested using the One-Step-COVID-2019-Test for IgM-IgG antibodies without distinction, as well as an in-house ELISA for IgG against the spike protein. Statistical analysis accounted for sampling weights, with accuracy assessed through sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), Youden index, and kappa coefficient, using ELISA as the reference standard. McNemar's test identified significant differences between the test results. Results: The ELISA-based prevalence of infection was 11.1%. The One-Step-COVID-2019-Test showed low sensitivity (27.0–30.8%) but high specificity (89.9–96.6%), with poor agreement (kappa: 0.290–0.337), particularly among asymptomatic individuals. Conclusions: The One-Step-COVID-2019 rapid test for COVID-19 demonstrated inadequate performance, characterized by low sensitivity and poor reliability, making it unsuitable for effective serological surveillance.

Keywords: SARS-CoV-2; serology; COVID-19 serological tests; rapid diagnostic units; pandemics

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1. Introduction

The global community grappled with the COVID-19 pandemic, instigated by the novel SARS-CoV-2 coronavirus, from late 2019 to early May 2023. This disease precipitated profound health, social, and economic repercussions. Consequently, population testing emerged as a pivotal strategy for controlling transmission, monitoring the epidemiological landscape, and evaluating the collective immune response, particularly during the initial stages of the pandemic [1].

Serological tests identify specific antibodies against pathogens, including SARS-CoV-2, in blood or other bodily fluids, indicating prior exposure to the virus. These tests

serve various purposes [1,2]. However, the validity of serological tests for COVID-19 is influenced by numerous factors [3]. Additionally, various serological tests, each with different methodologies, antigenic targets, sensitivity, and specificity, have emerged in the market [4].

The enzyme-linked immunosorbent assay (ELISA) serology test, an enzyme immunoassay, effectively detects antibodies of the immunoglobulin class. It boasts high sensitivity and specificity for COVID-19 antibody detection [4]. However, this test necessitates specialized laboratories and a longer duration for result acquisition. In contrast, rapid tests for COVID-19, such as the One-Step COVID-2019, are more cost-effective and simpler to administer. These tests are faster and can be conducted in less specialized settings, including pharmacies and healthcare facilities. Nevertheless, the diagnostic performance of these rapid tests is debatable, and their accuracy depends on the manufacturer and the demographics of the population tested [4,5].

It is imperative to evaluate the accuracy of rapid COVID-19 serological tests through population-based studies. These studies ought to reflect the diversity and heterogeneity of the general populace, taking into account both symptomatic and asymptomatic individuals [4,5]. These individuals could constitute a substantial fraction of COVID-19 cases and contribute to the virus's propagation. These data are crucial in ascertaining the efficacy of rapid serologic tests as a screening, diagnostic, or monitoring instrument for infectious diseases like COVID-19.

This study sought to evaluate the diagnostic efficacy of the One-Step COVID-2019 rapid serological test in identifying antibodies against SARS-CoV-2 among symptomatic and asymptomatic adults in a city in Minas Gerais, Brazil. The results were compared with those obtained using the ELISA serological test, which served as the reference standard.

2. Materials and Methods

2.1. Study Design

This is a cross-sectional panel-based survey with 593 adults who were evaluated in the study "COVID-Inconfidentes (Epidemiological surveillance of COVID-19 in the region of Inconfidentes/MG)", whose objective was to determine the prevalence of SARS-CoV-2 infection and health-related aspects of the population [6]. This was a population-based seroepidemiological survey, carried out in the months from October to December 2020, before the initiation of mass vaccination and during a period when rapid tests were scarcely available, in the city of Ouro Preto, located in the Iron Quadrangle region, Metallurgical Zone of Minas Gerais, Brazil. This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Federal University of Ouro Preto, Brazil (Ethics Submission Certificate no. 32815620.0.1001.5149).

The survey was carried out in three surveys with intervals of 21 days, considering the incubation period of the SARS-CoV-2 virus. This study design followed the recommendations of the seroepidemiological investigation protocol for SARS-CoV-2 infection by the World Health Organization [7]. All the procedures adopted by this study followed the Declaration of Helsinki and the Brazilian guidelines and standards for research involving humans.

