



Sensitivities of a Rapid Test Versus an ELISA Kit for Detecting Anti-SARS-CoV-2 IgM/IgG in Sera from an Egyptian Cohort

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Abstract

This study aimed to compare diagnostic sensitivities of a rapid test (Rt) and an ELISA kit for detecting anti-SARS-CoV-2 IgM/IgG in virus-RT-PCR-positive (VPP) and virus-RT-PCR-unchecked (VPU) subjects in an Egyptian cohort during the first wave of SARS-CoV-2 infection. The results revealed higher sensitivity of the Rt for detecting IgM/IgG in the VPP subjects. Both the Rt and ELISA showed identical sensitivities for IgM detection in the VPU subjects. The ELISA was more sensitive for detecting IgG in the VPU subjects. Generally, within both the VPP and the VPU groups, Rt was more sensitive for detecting IgM/IgG among the symptomatic (S) compared to asymptomatic (AS) subjects than ELISA. Within the VPP group, the Rt was more sensitive for detecting both IgM/IgG among the AS subjects than ELISA. In the VPU group, the Rt was more sensitive for detecting IgM among the S subjects than ELISA. The ELISA was more sensitive for detecting IgM/IgG among AS subjects than the Rt. From these results we concluded that, despite the limitation of sample size, this study indicates suitability of the used Rt for detecting anti-SARS-CoV-2 IgM/IgG among S subjects and sheds light on possibility of relying on the used ELISA for IgG detection among AS human subjects.

Abbreviations

Rt	Rapid test
ELISA	Enzyme linked immune sorbent assay
VPP	Virus-RT-PCR-positive

VPU	Virus-RT-PCR-unchecked
S	Symptomatic
AS	Asymptomatic
LSS	Low socioeconomic standards
HSS	High socioeconomic standards

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Introduction

SARS-CoV-2 incubation period lasts 4–6 days and in 95% of cases the onset of symptoms occurs within 14 days [1]. It induces asymptomatic (AS), mild, moderate, severe with critical pneumonia, septic shock, and acute respiratory distress syndrome infections [2, 3]. The gold standard test for SARSCoV-2 diagnosis is RNA detection using RT-PCR, yet, its readouts are influenced by the specimen types, the used reagents, the sampling time and transportation conditions [2, 4]. Rapid tests, ELISA, and chemiluminescence immunoassays have been used for serodiagnosis of SARS-CoV-2 [5–9]. Microbes including viruses stimulate the host immune system to produce various Ig classes including IgM/IgG which circulate for different durations according to their titers [10–12]. Antibody detection diagnostics relying are more preferable than antigen detection kits due to their better sensitivity, feasibility, and simple development

[13]. Anti-spike (S) and nucleocapsid (N) IgM and IgG are targets for SARS-CoV-2 serodiagnosis [14, 15]. Therefore, the majority of commercially available SARS-CoV-2 serodiagnostics are tailored for IgM and IgG detection [16, 17]. Multiple detection of anti-SARS-CoV-2 antibodies enhanced both sensitivity and accuracy of diagnosis [18]. Spike protein subunit 1 (S1) or receptor binding domain (RBD) has been widely recommended to be included in antibody-detection-based serodiagnostics to increase the detection specificity [19–22].

Additionally, prevalence of anti-SARS-CoV-Ig-classes may report on the infection stage (Early/late or acute/chronic). After 1 week of symptom onset, 50% of COVID-19 patients turn IgM/IgG positive [23], and as the time passes, antibodies titer shows significant elevation while virus load shows decrease [24, 25]. This proposes the relevance of combining sensitive serodiagnostic assay with RT-PCR for better diagnosis of SARS-CoV-2, particularly, for detection of the intermediate/late stages of infection and for reporting on levels of disease severity [26–28].

