

Diagnostic Performance of Five Rapid Serological Tests for SARS-CoV-2

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Abstract

We conducted a study to evaluate the sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) of five serologic tests. Subjects with negative or positive COVID-19 polymerase chain reaction (PCR) test results were tested with each of the serological tests. The results were compared with the reference PCR test. For the five tests evaluated, the Se ranged from 55.0% to 70.0% and the Sp ranged from 67.2% to 86.2%. PPV ranged from 53.2% to 80% and NPV from 75.0% to 86.2%. One test, the Wantai, had better specificity and sensitivity. None of the five tests had performance values of more than 90% in the entire sample. In symptomatic positive cases, the Wantai test reported excellent sensitivity. Overall, the low level of diagnostic performance of these tests does not support their use as an alternative to PCR for COVID-19 diagnostic. Test with better performance can be used for mass screening in low prevalence populations, to limit the indiscriminate use of PCR in context of resource-limited countries. Given the excellent sensitivity of Wantai in symptomatic cases, this test could be used as a referral test only in health facilities to discriminate suspected cases before PCR confirmation.

Keywords

SARS-Cov-2, Seroprevalence, Antibodies, COVID-19, Congo

1. Introduction

In December 2019, the first cases of Coronavirus Disease 2019 (COVID-19) were recorded and a pandemic was declared on March 11, 2020 [1]. Since then, the

whole world is facing a health crisis due to a deadly coronavirus for humans, the Severe Acute Respiratory Coronavirus 2 (SARS-CoV-2) [2] [3]. The worldwide epidemiological burden of COVID-19 on August 24, 2022 is heavy: 595,219,966 cumulative cases and 6,453,458 deaths [4] and for Africa 252,544 cases and 6779 deaths [5]. Through human-to-human transmission, COVID-19 primarily affects men, the elderly and those with underlying chronic diseases such as diabetes and cardiovascular disease [6]. Known or unaware, symptomatic or asymptomatic COVID-19 positive persons are the main sources of infection. It is transmitted by contact with the nose, mouth, or eyes through respiratory droplets, and manifests as fever, headache, dry cough, myalgias, and dyspnea. The advanced stage of the disease is often characterized by severe acute respiratory syndrome [6] [7]. The disease is fatal in severe cases [8].

The gold standard for the diagnosis of COVID-19 infection is the polymerase chain reaction (PCR), which allows the detection of viral ribonucleic acid (RNA) from a nasal or oropharyngeal swab [9]. However, it is expensive and requires a well-equipped biological laboratory with a sufficient number of trained personnel. All of these conditions limit its widespread use in resource-limited countries [10]. Seroepidemiological studies conducted in several countries and particularly in African countries show that positive case statistics based entirely on screening PCR underestimate the true extent of SARS-CoV-2 virus circulation in populations [11]-[16]. On the other hand, a meta-analysis study conducted in 2020 to describe the performance of different antigenic and serological tests in the diagnosis of COVID-19, including 25 diagnostic tests out of a total of 2247 participants, showed that only one diagnostic test had better specificity and sensitivity [17]. The weak performance of serological tests may contribute to the paradox between the number of cases reported in Africa and the estimates of seroprevalence studies [11]-[16]. The best serological tests for the detection of COVID-19 disease should be those that have the ability to identify positive (sensitivity) and negative (specificity) subjects. For this reason, any “new” test should be evaluated in a real field situation, in order to estimate the diagnostic performance, before being used in routine screening [18] [19].

In Congo, the first confirmed case of COVID-19 was recorded on March 14, 2020, and the number of cases has gradually increased to reach a total of 24,690 cases and 356 deaths by June 15, 2022. Since then, several serological tests are used to screen for COVID-19 throughout the country [20] [21]. In this context, it would be important to have serological tests with good diagnostic performance to be able to identify people who are infected with SARS-CoV-2 [22] [23]. This study was designed to evaluate the performance (sensitivity and specificity) and predictive values (positive and negative) of five rapid serological tests used for COVID-19 screening.

