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ORIGINAL ARTICLE

Random SARS-CoV-2 antibody screening in Egypt during the COVID-19 third wave

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ABSTRACT

Background: *Since its first hit in 2019, COVID-19 pandemic has caused devastating consequences all over the globe. Serological testing can assess the level of humoral immune response and can guide for appropriate health decisions. Objective: This work aimed to test performance agreement between rapid tests and ELISA in serological detection of COVID-19 antibodies among generally randomized Egyptian participants.* ***Methodology:*** *Total 238 randomized Egyptian participants were serologically screened for SARS-CoV-2 IgM and IgG using COVID-19 IgM/IgG Combo rapid test and NovaLisa SARS-CoV-2 IgM and IgG ELISA kit in the period from March 2021 to June 2021 (pandemic third wave).* ***Result:*** *COVID-19 antibodies showed seroprevalence rate of 47.47%, distributed among symptomatic and asymptomatic individuals by rates of 51% and 42.5%, respectively. IgM and IgG antibodies had rates of 8.8% and 35.6%, while rates of 10.9% and 28.2% by ELISA respectively. The agreement between ELISA and rapid test was none to slight for IgM ($p = 0.35$), while fair for IgG; ($p < 0.001$).* ***Conclusion:*** *COVID-19 antibodies were positive in nearly half of enrolled participants. Rapid test showed fair agreement for IgG, while none to slight agreement for IgM with ELISA, thus can not replace ELISA in serological testing.*

INTRODUCTION

COVID-19 infection by SARS-CoV-2 virus; a novel corona virus is a violent global crisis that first hit Wuhan, China in 2019, then turned to a pandemic in 2020 affecting 216 countries worldwide[1]. Since its emergence, COVID-19 has increased in accelerating rates with significant deaths and devastating health and socioeconomic consequences all over the globe[2]. According to the WHO, the global latest estimate for confirmed cases of COVID-19 was 752,517,552 including 6,804,491 deaths[3]. In Egypt, the total number of COVID-19 confirmed cases were estimated at 515,609 with 24,805 deaths[3]. The SARS-CoV-2 virus causes respiratory illness of variable severity and is characterized by high transmissibility and adverse complications, especially in immunocompromised or patients with underlying chronic diseases[1].

What is confusing about this disease is the overlap of its symptoms with other respiratory illnesses which makes highly accurate laboratory tests inevitably necessary to establish confident diagnosis[1]. At current, most of confirmed COVID-19 reports come from tests using polymerase chain reaction (PCR) that can early detect the viral ribonucleic acid (RNA) in different respiratory specimens and identify actively infected people who need isolation[4]. However, although being rapid and sensitive, PCR tests are impacted by several factors that affect their sensitivity related to the type of samples, collection method and transport, besides their need for well-trained laboratory personnel and specialized equipment[5]. PCR tests are unable to determine who had been previously exposed to the virus, or give information on the developed immunity against SARS-CoV-2, thus considered inconvenient tool for mass screening of COVID-19 among general population including asymptomatic carriers[4]. This has driven attention to the necessity of developing complementary immunoassays that use finger prick, blood or serum samples to detect viral IgM and IgG antibodies. IgM is an early prototype that appears about 5 days after the start of infection and has an intermediate binding potency to the virus, while IgG antibody has a higher binding strength to the virus and appears 8-10 days after infection[6]. Antibody tests can be used as surveillance tools being able to distinguish immune individuals who can safely resume their work and social activities from those susceptible to infection, and recognize hotspots in population with low immunity[5,7].

By entering the third wave of the Covid-19 pandemic in 2021, it will have been a year since the beginning of the pandemic in 2020. During this period, the rapid spread of the virus and the high rates of infection with intensive vaccination campaigns coupled with the theory of 'herd immunity' all make it expected that there would be increasing immunity among the general population over time. From this perspective, we aimed to assess the performance of lateral flow immunoassay rapid diagnostic tests (RDT) compared to enzyme-linked immunosorbent assay (ELISA) in estimating the seroprevalence of COVID-19 antibodies, in an attempt to assess the magnitude of immunity developed against SARS-CoV-2

