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**RESEARCH LETTER**

# Serological identification of past and recent SARS-CoV-2 infection through antibody screening in Luanda, Angola

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## 1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in China and spread to the world.<sup>1</sup> Pneumonia caused by SARS-CoV-2 was named coronavirus disease 2019 (COVID-19) and is currently a public health concern.<sup>2,3</sup> From December 2019 to December 2020, more than 60.1 million cases of infection and 1.4 million deaths, have been reported worldwide.<sup>4</sup> Until December 2020, the Ministry of Health of Angola reported more than 15 000 cases and 345 deaths.<sup>4</sup>

The timely diagnosis of SARS-CoV-2 infection is fundamental to ensure controlling of the COVID-19 pandemic, the provision of treatment, and to avoid the worsening of the disease.<sup>5-7</sup> In practice, RT-PCR is the only way to confirm the SARS-CoV-2.<sup>8</sup> There are individuals that despite being epidemiologically related and presenting pulmonary radiological findings compatible with SARS-CoV-2, remain RT-PCR negative.<sup>9</sup> Factors such as the sample collecting and processing procedure may affect the result of the PCR assay.<sup>10</sup> Also, RT-PCR does not distinguish virus with active replication from residual RNA, which could cause false results, especially in asymptomatic individuals.<sup>10</sup> Therefore, serologic tests offer an alternative to assess the degree of exposure amongst different population groups.<sup>11</sup> There are no published studies that assessed the rate of exposure and immune response to SARS-

CoV-2 in Luanda, the capital city of Angola. Herein, we used serological assay to screen IgM and IgG antibodies against SARS-CoV-2 in individuals from Luanda, to support the Ministry of Health of Angola in the management of the COVID-19.

## 2 | MATERIALS AND METHODS

### 2.1 | Study design and setting

This was a cross-sectional study carried out with 660 individuals screened for antibodies against SARS-CoV-2 between July and September 2020 at Instituto Nacional de Investigação em Saúde (INIS), located in Luanda, the capital city of Angola. The INIS is a public institution of the Ministry of Health of Angola, which has as its main objective, to develop scientific research on health and its determinants for strengthening public health policies. The research team collected sociodemographic data through a standardized questionnaire in all individuals who freely agreed to participate in the investigation. The study was approved by the National Ethics Committee of Angola (nr.25/2020). Besides that, participants or legal guardians of each minor were informed of the study, and verbal consent was obtained before being included in the study.

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## 2.2 | Sample collection and serological testing

An estimate of 5 ml of whole blood was collected from each participant in a tube with a clot activator. Then, the tubes containing the blood samples were centrifuged and human serum aliquoted and stored between 2 and 8°C. The blood sample preparation was carried out at the Laboratory of the immunoserology at INIS. Commercially available enzyme-linked fluorescent assay (ELFA) was used for the qualitative detection of IgM and IgG antibodies against SARS-CoV-2 (bioMérieux SA, France) in human serum samples from each participant, following the manufacturer's instructions.<sup>12,13</sup> Briefly, the principle of this serological assay combines a two-step sandwich enzyme immunoassay method that ends with fluorescence detection. The reagents used are pre-disposed on sealed disposable reagent strips and are ready to use. All test steps were performed automatically on the mini VIDAS machine (bioMérieux SA, France). First, the human serum samples were diluted and the IgG/IgM antibodies captured through recombinant antigens found coated inside each strip. Second, the samples were washed to remove unbound components, which allowed anti-human antibodies labeled with an alkaline phosphatase specifically bind to the IgG/IgM antibodies. Third, the substrate 4-methyl-umbelliferyl phosphate was cycled in and out of the strips and the conjugated enzyme catalyzes the hydrolysis of this substrate in a fluorescent product (4-methyl-umbelliferone), which was measured at 450 nm. Finally, the results of each serum sample were automatically calculated by the