2. Methods

2.1. Study Design and Population

A prospective data collection diagnostic validation study was conducted from

May 5 to June 6, 2020 in the COVID-19 screening units and the COVID-19 patient management centre in Brazzaville. The COVID-19 test authorized in Congo is PCR. Brazzaville is the epicentre of the new coronavirus disease in the Congo and at this time has two analysis laboratories (National Public Health Laboratory and the private laboratory of the Congolese Foundation for Scientific Research) and several sampling sites and 3 dedicated management sites: the University Hospital of Brazzaville, the Leyono Clinic for the management of symptomatic cases and the Hotel Hospital la Concorde of Kintélé for the management of asymptomatic cases in Brazzaville. The study took place at the National Public Health Laboratory, the Leyono municipal clinic, the Kintélé Hotel-Hospital and the screening centre of the Public Health Emergency Operations Centre. During the study period, individuals with a negative or positive PRC test were asked to participate in the study. Individuals who tested negative were included at the time of their result withdrawal. Covid-19 patients were recruited from two others study sites. Only COVID-19 positive cases who had not yet started treatment were included in the study.

2.2. Sample Size

We adopted as the sample size formula: $N = [U\alpha^2 * p(1 - p)]/i^2$, with: N = sample size, P = prevalence; i = margin of error: 5%. The unknown prevalence was estimated to be $P=0.5$. Using a proportion of 10% of the study population, recommended for pilot studies [24], we obtained a minimum sample size of $N = [(1.962 * 0.52)/0.052] * 10/100 = 38$ persons.

2.3. Sample Collection and Analysis Procedures

Two categories of samples were collected: blood samples from patients with a negative PCR test and blood samples from individuals with a positive PCR test. The collection of blood samples from PCR-negative subjects took place at the collection unit of the *Centre des Opérations des Urgence de Santé Publique* in Brazzaville, when the test results were withdrawn. For confirmed positive cases, samples were taken at the COVID-19 patient management sites. Blood samples were collected and placed in EDTA tubes by qualified biologists wearing personal protective equipment, accompanied by epidemiologists to fill out the survey forms, then transferred and stored in the molecular biology department of the National Public Health Laboratory (LNSP). Only the first reading of the test result, within the reading time recommended by the manufacturer, was considered a valid result. Information on the sociodemographic and clinical characteristics of the participants and the results of the diagnostic tests used were collected using a standardized questionnaire.

2.4. Statistical Analysis

Epi-info software was used for statistical analysis and Excel for the production of tables. The qualitative variables were summarised in numbers and percentages.

Quantitative variables were summarised as minimum, maximum, mean and standard deviation. We compared the results obtained with the serologic tests with those obtained by PCR. Sensitivity, specificity and likelihood values were used as indicators of test accuracy. The definitions are as follows:

- Sensitivity (Se) is the proportion of COVID-19 positive “sick” subjects by serologic test, among all positive “sick” subjects by PCR.
- Specificity (Sp) is the proportion of COVID-19 negative “non-diseased” subjects by serologic test, among all PCR negative subjects.
- Positive predictive value (PPV) is the probability of being ill when the serologic test is positive.
- Negative predictive value (NPV) is the probability of not being ill when the serologic test is negative.

Table 1 shows the details of the calculation of these four parameters, using the classification of participants as sick and not sick.

A positive serological test was considered as the presence of at least 1 of the two immunoglobulins tested: IgG or IgM. We considered as “true positives” all subjects in both serological and PRC tests were positive. Patients with a negative serological test and have a positive PCR result were considered as “false negatives”.

2.5. Ethical Issues

The Laboratory and Research Commission of the National Technical Committee for the Response to Covid-19 gave its approval for the study. All participants gave informed consent prior to enrolment in the study. For reasons of confidentiality, each of the serological tapes was marked with a registration number. The identity of the study subjects and their PCR results were linked to an identifier and known only to the study investigators. The read cassettes were kept for archiving at the LNSP.

2.6. Screening Tests Characteristics

The study included five (5) tests, the main characteristics of which are described below:

- *Ichroma™ COVID-19 Ab (ICHROMA)*, *Boditech Med Incorporated*

This is a test that detects IgM and IgG antibodies in serum, plasma and whole blood. It consists of an automatic result reader, a cassette with a 25-test ID, a diluent, and a tube containing a pellet. The procedure is as follows: introduce 150 µl of the diluent into the tube containing the granule, mix until it is completely

Table 1. Diagnostic test evaluation contingency table and performance index formulas.