The sample size was calculated using the OpenEpi tool, with the 2019 population estimate by the demographic census for the urban areas, 95% confidence level, design effect equal to 1.5, the estimated proportion of infection, and precision. The sample size calculation estimated that 409 individuals would have to be evaluated [8]. To maintain the accuracy, the sample was increased to compensate for eventual losses due to refusals, the absence of the resident drawn for the study, and the existence of closed houses, adding a percentage of 20% of recomposition to the sample size [8,9]. Therefore, the minimum sample size was 491 individuals.

The sample was selected in three stages: census sector (probability proportional to the number of households), household (systematic sampling), and resident (randomly) [9–11]. This design was based on large national household surveys, such as the National House-

hold Sample Survey (PNAD) [11]; Household Budget Survey (POF) [12]; "Saúde em Beagá" survey [10]; and, more recently, the "EPICOVID19" study [13,14]. For the selection of the sampling units, prior stratification was performed, considering the average income, according to IBGE data. Therefore, the representativeness of the different socioeconomic strata (<1 minimum wage, 1 to 3 minimum wages, and ≥4 minimum wages) was guaranteed in the final sample.

2.2. Serological Tests

To evaluate the existence of anti-SARS-CoV-2 antibodies in the blood of the participants, 7.5 mL of serum was gathered in an S-Monovette serum gel tube (Sarstedt, Nümbrecht, North Rhine-Westphalia, Germany). Two distinct serological tests were conducted: a rapid test and a reference test (ELISA). The serum served as the biological sample in both tests to enhance the precision of the outcomes. The rapid test employed was the One-Step-COVID-2019-Test®, which qualitatively identifies IgM and IgG antibodies against SARS-CoV-2 without differentiation. The reference test utilized was the in-house ELISA, which identifies IgG antibodies against SARS-CoV-2.

2.2.1. One-Step-COVID-2019-Test

The One-Step-COVID-2019-Test utilizes the capture immunoassay principle to detect SARS-CoV-2 IgG/IgM antibodies in human whole blood, serum, or plasma. When a sample is added to the reaction well of the test device, it is absorbed by capillary action, mixes with the SARS-CoV-2 antigen–dye conjugate, and flows through the pre-coated membrane. If the sample contains SARS-CoV-2 antibodies at or above the test's detection limit (cut-off), these antibodies bind to the antigen–dye conjugate and are captured by the human anti-IgG antibodies and anti-μ chain antibody complex immobilized in the test region (T) of the device. This binding results in a colored test band, indicating a positive result. Conversely, if the concentration of SARS-CoV-2 antibodies in the sample is zero or below the detection limit, no visible colored band appears in the test region (T), indicating a negative result. As a procedural control, a colored line will appear in the Control Region (C) if the test is performed correctly. This control line confirms sufficient sample volume, adequate membrane absorption, and correct procedural technique.

The results are interpreted as follows: a positive result occurs when two colored bands appear in both the test (T) and control (C) regions, indicating the presence of SARS-CoV-2 antibodies. A negative result is identified when only one colored band appears in the control line (C), indicating that the concentration of SARS-CoV-2 antibodies is zero or below the detection limit. An invalid result is observed when no visible colored band appears in the control line (C), suggesting a test or procedural failure, necessitating a repeat of the sample analysis. The One-Step-COVID-2019-Test includes internal quality control, ensuring that the presence of a colored line in the control region (C) verifies that the sample volume was sufficient, the membrane absorption was adequate, and the procedure was executed correctly.

2.2.2. ELISA Test

This test employs the anti-spike (S) protein as an antigen (kindly supplied by CT-vaccines, UFMG, Belo Horizonte, Minas Gerais, Brazil) "Kit Elisa COVID-19 IgG". For antigen production, the full-length coding region of the nucleocapsid (N) gene of SARS-CoV-2 (GenBank accession number: MT126808.1) was optimized for codon usage in E. coli and inserted into the pET-24a- (+) expression vector. This modified plasmid was introduced into E. coli BL21(DE3) cells. Successfully transformed bacterial clones were grown in LB medium, induced to express the nucleocapsid protein with 0.5 mM IPTG for 4 h, and the recombinant protein was purified using nickel-affinity chromatography with an AKTAprime plus system (GE Healthcare, Chicago, IL, USA). The positive samples were selected based on the development and validation of the ELISA test as described by Bagno et al. (2022). Negative sera obtained before 2020 were from healthy donors, and

those obtained after 2020 were from individuals who tested negative via qRT-PCR. Positive samples were confirmed either by positive SARS-CoV-2 PCR nasal swab or rapid test (DPP COVID-19 IgM/IgG). The ELISA, validated by UFMG's CT Vacinas group, showed high sensitivity and specificity, supporting the reliability of our study's findings [15].