machine, and all those samples with a test value <1 were considered negative, while those samples with a test value equal or more than one were considered positive. All antibody tests were performed in the presence of positive and negative control, both provided by the manufacturer. None external control, such as samples known as positive or negative for IgG or IgM against SARS-CoV-2 infection was included in these assays. The results were grouped as follows: noninfection (IgG-/IgM-), past infection (IgG+/IgM-), and recent infection (IgG-/IgM+ or IgG+/IgM+). Additionally, samples for all individuals with a reactive serological result for IgG or IgM were forwarded to the molecular biology laboratory of INIS, to detect the presence of viral RNA on upper respiratory tract specimens using a protocol previously described for the detection of 2019 novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing; Daan Gene, China).<sup>14</sup>

## 2.3 | Statistical analysis

The statistical analysis was conducted using SPSS v25 (IBM SPSS Statistics). The frequencies and percentages are presented in the descriptive analysis. Mean and the SD was presented for normal distribution data. All categorical variables were dichotomized and analyzed with the chi-square ( $\chi^2$ ) test and logistic regression. Moreover, odds ratio (OR), adjusted odds ratio (AOR), and their 95% confidence intervals (CIs) were also calculated to check interactions between categorical variables. The *P* value was deemed significant when *P* < .05.

**TABLE 1** Sociodemographic characteristics and identification of past and recent infection with SARS-CoV-2 by antibody test in Luanda, Angola

Characteristics	N (%)	Antibody test		Noninfection		P value	Past infection		P value	Recent infection		P value
		IgG+ (%)	IgM+ (%)	No (%)	Yes (%)		No (%)	Yes (%)		No (%)	Yes (%)	
Overall	660 (100)	7 (1.1)	15 (2.3)	20 (3.0)	640 (97.0)		655 (99.2)	5 (0.8)		645 (97.7)	15 (2.3)	
Age groups												
<40 years	331 (50.2)	3 (0.9)	7 (2.1)	10 (3.0)	321 (97.0)	.989	328 (99.1)	3 (0.9)	.658	324 (97.9)	7 (2.1)	.785
≥40 years	329 (49.8)	4 (1.2)	8 (2.4)	10 (3.0)	319 (97.0)		327 (99.4)	2 (0.6)		321 (97.6)	8 (2.4)	
Gender												
Female	88 (13.3)	0 (0.0)	3 (3.4)	3 (3.4)	85 (96.6)	.824	88 (100)	0 (0.0)	.379	85 (96.6)	3 (3.4)	.442
Male	572 (86.7)	7 (1.2)	12 (2.1)	17 (3.0)	555 (97.0)		567 (99.1)	5 (0.9)		560 (97.9)	12 (2.1)	
Province												
Outside Luanda	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)	.860	1 (100)	0 (0.0)	.930	1 (100)	0 (0.0)	.879
Luanda	659 (99.8)	7 (1.1)	15 (2.3)	20 (3.0)	639 (97.0)		654 (99.2)	5 (0.8)		644 (97.7)	15 (2.3)	
Place of residence												
Rural area	352 (49.2)	2 (0.6)	7 (2.2)	9 (2.8)	316 (97.2)	.700	323 (99.4)	2 (0.6)	.678	318 (97.8)	7 (2.2)	.840
Urban area	335 (50.8)	5 (1.5)	8 (2.4)	11 (3.3)	324 (96.7)		332 (99.1)	3 (0.9)		327 (97.6)	8 (2.4)	
Occupation												
Unemployed	60 (9.1)	1 (1.7)	2 (3.3)	3 (5.0)	57 (95.0)	.351	59 (98.3)	1 (1.7)	.394	58 (96.7)	2 (3.3)	.563
Employed	600 (90.9)	6 (1.7)	13 (2.2)	17 (2.8)	583 (97.2)		596 (99.3)	4 (0.7)		587 (97.8)	13 (2.2)	

Note: Noninfection (IgG-/IgM-); past infection (IgG+/IgM-); and recent infection (IgG-/IgM+ or IgG+/IgM+).