METHODOLOGY

Our study was conducted in the period from March 2021 to June 2021 (third wave COVID-19 strike as announced in Egypt), on randomized 238 Egyptian participants, where demographic data (age and sex) as well as history of symptoms and underlying chronic illnesses were collected through direct questioning of enrolled participants. "Symptomatic" individuals have been defined as those who suffer from symptoms suggestive of COVID-19 such as: cough, dyspnea, sore-throat, anosmia, diarrhea, fatigue, headache either during the past 6 months or currently since >5 days from date of sample collection. Individuals who started their symptoms on the day of testing or as early as < 3-4 days from date of testing were excluded as antibodies would not have risen enough for being detected in serum. Individuals who have not given any history of relevant symptoms for the past six months were defined as "asymptomatic". Two ml blood samples were collected from each participant in the period from March 2021 to June 2021. Samples were screened for IgM and IgG antibodies using 2 serological methods: rapid COVID-19 IgM/IgG Combo kit (SD Biosensor, Inc., MT Promedt Consulting GmbH, Germany, REF: Q-NCOV-01C) and Enzyme linked immunosorbent assays (ELISA); NovaLisa®SARS-CoV-2 (Covid19) IgM and IgG (NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany, product no.: COVM0940 & COVG0940).

SD Biosensor COVID-19 IgM/IgG Test is a rapid immunochromatographic assay for qualitative detection of specific SARS-CoV-2 antibodies (nucleocapsid(N) protein antibodies and Spike RBD antibodies) in human plasma, serum or whole blood, with a test time of 10-15 minutes[8]. This test is intended for use as an initial screening tool to diagnose COVID-19 infection in convalescent phase of patient[8].

ELISA assay; NovaLisa®SARS-CoV-2 (Covid19) IgM and IgG was used for semi-quantitative detection of IgM and IgG and was performed according to the manufacturer procedure. In brief, samples, blanks and standards were added with a volume of 100 µl in different wells of the plate, sealed by foil and incubated at 37° ± 1°C for one hour ± 5 min. Afterwards, a washing buffer (300 µl) was added to wash wells for 3 times. A conjugate was added with a volume of 100 µl to wells except for the blank well, then incubated at room temperature for 30 minutes, followed by washing (3 times) with washing buffer (300 µl). Substrate solution (100 µl) was added to all wells, and incubated at room temperature for 15 minutes in dark. To stop the enzyme reaction, stop solution (100 µl) was quickly added into each well, then the plate was read on an ELISA reader at a wave length 450/620 nm within 30 minutes after adding the stop solution[9]. Results were expressed in the form of NovaTec Units (NTU), calculated as a ratio of absorbances value of sample to cut-off controls and interpreted as positive: > 11, equivocal:9-11, and negative: < 9[9,10].

Ethical Statement

This study was reviewed and approved by the Research Ethics Committee (REC) of Faculty of medicine-Cairo University (approval number: N-108-2020)

Statistical analysis

Statistical tests were carried out using SPSS Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA version 15 for Microsoft Windows (Microsoft Corp., Redmond, WA). Categorical agreement in performance between the 2 serological methods was calculated with Cohen's kappa coefficient and interpreted as follows: no agreement (values ≤ 0), none to slight (0.01–0.20), fair (0.21–0.40), moderate (0.41– 0.60), substantial (0.61–0.80) and perfect (0.81–1.00)[11].

RESULTS

The present study was conducted with serum samples collected from a total number of 238 participants who had ages with mean +/- SD of 38.23 ± 14.2 and median (IQR) of 38 (28-48). Male and female gender were distributed at 31.9% and 68.1%, respectively. Symptomatic participants who gave history of symptoms relevant to COVID-19, either in the last 6 months or began showing symptoms since > 7days of serological testing, accounted for 54.6% (130/238) of all enrolled participants. All symptoms given by participants were described in **Table 1**, showing the highest rates for weakness (66.15%) and bony pains (65.3%). No history of symptoms was given in 108/238 (45.3%) of participants i.e. asymptomatic. Underlying comorbidities were stratified as illustrated in **Figure 1**, demonstrating the predominance of hypertension, diabetes, chest and cardiac diseases. Four participants gave history of underlying autoimmune diseases (1.7%)

Table 1: Clinical symptoms among symptomatic participants. (n= 130)