	Disease+ (PCR positif)	Disease- (PCR negatif)	Total
Test+	a	b	a + b
Test-	c	d	c + d
Total	a + c	b + d	a + b + c + d

dissolved, then add 10 µl of the whole blood/serum/plasma to the solution. Then initialise the cassette in the reader, place 75 µl of the mixture in the “sample” well and press the “start” icon on the reader to initiate the reading procedure. The result obtained after 15 minutes is both quantitative and qualitative in three modalities, according to the following threshold: <0.9 for “Negative” [0.9 - 1.1] for “Indeterminate” and [1.1 - 200] for “Positive”.

- *One Step Test for Novel Coronavirus (2019-nCoV) IgM/IgG Antibody (GB)*, Getein Biotech, Inc, China

Immuno-chromatographic test for IgM and IgG antibodies in serum/plasma/total blood. It consists of a cassette and a diluent. The procedure is to place 20 µL of whole blood or 10 µL of serum/plasma into the sample well of the cassette and immediately add 3 drops of diluent. The test is positive when a band appears near the IgG or IgM area. Qualitative result obtained between 10 and 20 minutes, and validated by the presence of a band at the “control” zone. IgG/IgM zones do not appear clearly on the cassette: need to refer to the manufacturer’s instructions.

- *Wantai SARS-CoV-2 Ab Rapid Test (WANTAI)*, Beijing Wantai Biological Pharmacy Enterprise Co, Ltd, China

An immuno-chromatographic test that detects antibodies in serum/plasma/total blood, without differentiation between IgM and IgG. It consists of a cassette and diluent. The procedure is as follows: place 10 µL of whole blood/serum/plasma in the “sample” well of the cassette and immediately add 2 drops of diluent. The positivity of the test is similar to that of GP, the qualitative result obtained after 15 minutes, and validated by the presence of a band at the “control” zone

- *Rapid IgG/IgM test 2019-nCoV (MULTI-G)*, Multi-G bvba, Belgium

Immuno-chromatographic test for IgM and IgG antibodies in serum/plasma/total blood. It consists of a cassette and a diluent. The procedure is as follows: place 20 µL of whole blood or 10 µL of serum/plasma in the “sample” well of the cassette and immediately add 2 drops of diluent. The positivity of the test is similar to that of GP, the qualitative result obtained after 10 minutes, and validated by the presence of a band at the “control” area. Manufacturer:

- SARS-CoV-2 Antibody Test Strip (Colloidal Gold Method) (SINOCARE), Changsha Sinocare Inc.

This is an immuno-chromatographic test that detects IgM and IgG antibodies in serum/plasma/total blood. It consists of a cassette with a diluent and a micro-pipette. The procedure is as follows: place a drop of serum or plasma in the “sample” well of the cassette and immediately add 2 to 3 drops of diluent. The positivity of the test is similar to that of GP, the qualitative result obtained after 10 minutes, and validated by the presence of a band in the “control” area.

3. Results

3.1. Main Characteristics of Participants

A total of 130 people were surveyed, 70% were male and the mean age was 39.4

± 13.0 years, 41 (31%) had a positive Covid-19 PCR test and 89 (69%) 41 a negative PCR test, 86% of subjects were asymptomatic. The participants in the survey came from the nine districts of Brazzaville. Details of the main characteristics of the participants are presented in **Table 2**.

3.2. Diagnostic Performance Indices of Serological Tests

The detailed results regarding the diagnostic performance of the five tests assessed are presented in **Table 3**. Below is the key information on the performances of each test:

Table 2. Main characteristics of study respondents, according to PCR results.

	PCR. n (%)		
	n (%)	Negatif. n = 89	Positif. n = 41
Sociodemographic characteristics			
Gender			
<i>Woman</i>	39 (30.0)	26 (29)	13 (32)
<i>Male</i>	91 (70.0)	63 (71)	28 (68)
Age			
(m \pm standard derivation)	39.4 \pm 13.0	38.5 \pm 13.0	42.0 \pm 13.0
District			
<i>Makélékélé</i>	14 (10.7)	4 (4)	10 (24)
<i>Bacongo</i>	3 (2.3)	2 (2)	1 (2)
<i>Poto-Poto</i>	11 (8.5)	9 (10)	2 (5)
<i>Moungali</i>	27 (20.7)	20 (22)	7 (17)
<i>Ouenze</i>	11 (8.5)	7 (8)	4 (10)
<i>Talangai</i>	29 (22.3)	18 (20)	11 (27)
<i>Mfilou</i>	11 (8.5)	10 (11)	1 (2)
<i>Madibou</i>	7 (5.4)	6 (7)	1 (2)
<i>Djiri</i>	17 (13.1)	13 (15)	4 (10)
Clinical characteristics			
Presence of COVID-19 symptoms			
<i>Yes</i>	112 (86.2)	77 (87)	35 (85)
<i>No</i>	18 (13.8)	12 (13)	6 (15)
Frequency of symptoms (n = 18)			
<i>Fever</i>	6 (15.0)	3 (17)	3 (14)
<i>Cough</i>	6 (15.0)	2 (11)	4 (19)
<i>Shortness of breath</i>	7 (18.0)	1 (6)	6 (29)
<i>Sore throat</i>	1 (3.0)	0 (0.0)	1 (5)
<i>Headache</i>	10 (26.0)	3 (17)	7 (53)
<i>Muscles soreness</i>	9 (23.0)	9 (50)	0 (0)