Upon emergence/resurgence of pandemics, cocktails of diagnostics are released to the market with each manufacturer tries to claim the usefulness of his product to have the best sensitivity/specificity to diagnose infection. In particular, for pandemics caused by rapidly spreading viruses like SARS-CoV-2 appropriate rapid decision on the infection, especially for the AS subjects is needed. It is known that the first host response to infection is to elicit antibodies to pathogen antigens. This dramatically varies according to the pathogen antigen used to detect the antibody response(s) in a given host. The ideal case scenario is to rely on a rapid serodiagnostic that can detect infection as early as possible, differentiate between early/late and acute/chronic infections with readouts that might correlate with symptoms intensities. Therefore, here we compared the sensitivities of two different serodiagnostic assays to detect anti-SARS-CoV-2 IgM/IgG in virus qRT-PCR positive (VPP) and virus qRT-PCR unchecked (VPU) Egyptian human cohorts after the first wave of the SARS-CoV-2 infection.

Of note, the VPU group was intentionally included as a control cohort for the VPP group with the aim to see how useful the used serological assays are to report on infection independent of qRT-PCR results. This is of particular economic importance particularly for a developing country like Egypt where not everybody is capable to afford costs of qRT-PCR. The VPU group is composed of individuals working at the same institution, yet, of different occupational nature (daily duties), education and awareness levels. Accordingly, the VPU cohort was classified into individuals of low socioeconomic standards (LSS) and of high socioeconomic standards (HSS). The rationale behind including these two groups, is to see if our serological results will reveal

differential risk of exposure to SARS-CoV-2 infection due to differences in the socioeconomic levels.

Materials and Methods

The Study Cohorts

Age range of the study cohorts was 25–60 years, and their detailed descriptions are presented in the Supplementary Table 1. A total of 45 VPP human subjects were included, of whom, 22 were females and 23 were males. Of the 22 females 17 were symptomatic (S) and 5 were AS, whereas, of the 23 males 20 were S and 3 were AS. A total of 90 VPU subjects were included, of whom, 47 were females and 43 were males. Of the 47 females 27 were S and 20 were AS, whereas, of the 43 males 26 were S and 17 were AS.

Based on their socioeconomic standards, the VPU group included individuals of LSS and of HSS and the classification criteria were mentioned in detail in our earlier report [29]. The LSS humans were 51 including 30 females and 21 males, while, the HSS individuals were 55, including 24 females and 31 males.

Sample Collection and Antibody Detections

Collection of blood samples from the human subjects was approved by the Medical Ethical Committee at the National Research Centre, Egypt (Meeting date: 5.11.2020, Decision number: 20166) and was done in compliance with the relevant laws, institutional guidelines and according to the ethical standards mentioned in the declaration of Helsinki.

Blood samples of the VPP humans were kindly collected by our coauthors from Egypt Center for Research and Regenerative Medicine, Cairo, Egypt. SARS-CoV-2 RNA was quantified in the collected swaps from the same individuals using the TaqPath COVID-19 CE-IVD RT-PCR Kit (A51738; Thermo Fisher Scientific) according to the manufacturer instructions.

Serum samples were separated and freshly applied on the same day of collection to the AMP rapid SARS-CoV-2 IgG/IgM detection test (AMEDA Labordiagnostik GmbH; Graz, Austria) as described by the manufacturer. This test relies on both the recombinant viral spike 1 (S1) subunit and nucleocapsid (N). The rest of serum samples were kept frozen until being used in IgM/IgG quantification using the anti-2019 nCoV (N) ELISA kits (Wuhan Fine Biotech; Cat Nrs. EH4396 and EH4395) according to the manufacturer instructions. The changes in the optical densities (OD) in ELISA were measured using a multi-well plate reader (Tecan, Switzerland) at λ_{\max} 450 nm and reference wavelength 680 nm.

Statistical Analysis

We did a pre-design sample size calculation on (<https://sample-size.net/correlation-sample-size/>) to determine the minimum number of samples required to conduct comparison between the two studied group using the independent t test of unequal variance analysis, bearing in mind that the statistical power (1-beta) should not be less than 0.8, significance level threshold (alpha) should be 0.05 and results to reach this the minimum sample size of each group should be 63.