The cut-off value was determined using 88 samples from healthy donors (obtained before 2020), calculated as the mean absorbance of the negative controls plus three standard deviations using the formula: cut-off = (mean absorbance of the negative control + 3 standard deviations). Positive and negative controls were prepared using heat-inactivated samples (56 °C for 30 min). Furthermore, internal evaluation of accuracy included testing 135 serum samples: 11 from patients admitted to a hospital in Belo Horizonte (Minas Gerais, Brazil) with positive PCR for COVID-19; 43 from non-hospitalized individuals (symptomatic or oligosymptomatic) with positive PCR for COVID-19, collected 13 ± 2.2 days post-PCR confirmation; 38 from healthy donors who tested negative for SARS-CoV-2 nasal swab PCR; and 43 from healthy donors who donated blood before the emergence of COVID-19 (before 2020). *p* values were determined using an unpaired, two-sided Mann–Whitney U-test. For all validation assays, an index (I) for each sample was calculated, with results classified as non-reactive ($I < 0.8$), borderline ($0.8 \leq I < 1.1$), or reactive ($I \geq 1.1$).

All analyses were performed according to the manufacturer's protocol and recommendations, as detailed by Bagno et al. (2022) in their study on the development and validation of an enzyme-linked immunoassay kit for COVID-19 diagnosis and surveillance [15].

2.3. Statistical Analysis

The study sample was described using statistical analyses, which took into account the sampling weighting factors. These analyses were performed using the 'svy' command in the Stata® software, version 15.0. The results were presented as relative frequencies and 95% confidence intervals and evaluated using Pearson's chi-square. The accuracy of the One-Step-COVID-2019 Test was assessed by calculating sensitivity [true positives/(true positives + false negatives)], specificity [true negatives/(true negatives + false positives)], positive predictive value (PPV) [sensitivity × prevalence/(sensitivity × prevalence) + (1 − specificity) × (1 − prevalence)], negative predictive value (NPV) [(specificity × (1 − prevalence)/specificity × (1 − prevalence) + (1 − sensitivity) × prevalence], area under the curve (AUC), Youden index (sensitivity + specificity − 1), and kappa coefficient [$\kappa = (p_0 - p_e)/(1 - p_e)$], where '$p_0$' is the observed agreement and 'p_e' is the expected agreement by chance. All tests were realized with the ELISA test serving as the reference standard. McNemar's test was employed to identify significant differences in the proportion of positive or negative results between the tests [$\chi^2 = (b - c)^2/b + c$], where 'b' is the number of discordant pairs where the ELISA is positive but the One-Step Test is negative, and 'c' is the number of discordant pairs where the ELISA is negative but the One-Step Test is positive.

Furthermore, socioeconomic and demographic variables (sex, age, skin color, marital status, education level, family income, working status, work from home) are included to provide a comprehensive characterization of the study sample. These variables are essential for analyzing the relationship between positive serology results and individual characteristics across the two serological methods used in the study. By examining these factors, we can identify patterns and disparities in seroprevalence, offering valuable insights into the epidemiological context of COVID-19. A significance level of 0.05 was used in all analyses.

3. Results

In this study, 633 serum samples were collected from various individuals. However, 40 samples, representing 6.3% of the total, were excluded from the analysis due to ambiguous ELISA results. Consequently, 593 serum samples were subjected to two anti-SARS-CoV-2 serological tests, namely the One-Step-COVID-2019-Test and ELISA (Figure 1).