Characteristics		N (%)
Fever	Present	50 (38.4%)
	Absent	80 (61.5%)
Bony pains	Present	85 (65.3%)
	Absent	45 (34.6%)
Weakness	Present	86 (66.15%)
	Absent	44 (33.8%)
Throat pain	Present	25 (19.2%)
	Absent	105 (80.7%)
Dyspnea	Present	64 (49.2%)
	Absent	66 (50.7%)
Cough	Present	57 (43.8%)
	Absent	73 (56.1%)
Diarrhea	Present	66 (50.7%)
	Absent	64 (49.2%)
Headache	Present	35 (26.9%)
	Absent	95 (73%)

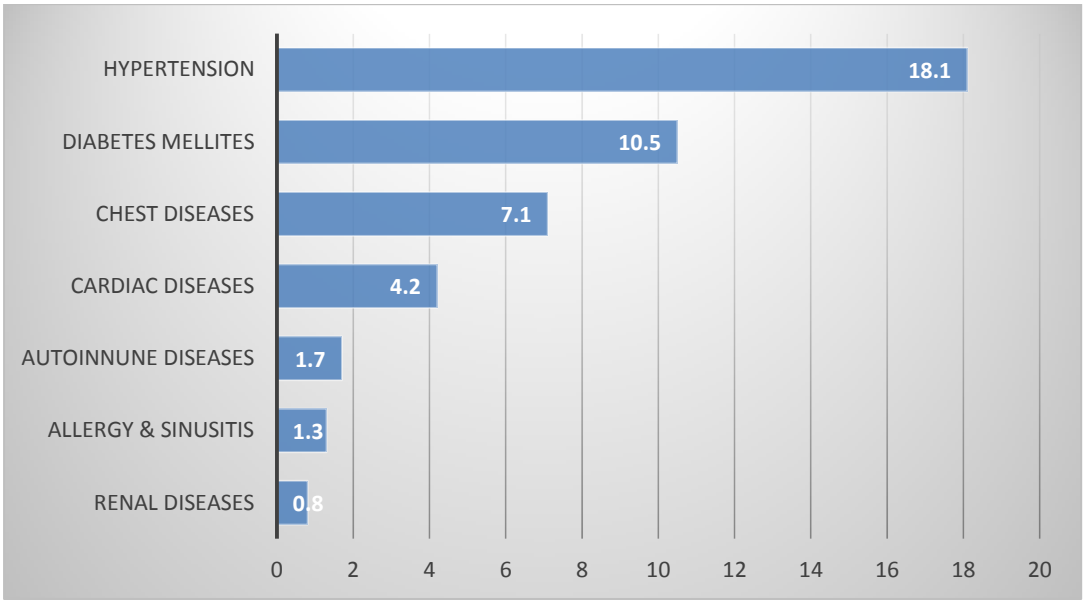

Fig.1: Underlying comorbidities among participants.

Out of total enrolled participants (n=238), serological screening for COVID-19 antibodies (IgM and IgG) was performed to 174 by both rapid tests and ELISA, while done to 64 cases by rapid tests only. Screening results by rapid tests and ELISA are detailed in **Table 2**. As for rapid tests, screening results showed that positive IgM was clearly identified at 21/238 (8.8%) and positive IgG at 85/238 (35.6%). Screening by ELISA showed positive detection of each of IgM and IgG in 10.9% and 28.2%, respectively, among tested population (n=174). Equivocal results for screened antibodies by each of rapid tests and ELISA are described in **Table 2**.

Table 2: Results of Rapid Diagnostic Test (RDT) & ELISA assay among participants.

		Results of RDT (n=238)		Results of ELISA (n= 174)*	
		no.	%	no.	%
IgM	Negative	182	76.5	127	73.4
	Equivocal	35	14.7	26	15
	Positive	21	8.8	20	11.6
IgG	Negative	138	58	115	66.1
	Equivocal	15	6.3	10	5.7
	Positive	85	35.7	49	28.2

RDT: rapid diagnostic test by COVID-19 IgM/IgG Combo kit (SD Biosensor), ELISA: Enzyme linked immunosorbent assay (NovaLisa®SARS-COV-2 (Covid19) IgM and IgG, (*): ELISA was performed only on 174 out of all serum samples of enrolled participants.

Results of performance agreement between rapid test and ELISA in detecting each of IgM and IgG among 174 bi-screened cases are displayed in **Table 3**. None to slight agreement between the 2 tests was shown regarding IgM; $\kappa = 0.054$, $p = 0.35$, while fair agreement regarding IgG; $\kappa = 0.387$, $p < 0.001$.

Table 3: Analytical agreement between results of RDT and ELISA among bi-screened 174 participants (n=174).