**TABLE 2** Sociodemographic characteristic related to recent infection with SARS-CoV-2 in Luanda, Angola

Characteristics	Univariate analysis		Multivariate analysis <sup>a</sup>	
	OR (95% CI)	P value	AOR (95% CI)	P value
Age groups				
<40 years	1.00	–	1.00	–
≥40 years	1.15 (0.41-3.22)	.785	1.20 (0.42-3.39)	.735
Gender				
Female	1.00	–	1.00	–
Male	0.61 (0.17-2.20)	.447	0.64 (0.18-2.34)	.500
Province				
Outside Luanda	0.0 (0.0-0.0)	1.000	0.0 (0.0-0.0)	1.000
Luanda	1.00	–	1.00	–
Place of residence				
Rural area	1.00	–	1.00	–
Urban area	1.11 (0.40-3.10)	.840	1.19 (0.41-3.46)	.749
Occupation				
Unemployed	1.00	–	1.00	–
Employed	0.64 (0.14-2.92)	.566	0.62 (0.12-3.08)	.557

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; OR, odds ratio.

<sup>a</sup>Adjusted for all the explanatory variables listed.

### 3 | RESULTS

#### 3.1 | Sociodemographic characteristics

The sociodemographic characteristics and identification of SARS-CoV-2 by antibody test are summarized in Table 1. A total of 660 individuals were screened for antibodies against SARS-CoV-2 between July and September 2020. Of these, 572 (86.7%) were male and 88 (13.3%) were female. The age range varied between 12 and 67 years, with an average of  $40.3 \pm 9.3$  years old. Besides that, the studied population was predominated by individuals under the age of 40 (50.2%, 331/660), residents in the province of Luanda (99.8%, 659/660), in urbanized areas (50.8%, 335/660), and employed (90.9%, 600/660).

#### 3.2 | Seroprevalence and determinants of SARS-CoV-2 infection

Of the 660 individuals screened for antibody against SARS-CoV-2, 7/660 (1.1%) were reactive for IgG and 15/660 (2.3%) were reactive for IgM (Table 1). Past infection (IgG+/IgM–) was observed in 5/660 (0.8%) individuals, whereas recent infection (IgG–/IgM+ or IgG+/IgM+) was observed in 15/660 (2.3%; Table 1). Although no statistical significance was observed ( $P > .05$ ), the data indicated that the recent infection (IgG–/IgM+ or IgG+/IgM+) was more frequent in the age group above 40 years (2.4%), in females (3.4%), in Luanda province

(2.3%), in individuals residing in urbanized areas (2.4%), and unemployed individuals (3.3%; Table 1). Besides that, we also found that the risk of exposure and recent infection to SARS-CoV-2 was higher in individuals over 40 years old (OR: 1.15 [95% CI: 0.41-3.22],  $P = .785$ ) and residents in urbanized areas (OR: 1.11 [95% CI: 0.40-3.10],  $P = .840$ ), whereas, the risk of exposure and recent infection was lower in male (OR: 0.61 [95% CI: 0.17-2.20],  $P = .447$ ) and employed individuals (OR: 0.64 [95% CI: 0.14-2.92],  $P = .566$ ; Table 2).

### 4 | DISCUSSION

This study demonstrated that SARS-CoV-2 has induced antibody responses in COVID-19 patients from Angola. Previous studies have shown that the seroconversion and the levels of antibodies increase during the first 2 weeks and that seropositivity is 50% until the 11th day and 100% on the 39th day after the infection.<sup>10</sup> Total antibodies are the first to be detected, followed by the IgM and IgG antibodies.<sup>10</sup> Our study showed that about 1.1% and 2.3% of the studied population were exposed to SARS-CoV-2 and had IgG and IgM antibodies, respectively (Table 1). These results indicate a low rate of exposure to SARS-CoV-2 and that exposed individuals in Angola, could be identified with antibody test in the first few weeks after exposure. Although the IgG is specific and provides evidence of previous SARS-CoV-2 infection, this does not guarantee the existence of protective and lasting immunity.