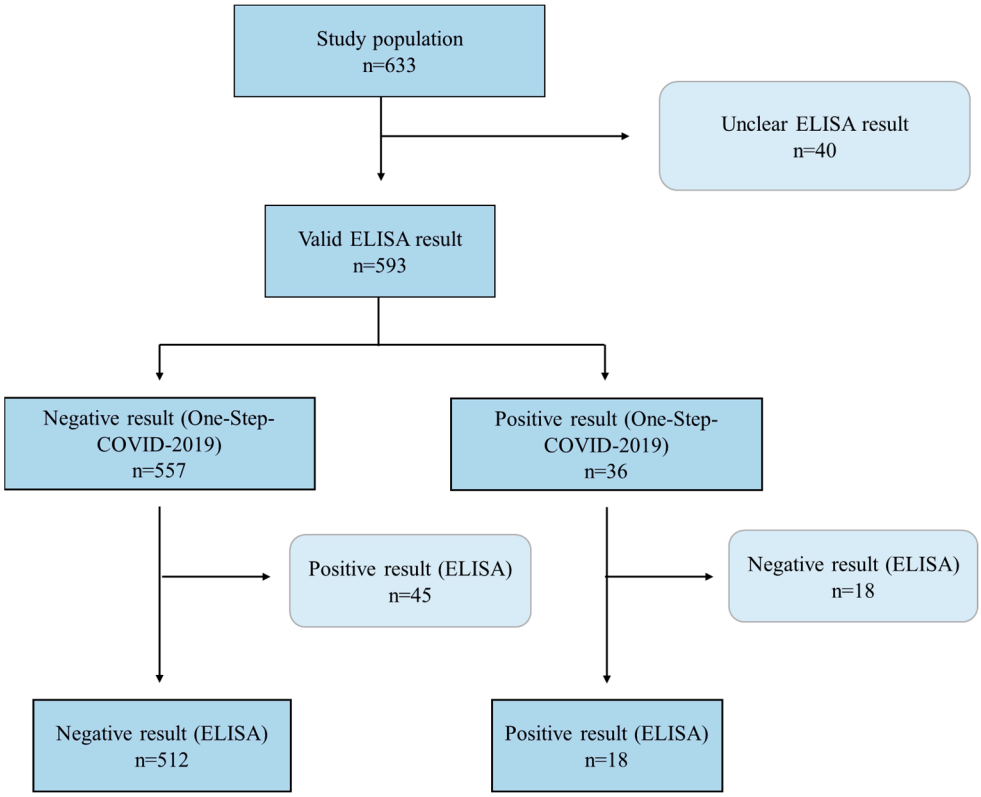

Figure 1. Flowchart of study participants.

Using the ELISA test as a reference, the overall results for all individuals were as follows: the One-Step-COVID-2019-Test demonstrated a sensitivity of 28.6%, a specificity of 96.6%, a PPV of 50.0%, and an NPV of 91.9%. When the results were stratified based on the presence or absence of COVID-19 symptoms in the past 15 days, it was observed that the One-Step-COVID-2019-Test had marginally higher diagnostic accuracy in symptomatic individuals (sensitivity: 30.8%; specificity: 96.4%; PPV: 57.1%; NPV: 89.9%; AUC: 0.636; kappa: 0.337) compared to asymptomatic individuals (sensitivity: 27.0%; specificity: 96.7%; PPV: 45.5%; NPV: 92.8%; AUC: 0.619; kappa: 0.290). However, when evaluating the kappa index across all analyses, the One-Step-COVID-2019-Test needs to show better agreement with the ELISA (k < 0.4) (Table 1).

Table 1. Diagnostic accuracy of the One-Step-COVID-2019-Test for assessing the seroprevalence of anti-SARS-CoV-2 antibodies concerning the reference ELISA test.

	N	TP	FP	TN	FN	Sens. (%)	Spec. (%)	PPV (%)	NPV (%)	AUC	Kappa	Youden	*p*-Value [a]
Total	593	18	18	512	45	28.6	96.6	50.0	91.9	0.626	0.310	0.252	**<0.001**
Symptoms of COVID-19													
Non-symptomatic	398	10	12	349	27	27.0	96.7	45.5	92.8	0.619	0.290	0.237	**0.016**
Symptomatic	192	8	18	160	6	30.8	96.4	57.1	89.9	0.636	0.337	0.272	**0.014**

N, number of individuals evaluated; TP, true positive; FP, false positive; TN, true negative; FN, false negative; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve. [a] McNemar test. Values in bold are the significant associations ($p < 0.05$).