IgM					IgG				
		ELISA					ELISA		
		Negative	Equivocal	Positive			Negative	Equivocal	Positive
RDT	Negative	94	20	12	RDT	Negative	79	3	10
	Equivocal	22	5	4		Equivocal	8	2	3
	Positive	11	1	4		Positive	28	5	36

RDT: rapid diagnostic test by COVID-19 IgM/IgG Combo kit (SD Biosensor), ELISA: Enzyme linked immunosorbent assay (NovaLisa®SARS-COV-2 (Covid19) IgM and IgG

Among total screened participants (n=238) by rapid tests, overall seropositive cases for COVID-19 antibodies accounted for 113 (47.47%), distributed among symptomatic and asymptomatic individuals with rates of 51% (67/130) and 42.5% (46/108), respectively. No serum antibodies were detected in 125 (52.5%) of all tested participants, of which 63 (50.4%) and 62 (49.6%) were symptomatic and asymptomatic, respectively.

Among asymptomatic individuals (n=108), 25 and 38 revealed positive each of IgM and IgG by rapid test with prevalence rates of 23.14% and 29.2%, respectively, including cases with equivocal results (weak positivity). Symptomatic individuals gave positive detection of IgM in 31/130 and IgG in 58/130 by rapid tests, with rates of 23.8% and 44.6%, respectively. All findings of tested IgM and IgG by rapid tests among symptomatic and asymptomatic cases are detailed in **Table 4**. Among seronegative participants with absent antibodies in serum samples, 22.4% (28/125) had history of underlying comorbidities, including 2 cases with autoimmune diseases (**Figure 2**).

Table 4: Distribution of positive and negative antibody detection results by rapid diagnostic test (RDT) among symptomatic and asymptomatic participants.

	Total screened cases by RDT (n=238)								
	Positive antibody detection (n=113)								Negative antibody detection (n=125)
	IgM/IgG (+/-)	IgM/IgG (E/-)	IgM/IgG (-/+)	IgM/IgG (-/E)	IgM/IgG (+/+)	IgM/IgG (E/E)	IgM/IgG (E/+)	IgM/IgG (+/E)	IgM/IgG (-/-)
Symptomatic (n=130)	0	5	32	4	10	6	9	1	63
Asymptomatic (n=108)	1	7	18	3	8	8	6	1	62

RDT: rapid diagnostic test by COVID-19 IgM/IgG Combo kit (SD Biosensor), (+): positive antibody detection, (-): negative antibody detection, (E): Equivocal

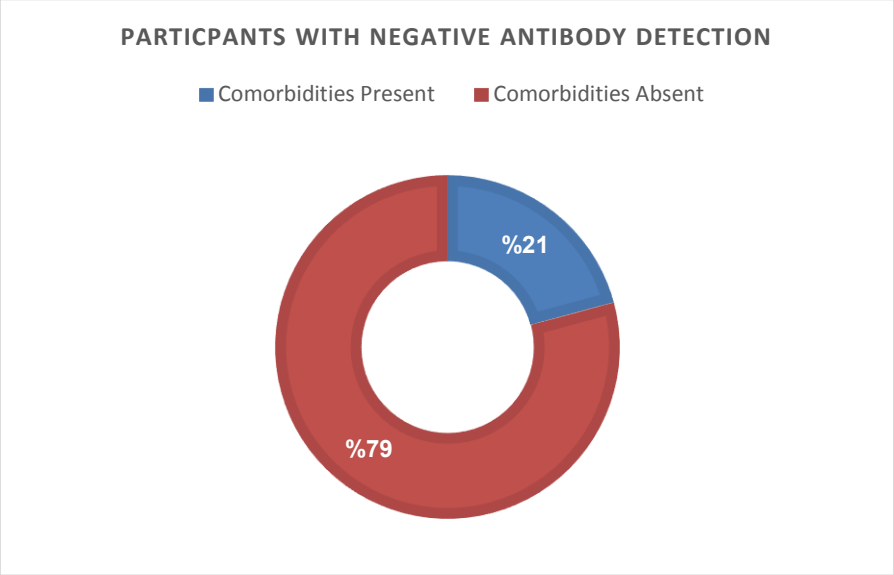

Fig. 2: Underlying comorbidities among seronegative participants by rapid diagnostic test