Table 3. Overall diagnostic performance indices for all five serological rapid tests.

	Ichroma	IgM/IgG Antibody GB	Wantai	Multi-G	Sinocare
Sensitivity % [IC95%]	62.5 [47.5 - 77.5]	55.0 [39.6 - 70.4]	77.8 [58.6 - 97.0]	65.0 [50.2 - 79.8]	70.0 [55.8 - 84.2]
Specificity % [IC95%]	67.2 [55.9 - 78.4]	81.4 [72.3 - 90.5]	86.2 [73.7 - 98.8]	81.2 [71.9 - 90.4]	86.3 [76.8 - 95.7]
Positive Predictive Value % [IC95%]	53.2 [38.9 - 67.5]	62.9 [46.8 - 78.9]	77.8 [58.6 - 97.0]	66.7 [51.9 - 81.5]	80.0 [66.7 - 93.3]
Negative Predictive Value % [IC95%]	75.0 [64.0 - 86.0]	76.0 [66.3 - 85.7]	86.2 [73.7 - 98.8]	80.0 [70.6 - 89.4]	78.6 [67.8 - 89.3]

Sensitivity (Se)

- WANTAI gives 77.8% true positives and 22.2% false negatives;
- ICHROMA gives 62.5% true positives and 37.5% false negatives;
- GB gives 55.0% true positives and 45.0% false negatives;
- MULTI-G gives 65.0% true positives and 35.0% false negatives;
- SINOCARE gives 70.0% true positives and 30.0% false positives.

Specificity (Sp)

- WANTAI gives 86.2% true negatives and 13.8% false positives;
- ICHROMA gives 67.2% true negatives and 32.8% false positives;
- GB gives 81.4% true negatives and 18.6% false positives;
- MULTI-G gives 81.2% true negatives and 18.8% false positives;
- SINOCARE gives 86.3% true negatives and 13.7% false positives.

Positive predictive value (PPV)

The probability of being ill when the test is positive is 77.8% for WANTAI, 53.2% for ICHROMA, 66.7% for MULTI-G, 62.9% for GB and 80.0% for Sinocare.

Negative predictive value (NPV)

The probability of having a negative test when not ill is 86.2% for WANTAI, 75.0% for ICHROMA, 80.0% for MULTI-G, 76.0% for GB and 78.6% for Sinocare.

Specific case of symptomatic persons

Analyses restricted to symptomatic cases with negative or positive PCR show much better results with sensitivities and negative predictive values all above 80%. Only the WANTAI test has optimal diagnostic performance (Se = 100% and NPV = 100%). The detailed results are presented in **Table 4**.

4. Discussion

We aimed to evaluate the diagnostic performance of five rapid Serological tests for the diagnosis of COVID-19. Using a population of patients with a known COVID-19 disease status by PCR test, we determined for each test the true positive and true negative rates, as well as the probability of a person being ill when the test is positive and the probability of a person not being ill when the test is negative. Overall, the performance indices of the tests evaluated were not better. The study took place between May and June 2020, just two months after the official notification on 14 March 2020 of the first case of COVID-19 in Congo. It

Table 4. Diagnostic performance indices of the five serological tests in symptomatic COVID-19 cases.