This was followed in the VPU group, while, although a smaller sample size ($n=45$) was included in the VPP group, it is still statistically acceptable and does not affect our conclusions. GraphPad PRISM version 5 software was used to carry out the statistical data analysis. We analyzed each serum samples in triplicate. The student's t test was used to calculate the values of significance by comparing mean values \pm standard deviations (SD) of the studied groups. Differences were considered significant when the P value was <0.05 . Correlation analysis was carried out by calculating the square value of the correlation coefficient (r^2) for a nonparametric and non-normally distributed data. All plots were generated using Python Seaborn package (Version 0.10.1). The Pandas library (Version 1.1.1) was used to load data sets from excel sheets into data frame objects. All analysis was carried out in a Jupyter notebook (Version 6.0.3) with a Python 3.7.7 work environment.

Results

Comparing the Rt and ELISA Sensitivities for Detecting anti-SARS-CoV-2 IgM/IgG Among the VPP and VPU Groups

Using the Rt, of the 45 VPP subjects, 28 (62.2%) were IgM and 33 (73.3%) were IgG positive, whereas, of the 90 VPU subjects, 17 (18.9%) were IgM and 51 (56.7%) were IgG positive. In the ELISA, of the 45 VPP subjects, 11 (24.4%) were IgM and 29 (64.4%) were IgG positive, whereas, of the 90 VPU subjects, 17 (18.9%) were IgM and 59 (65.5%) were

IgG positive (Table 1). From these results we can conclude that, 1) the rapid test generally showed better sensitivity for detecting IgM/IgG among the VPP group, 2) both the rapid test and ELISA showed identical sensitivities for detecting IgM among the VPU group 3) the ELISA showed better sensitivity for IgG detection among the VPU group. Of note, the cut-off value for the rapid test was zero, i.e., all values above (0) were considered positive, whereas, for ELISA the cut off values, 0.498 for IgM and 0.542 for IgG, were calculated according to the manufacturer instructions.

Analysis of IgM/IgG fold changes based on the Rt and ELISA readouts within the VPU group is presented in Fig. 1a. Within the VPU group, the percentages of IgM negative and positive subjects who show fold changes above the Rt and ELISA cut-offs are shown in Fig. 1b. Within the VPU group, the ratios of the IgG negative and positive subjects who show fold changes above the Rt test and ELISA cut-offs are presented in Fig. 1c.

Analysis of IgM/IgG fold changes based on the Rt and ELISA readouts within the VPP and VPU groups is shown in Fig. 2a. Within the VPP and VPU groups, the ratios of IgM negative and positive subjects who show fold changes above the ELISA cut-offs are presented in Fig. 2b. Within the VPP and VPU groups the percentages of IgG negative and positive subjects who show fold changes above the ELISA cut-offs are presented in Fig. 2c.

Comparing the Rt and ELISA Sensitivities for Detecting Anti-SARS-CoV-2 IgM/IgG Among Subjects of the Two Different Socioeconomic Standards Within the VPU Group