DISCUSSION

Molecular and serological testing are two main approaches for diagnosis of COVID-19, whereas serological antibody testing can reflect the immune status of population against SARS-CoV-2. The present work aimed to investigate the performance of rapid antibody tests against ELISA assays in testing the seroprevalence of COVID-19 antibodies among 238 recruited population, where rapid tests were conducted on all serum samples (n=238), while ELISA was performed on 174 samples. It is worth to note that we had no available access to documents on the vaccination status for enrolled participants. Our study reported a total seropositivity rate of 47.4% for COVID-19 antibodies, which aligns well with previously reported rates with range of 50-95% by several studies[12-14]. Compared to our study, higher seropositivity rates in some studies, which may reach as high as 100%[15] can be explained by their studies conduct with laboratory confirmed COVID-19 patients, as well as performing serological testing at no earlier than 2 weeks from onset of symptoms, enabling for higher likelihood of positive antibody detection. This is in contrast to our study, where COVID-19 antibodies were screened among general population without any prior laboratory confirmation of COVID-19 and serological testing was performed as early as 7 days from onset of symptoms. On the other hand, the overall seroprevalence rate in our study is considerably higher than estimates reported by studies in other countries as in US (2.8%-14%)[16–18] and in Spain (5·0%)[19]. The observed variations in estimates among different studies in different countries might be due to different study populations, testing methods with varying sensitivities and specificities. Moreover, another key factors that cannot be ignored are different epidemic conditions and applied health protocols that were initiated earlier in some countries than others[18–20].

The participants enrolled in our study had median age of 38 (IQR: 28-48) and were predominantly females (68.1%), which was close to demographic data described in various studies[14]. Our study adopted a random enrollment of participants from general population for COVID-19 serological screening and was not only confined to laboratory-confirmed COVID-19 patients by PCR. This is based on the fact that some SARS-CoV-2 infected patients do not express any symptoms or signs, yet can transmit the virus to the surrounding population[21–23]. Symptomatic and asymptomatic groups were found in comparable rates of 54.6% and 45.3%, respectively, with the symptomatic group predominant, nevertheless asymptomatic group accounted for a considerable rate. This was supported by an Italian study that found 44% of laboratory-confirmed cases of SARS-CoV-2 not suffering any symptoms[24]. As referred to several studies, the most commonly reported symptoms of COVID-19 were fever, fatigue and cough, while diarrhea, nasal congestion and headache were reported less commonly[25–27]. This agreed well with the findings in our study, apart from that fever was less ranked beyond the observed higher top symptoms (fatigue, bony pains, cough and sore throat), unlike the above studies, where fever was the dominant feature among symptomatic COVID-19 patients. Nearly half of asymptomatic group were found as seropositive at an estimated rate of 42.5%, which is consistent with reported rates by previous meta-analysis studies and is in line with the fact of might getting infected with SARS-CoV-2 without any explicit symptoms[21,28,29]. This sheds light

on the importance of adherence to epidemic protocols and regulations for wearing masks and physical distancing among the general population, not only among contact individuals to COVID-19 patients, in order to mitigate transmission of SARS-CoV-2 in the community[28]. In our study, the seronegative group showed nearly equal proportions of symptomatic and asymptomatic individuals. Absent COVID-19 antibodies do not necessarily rule out COVID-19 infection, however there are various possibilities that may explain negative antibodies in SARS-CoV-2 infected patients including: i) delayed production of antibodies at the beginning of illness or their early disappearance in blood by the end of the disease course, ii) low antibody concentrations in serum below the detection limit by the testing serological methods, especially those with poor diagnostic sensitivity, iii) presence of underlying comorbidities or autoimmune diseases which impairs immune response and antibody production[30]. In our study, comorbidities were noted in 22.4% (28/125) of seronegative individuals including 2 cases with underlying autoimmune disease.

Although SARS-CoV-2 PCR assay is currently the standard diagnostic method for COVID-19 being specific and efficient, yet it suffers several limitations including intensive labor, long turn-around time, need for well trained technicians added to cost barriers, which all stand against the use of PCR in the rapid screening of COVID-19 in a large population[30,31]. Moreover, PCR assays might be challenged by false negative results because of inadequate sampling or inappropriate testing time from the time of symptoms onset[21]. These limitations urged the employ of serological methods that enable quick identification of COVID-19 through serum detection of specific SARS-CoV-2 antibodies[30]. Compared to PCR assay, serological tests are simpler and more time-saving, thus can be more convenient to use in large-scale screening especially among asymptomatic carriers[22,23], due to a broader window of SARS-CoV-2 detection[21].