	Ichroma	IgM/IgG Antibody GB	Wantai	Multi-G	Sinocare
Sensitivity % [IC95%]	83.3 [44.2 - 98.1]	83.3 [44.2 - 98.1]	100.0 [67.0 - 100.0]	83.3 [44.2 - 98.1]	83.3 [44.2 - 98.1]
Specificity % [IC95%]	87.5 [54.6 - 98.6]	87.5 [54.6 - 98.6]	66.7 [17.7 - 96.1]	75.0 [40.8 - 94.4]	87.5 [54.6 - 98.6]
Positive Predictive Value % [IC95%]	83.3 [44.2 - 98.1]	83.3 [44.2 - 98.1]	85.7 [49.9 - 98.4]	71.4 [35.2 - 93.5]	83.3 [44.2 - 98.1]
Negative Predictive Value % [IC95%]	87.5 [54.6 - 98.6]	87.5 [54.6 - 98.6]	100.0 [33.0 - 100.0]	85.7 [49.9 - 98.4]	87.5 [54.6 - 98.6]

was later understood that the virus circulating at that time was the parent strain. Since then, Congo has experienced four outbreaks due to mutant strains of SARS-CoV-2, which can theoretically lead to a loss of sensitivity of serologic tests [25].

Available data from serological tests allow an assessment of the level of circulation of the COVID-19 virus in the population [11]-[16]. In the field of epidemiology, it is important to know in advance the performance of new tests in the field, in order to increase the certainty of the presence or absence of the disease. For the detection of COVID-19, the best serological test would be the one that allows for a broad coverage, *i.e.* the one that produces more false positives that can later be confirmed by PCR. The interest here is to avoid a false negative result, so as not to leave people in the population likely to be ill, thus contributing to the maintenance of the pandemic. In this study, five tests from five different laboratories were evaluated. Our results show that the values of the four performance parameters measured differ from one test to another. This suggests that for conducting seroepidemiological surveys, the choice of a single test is necessary in order not to aggravate the usual ranking bias of serologic tests by using several tests at the same time. Our results show that out of five tests, only one had better sensitivity and specificity. In this respect, our results corroborate those of previous studies that reported poor performance of most rapid serological tests for COVID-19 [17]. Due to the low performance mentioned, these tests would not be eligible for the diagnosis of COVID-19 as alternatives to PCR. Ideally, only tests with minimum performance thresholds of 98% specificity and 90% - 95% sensitivity should be used. Given the excellent performance of the WANTAI test in symptomatic cases of COVID-19, it could be used as a rapid diagnostic test (TROD) in health facilities for patients with symptoms of COVID-19. This will have the advantage of discriminating between suspected cases of COVID-19. Thus, the positive WANTAI test should allow early management of patients awaiting PCR confirmation.

Overall, the low level of diagnostic performance of the tests evaluated does not allow their use as an alternative to PCR. Those with better performance can be used for mass screening in low prevalence populations, to limit the indiscriminate use of PCR when resources are limited. Given the excellent sensitivity of Wantai in symptomatic cases, this test could be used as a diagnostic test (TROD) only in health facilities to discriminate between suspected cases before confirma-

tion by PCR. As SARS-CoV-2 infection is a recent phenomenon, there is still a lack of experience with the use of serologic tests in the detection of COVID-19. For this reason, numerous population-based studies will have to be repeated in order to identify which of these new rapid tests have the best diagnostic performance.

Our results also show that the false positive rate differs from one test to another. For example, Wantai has a 13.8% false positive rate. It is true that these tests need further population-based evaluation studies to better determine their performance in real-life situations. A single study is not enough to definitively determine the performance of a serological test.

5. Conclusion

In conclusion, the low diagnostic performance of assessed tests does not allow them to be used as an alternative to PCR for the diagnosis of COVID-19. However, those with better performance can be used for mass screening in low-prevalence populations to limit the indiscriminate use of PCR in the context of resource-limited countries. Given the excellent sensitivity of Wantai in symptomatic cases, this test could be used as a reference test only in health facilities to discriminate between suspected cases before PCR confirmation.

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Authors' Contributions

Gilbert Ndziessi coordinated the study and mobilised resources, participated in the development of the research protocol, analysis and interpretation of the results and produced the draft of the article.

Fabien Roch Niama and Francine Ntoumi validated the results of all realized serological test.

Jospeh Axel Ngatse and Fresnovie Geladore Mbele supervised data collection and performed data analysis.

Mayengue Pembe Issamou coordinated the work of the biologists responsible for carrying out the tests at the National Public Health Laboratory.

Laure Stella Ghoma Linguissi and Henriette Poaty participated in the elaboration of the research protocol and supervised data collection

All authors approved the final version of article.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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