Serological tests revealed a higher prevalence of SARS-CoV-2 seropositivity among less educated individuals and those not working from home, as indicated by both ELISA and One-Step-COVID-2019-Tests ($p < 0.05$). A higher seroprevalence for COVID-19 was observed exclusively among women in the ELISA test ($p = 0.023$). Furthermore, individuals not currently employed exhibited a higher seroprevalence for COVID-19, but only in the One-Step-COVID-2019-Test ($p = 0.001$) (Table 2).

Table 2. Characteristics of study participants according to the serological tests evaluated.

		ELISA			One-Step-COVID-2019		
Characteristics	**Total % (CI95%)**	**Non-Reactive % (CI95%)**	**Reactive % (CI95%)**	p *	**Non-Reactive % (CI95%)**	**Reactive % (CI95%)**	p *
Total		88.9 (85.4–91.6)	11.1 (8.4–14.6)		93.9 (91.2–95.8)	6.1 (4.2–8.8)	
Sex							
Male	49.8 (42.7–56.8)	94.1 (87.9–97.2)	5.9 (2.8–12.0)	**0.023**	92.0 (88.9–94.2)	8.0 (5.8–11.1)	0.099
Female	50.2 (43.2–57.2)	83.8 (76.6–89.0)	16.2 (10.9–23.4)		95.8 (91.1–98.1)	4.2 (1.9–8.9)	
Age							
18–34 years	30.8 (25.3–36.8)	87.3 (75.0–94.1)	12.7 (5.9–25.0)		93.6 (88.7–96.5)	6.4 (3.5–11.3)	
35–59 years	49.2 (43.5–54.9)	91.3 (85.3–94.9)	8.7 (5.0–14.7)	0.514	95.3 (89.0–98.0)	4.7 (2.0–11.0)	0.514
≥60 years	20.0 (15.6–25.3)	85.6 (76.5–91.6)	14.4 (8.4–23.5)		90.8 (79.5–96.2)	9.2 (3.8–20.5)	
Skin color [1]							
White	29.9 (22.7–38.1)	90.8 (80.9–95.8)	9.2 (4.2–19.1)	0.560	96.0 (91.8–98.0)	4.0 (2.0–8.2)	0.267
BBYI	70.1 (61.9–77.3)	88.1 (83.7–91.4)	11.9 (8.6–16.3)		93.0 (88.6–95.7)	7.0 (4.2–11.4)	
Marital status [2]							
Married	52.2 (45.4–58.9)	88.9 (82.0–93.4)	11.1 (6.6–17.9)	0.990	93.2 (86.9–96.6)	6.8 (3.4–13.1)	0.648
Not married	47.8 (41.1–54.5)	88.7 (79.6–94.2)	11.1 (5.8–20.3)		94.6 (91.1–96.7)	5.4 (3.3–8.9)	
Education level							
0–8 years	24.4 (19.5–30.0)	80.1 (68.6–88.2)	19.9 (11.8–31.4)	**0.048**	87.8 (76.4–94.1)	12.2 (5.9–23.6)	**0.016**
≥9 years	75.6 (69.9–80.5)	91.5 (86.2–94.9)	8.5 (5.1–13.8)		95.8 (93.5–97.3)	4.2 (2.7–6.5)	
Family income [3]							
≤2 MW	39.3 (32.3–46.8)	85.5 (75.2–91.9)	14.5 (8.0–24.8)		94.7 (90.0–97.3)	5.3 (2.7–10.3)	
>2 to ≤4 MW	32.3 (27.0–37.9)	88.4 (79.4–93.8)	11.6 (6.2–20.5)	0.232	91.7 (83.0–96.1)	8.3 (3.9–17.0)	0.520
>4 MW	28.4 (20.0–38.6)	94.3 (89.0–97.1)	5.7 (2.9–11.0)		95.2 (89.6–97.8)	4.8 (2.1–10.4)	
Working [4]							
Not workers	45.1 (41.2–49.1)	86.4 (78.0–91.9)	13.6 (8.0–21.9)	0.165	91.7 (86.4–95.0)	8.3 (5.0–13.6)	**0.001**
Active workers	54.9 (50.9–58.8)	92.2 (86.6–95.6)	7.8 (4.4–13.4)		96.9 (94.6–98.3)	3.1 (1.7–5.4)	
Work from home [5]							
No	71.9 (65.7–77.3)	86.6 (82.2–90.1)	13.4 (9.8–17.8)	**0.033**	92.8 (89.8–94.9)	7.2 (5.1–10.2)	**0.045**
Yes	28.1 (22.6–34.3)	94.6 (88.5–97.6)	5.4 (2.4–11.5)		96.6 (92.2–98.6)	3.4 (1.4–7.8)	
Symptoms of COVID-19 [6]							
No	69.7 (64.9–74.2)	84.0 (77.7–88.8)	16.0 (11.2–22.3)	0.196	94.6 (91.2–96.7)	5.4 (3.3–8.8)	0.404
Yes	30.3 (25.8–35.1)	78.6 (71.3–84.5)	21.4 (15.5–28.7)		92.2 (84.9–96.1)	7.8 (3.9–15.1)	