The confirmatory diagnosis of SARS-CoV-2 depends on RT-PCR. In our study, none of the IgG reactive tested positive for RT-PCR, whereas, one of the IgM reactive tested positive for RT-PCR, which indicates that the patient was probably in the acute phase of the infection (results not shown). These results might indicate that individuals with IgG are recovering from SARS-CoV-2 and could benefit from epidemiological discharge, while individuals with IgM positive should perform an RT-PCR test for confirmation of acute infection.

The national screening coverage with RT-PCR is not satisfactory, mainly in a resource-limited setting. Besides that, the RT-PCR is an expensive tool, and during the waiting for SARS-CoV-2 confirmation, many suspect individuals do not receive treatment, and the decision to comply with the quarantine is not established. The aspects raised, can make the diagnosis of SARS-CoV-2 one of the biggest bottlenecks in the COVID-19 response. Therefore, our results showed that immunoassays could be a supplement in epidemiological studies to identify recent or past infections, humoral immunity, quarantine decision, and timely clinical decision.

About 2.3% and 0.8% of the studied population showed recent and past infections, respectively (Table 1). The higher frequency of recent infection could be attributed to the fact that Angola is experiencing a community transmission of SARS-CoV-2, especially in Luanda, which is the epicenter of the COVID-19 in Angola. Therefore, in a context of community transmission,<sup>15</sup> individuals with recent infection should also be given priority in public

health, to receive clinical care capable of interrupting the SARS-CoV-2 transmission. The issues raised deserve attention, especially in urbanized areas, which had a higher frequency of IgG (1.5%) and IgM (2.4%), compared to rural areas which presented 0.6% and 2.2%, for IgG and IgM, respectively. On the other hand, although the unemployed and women had a higher frequency of recent infection, urgent attention should be paid to individuals aged 40 years and over, who in addition to having a higher frequency of recent infection (Table 1), were also more likely to get the SARS-CoV-2 (AOR: 1.20,  $P = .735$ ; Table 2). Therefore, serological tests could be used for monitoring the adult population, especially in a community where there are limitations regarding molecular tests.

There are limitations to the present study. First, we did not determine the duration of the IgG or IgM antibodies. Second, we did not assess the cross-reactivity of SARS-CoV-2 with other coronaviruses. Third, there were no used other serological tests including the determination of total antibodies, to compare the effectiveness of detecting SARS-CoV-2. Finally, the low antibody prevalence may not allow a statistically significant to support the conclusions. The weaknesses showed that the presence of IgG or IgM antibodies against SARS-CoV-2 in Angola deserves further investigation. Even so, our results may reinforce ongoing strategies for diagnosis and control of the COVID-19 in Angola. Future studies should be carried out to determine the duration of IgG and IgM antibodies in COVID-19 patients from Angola.

In conclusion, our results showed that serological tests for IgG and IgM antibodies can be applied to assess SARS-CoV-2 attack rates in the general population and contribute to timely clinical management of COVID-19 patients in Angola.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## ETHICS STATEMENT

The study was approved by the National Ethics Committee of Angola (nr.25/2020). Participants or legal guardians of minors were informed of the study before being included in the study.

## AUTHOR CONTRIBUTIONS

Conceptualization: Cruz S. Sebastião, Zoraima Neto, Jocelyne Neto de Vasconcelos, and Joana Morais

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Writing—Original Draft Preparation: Cruz S. Sebastião

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All authors approved the final manuscript for publication.

All authors have read and approved the final version of the manuscript. The corresponding author had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis

## DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article.

## TRANSPARENCY STATEMENT

The lead author Cruz S. Sebastião affirms that no important aspects of the study have been omitted.

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