Since the beginning of coronavirus outbreak, the market has been flooded by many commercial serological antibody tests for COVID- 19[32] including enzyme-linked immunosorbent assay (ELISA), and lateral flow immunochromatographic assays (LFIA). In our study, SD-Biosensor LFIA was performed on all serum samples, while ELISA assay was used to test only 174 sera. Unfortunately, due to financial issues, ELISA kits could not have been afforded to complete testing for the rest of samples. LFIA are rapid diagnostic tests (RDT) characterized by simplicity, short time assay and high-throughput making it convenient as point-of care- tests (POC)[33]. On the contrary, ELISA has relatively longer time assay and needs well trained technicians and special equipment[33]. However, ELISA enables quantitative antibody detection offering an added value to identify donors of convalescent plasma for treatment and verify immune response to vaccines in the future[34]. According to the manufacturer, SD-Biosensor RDT has an overall sensitivity and specificity of 99.03% and 98.65%, respectively. As referred to previous studies, sensitivities and specificities of SD-Biosensor RDT were reported at 78.9% and 98.3% for IgM, while 94.5% and 96.6% for IgG, respectively[35]. For ELISA assay (NovaLisa), one study reported sensitivities of 94.4% and 48.7%, while specificities of 96.2%and 98.7%, for IgG and IgM, respectively[36]. Another study that used ELISA for only IgG reported sensitivity and specificity of 86.4%and 85.7%, respectively[37]. It was not possible for our study to evaluate analytical sensitivities and specificities of the performed RDT and ELISA, because our study did not involve PCR as a gold standard test for diagnosis of SARS-CoV-2 infection. Nevertheless, our study found significant moderate agreement between RDT (SD-Biosensor) and ELISA (NovaLisa) for IgG, while insignificant weak agreement for IgM. In general, technical variations in sensitivities and specificities between immunoassays have several explanations related to number and type of SARS-CoV-2–derived antigens[8,38,39].

In the present study, we attempted to assess the immune status and SARS-CoV2 antibody response among Egyptian population, which in turn can prime for establishing appropriate infection control policies and offer enlightened guidance for relevant public health decisions, however we were challenged by low number of recruited participants. It is worth note that our study faced several limitations, including : first, because of the financial barriers we encountered in our study, the ELISA test could not be afforded for all participants; second, the nature of one point-testing in the mass screening with no follow up samples, as well as the lack of data on time duration from symptoms onset to serological testing among symptomatic patients, which did not allow for assessing the timing of seroconversion of IgM and IgG and hindered the possibility of demonstrating dynamic variations along the disease time course. Third, as we aimed for primary screening of COVID-19 antibodies, we addressed the results of ELISA qualitatively, that were interpreted as positive, negative or equivocal, however quantitative values were not recorded to allow performing analytical correlation of antibody concentrations with disease severity. Although COVID-19 antibodies can be positive either due to previous infection or vaccination, yet our study was limited by inaccessible data on the vaccination status for the enrolled participants, moreover we aimed to apply a primary screening for COVID-19 antibodies to assess the overall immune state reached by general population in the third wave, whether the antibodies were present due to previous vaccination or infection

Serological tests can be employed in the following conditions:1) patients having clinical features

suggestive of COVID-19 with negative PCR test, 2) healthy contacts who may act as asymptomatic carriers of SARS-CoV-2, 3) distinguishing immune individuals from those susceptible to COVID-19 and recognize hotspots in population with low immunity. This can offer guidance for proper health and socioeconomic decisions, moreover can allow better allocation of resources to the most needed areas[14]. A good understanding of the role of serological testing for COVID-19 can allow them to be perfectly harnessed in epidemiological sero-surveys to assess the magnitude of viral spread in community and the consequent herd

CONCLUSION

Serological testing can be helpful in understanding SARS-CoV-2 epidemiology and the level of generated humoral immunity. The overall seropositivity for COVID-19 antibodies accounted for 47.47% of all enrolled participants, however with unavailable data on vaccination status. The performance agreement between rapid test and ELISA was found to be fair for IgG, while none to slight for IgM, which may not allow for the rapid antibody tests to replace ELISA in serological screening of antibodies.

Conflict of Interest:
No conflicts of interest to be declared

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