[1] BBYI: Black, brown, yellow, and indigenous. [2] Not married: widowed, divorced, single. [3] Value of the minimum wage at the time of data collection (2020): BRL 1045.00. [4] Not-workers: unemployed, pensioner, or retiree. [5] Percentage of active workers who were working at home. [6] At least one symptom in the 15 days before the interview (fever, sore throat, cough, dyspnea, tachycardia, diarrhea, vomiting, anosmia, ageusia, and fatigue). * Pearson chi-squared test. Values in bold are the significant associations ($p < 0.05$).

4. Discussion

The findings indicate that the rapid test evaluated, while beneficial, could have been more effective in diagnosing COVID-19 within the study population. It underestimated the infection prevalence, thereby compromising disease control and prevention measures. Rapid tests for infectious diseases, such as COVID-19, are crucial for epidemic diagnosis and monitoring. However, these tests require high sensitivity, meaning they must accurately detect individuals exposed to the virus who have subsequently developed antibodies. Tests with low sensitivity can yield numerous false-negative results, failing to identify diseased individuals. This can underestimate the infection prevalence, weaken control measures, and lead to triage failures for patients requiring medical attention. Consequently, tests with low sensitivity are unsuitable for studies aiming to screen patients and assess the disease's impact on the population.

The diminished sensitivity of the rapid test could be attributed to the nature and quality of the antigen utilized, the immunological window of SARS-CoV-2 infection, and individual differences in the immune response. Prior research has indicated that the sensitivity of serological tests fluctuates based on the testing method, immunoglobulin class, and duration since the onset of symptoms. Sensitivity peaks at least three weeks post-symptom onset (69.9–98.9%), as opposed to the initial week (13.4–50.3%). ELISA tests

exhibit the greatest sensitivity, followed by CLIAs and LFIAs [4,5]. Conversely, the high specificity of the rapid test could be due to minimal cross-reactivity with other prevalent coronaviruses or distinct respiratory infections [16]. Comparing the findings of this study with those of similar research uncovers substantial discrepancies in the accuracy of rapid tests for COVID-19. Such variation can be explained by factors like the type and brand of rapid test employed, the interval between symptom onset and test administration, the clinical and epidemiological characteristics of the study population, and the reference method implemented [4,5]. These elements can impact both the quality and interpretation of rapid test outcomes.

The One-Step-COVID-2019-Test was one of the first rapid tests approved in Brazil for the serological evaluation of COVID-19 and played an important role in detecting antibodies at the beginning of the pandemic when the availability of tests was limited [17]. However, although the use of the test has shown some usefulness in symptomatic individuals, its low sensitivity and high rate of false negatives compromise the accuracy of diagnoses and the effectiveness of control measures [18].

Despite the One-Step-COVID-2019-Test's low sensitivity, it can be utilized in conjunction with other data, such as disease symptoms, to enhance the probability of an accurate diagnosis. This was evident in our study, where the rapid test demonstrated a marginal increase in accuracy among symptomatic individuals, although the results remained unsatisfactory. Several studies suggest that rapid tests exhibit greater sensitivity in symptomatic individuals or those with prior exposure to the virus, as these individuals typically present a higher viral load and a more robust immune response [4]. However, we acknowledge that the diversity and non-specific nature of COVID-19 symptoms present significant challenges. Symptoms overlap with other respiratory infections, complicating differential diagnosis [19]. Additionally, during serological surveys, many individuals may have already recovered, which can affect the detection of antibodies. In such cases, the One-Step-COVID-2019-Test alone may not provide a reliable diagnosis and should be complemented with more specific methods, such as PCR tests or more sensitive serological assays.

Therefore, while the rapid test can be a useful tool, especially in symptomatic individuals, its limitations must be considered, and it should not be relied upon as the sole diagnostic method. This highlights the importance of using a combination of diagnostic approaches to improve the accuracy of COVID-19 diagnosis and surveillance. Consequently, rapid tests may be more suitable for these cases, assuming they are corroborated by a more specific method. However, for asymptomatic individuals or those with minimal exposure to the virus, rapid tests may not be the best choice due to their potentially lower viral load and immune response, which could hinder antibody detection.

Accurate testing for COVID-19 is crucial for identifying groups at risk of infection. If the test is not carried out correctly or has low accuracy, this can lead to an underestimation or overestimation of the prevalence of COVID-19 in specific population groups, thus prejudicing pandemic prevention and control measures. For example, in this study, a higher seroprevalence for COVID-19 was observed in women only when using the ELISA test, which is more sensitive and specific than the One-Step-COVID-2019-Test. This finding suggests that women may be at a high risk of SARS-CoV-2 infection, which could be missed by the One-Step-COVID-2019 test due to its high false-negative rate. Therefore, it is imperative to employ reliable and standardized tests for COVID-19 that can accurately distinguish between individuals with and without the disease, revealing the true risk factors.

The relevance of evaluating the One-Step-COVID-2019-Test lies in its potential utility for future public health emergencies. Understanding the limitations and strengths of rapid serological tests is essential for improving diagnostic strategies in any upcoming pandemic. This study's findings can inform public health policies and contribute to the development of more effective diagnostic tools, ensuring better preparedness and response. Learning from the limitations of previous methods is vital for enhancing future diagnostic accuracy and reliability, ultimately aiding in the control and management of infectious disease outbreaks.

This study's results were influenced by both its strengths and limitations. A significant limitation was the exclusive use of a single serological test to detect anti-SARS-CoV-2 IgG antibodies. If the IgM ELISA test had been conducted, the rapid test results could have been potentially worse due to an increased discordance between the tests. Excluding 40 samples with indeterminate ELISA results could have limited the rapid test's accuracy if they were later confirmed as positive or negative. Another important limitation was the inability to include RT-PCR testing due to the study's design constraints, which did not allow for nasopharyngeal swab collection. Consequently, only serological tests were conducted, limiting the scope of the comparison. Conversely, the study's strengths included the use of a representative population sample, adherence to WHO recommendations for evaluating COVID-19 diagnostic tests, and the collection of household samples in a pandemic context before initiating COVID-19 vaccination. Additionally, the comparison of the rapid One-Step-COVID-2019-Test with the ELISA test in a general population rather than solely in symptomatic individuals and/or potential contractors also added to the study's strengths. These factors likely enhanced the study's validity and impact.

We conclude that the rapid serological test evaluated in this study was beneficial during the initial stages of the pandemic. However, it was not suitable for screening the vulnerable population for COVID-19 within the health units of municipalities. The test's low sensitivity and accuracy could result in underreporting of cases and a false sense of security among those tested. Furthermore, the rapid test may fail to identify individuals requiring appropriate isolation and treatment, thereby facilitating the virus's spread. Consequently, we recommend that healthcare facilities employ more reliable methods for diagnosing COVID-19, such as ELISA or RT-PCR, and consider additional clinical and epidemiological factors when assessing patients.

This study underscores the significance of drawing insights from novel methodologies that could potentially undervalue the actual impact of infectious diseases, particularly when these methods are constrained by factors like sensitivity-dependent thresholds. Furthermore, this study posits that a positive case should serve as an alert, at least within the family unit where the virus can circulate more readily, thereby underscoring the need for social distancing measures. This was a practice only partially adopted during the pandemic, with the primary response being the isolation of the infected individual. Consequently, we hope this study will enhance the understanding and management of COVID-19 and future epidemics.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of the Federal University of Ouro Preto, Brazil (Ethics Submission Certificate no. 32815620.0.1001.5149).

Informed Consent Statement: Informed consent was obtained from all individual participants included for study participation.

Data Availability Statement: The datasets generated and/or analyzed as part of the current study are not publicly available due to confidentiality agreements with subjects. However, they can be made available solely for the purpose of review and not for the purpose of publication from the corresponding author upon reasonable request.

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