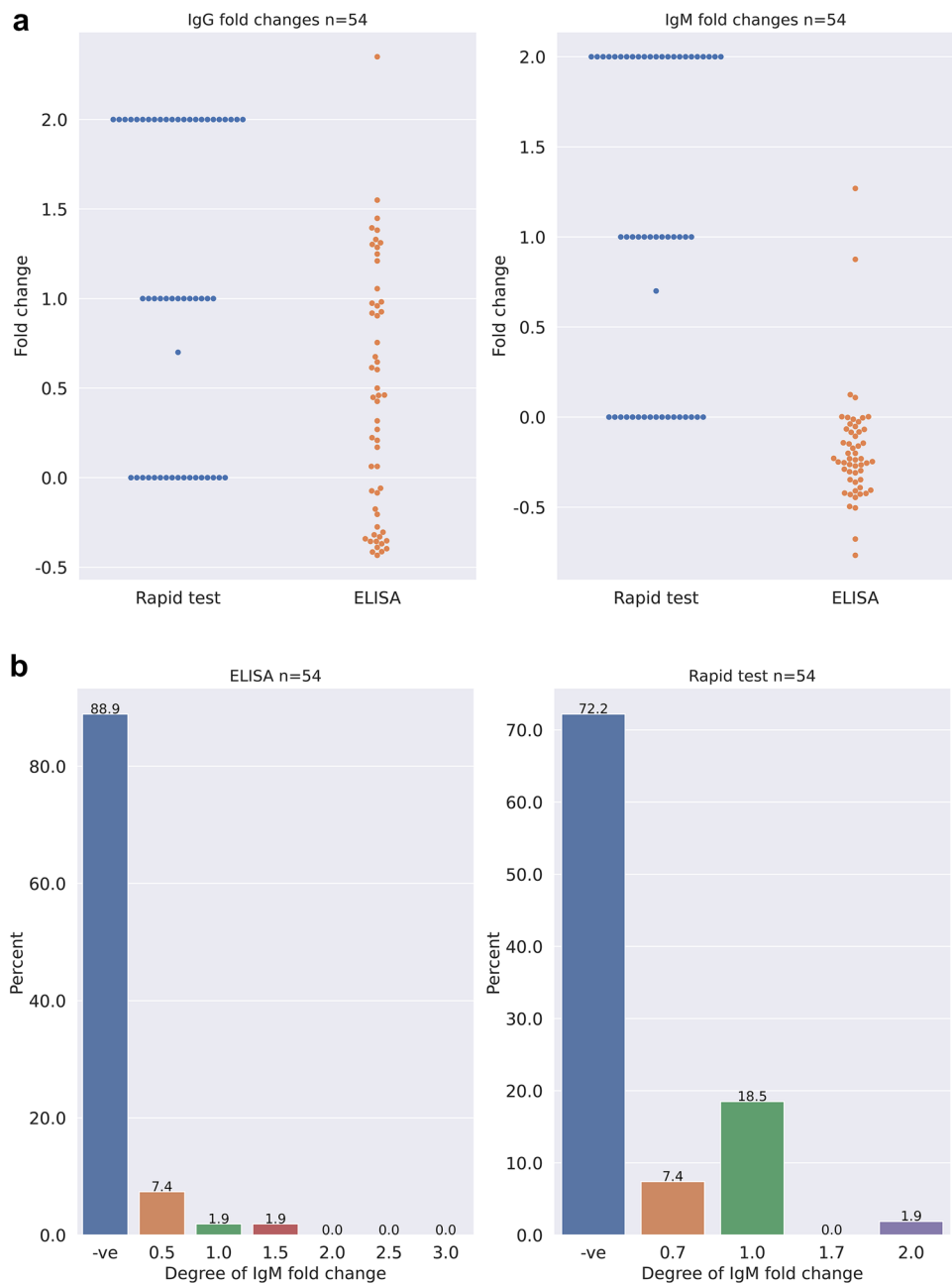
Within the VPU group, the ratios of IgM/IgG negative and positive subjects of L and H socioeconomic standards (SS) who show fold changes above the ELISA cut-offs are shown in Fig. 3. This set of results revealed that (1) higher sensitivity of ELISA for detecting anti-SARS-CoV-2 IgG among subjects of the two different SS and (2) interestingly, the highest ratio of IgG fold change was reported among the HSS subjects compared to the LSS one.

Table 1 Comparing sensitivities of the rapid test and ELISA for detecting anti-SARS-CoV-2 IgM/IgG among the virus-RT-PCR positive (VPP) and virus-RT-PCR unchecked (VPU) subjects

Test	Total ($n=135$)							
	Rapid test				ELISA			
	VPP ($n=45$)		VPU ($n=90$)		VPP ($n=45$)		VPU ($n=90$)	
Subjects	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
Prevalence (%)	28/45 (62.2)	33/45 (73.3)	17/90 (18.8)	51/90 (56.6)	11/45 (24.4)	29/45 (64.4)	17/90 (18.8)	59/90 (65.5)

n number of samples; VPP virus-RT-PCR positive; VPU virus-RT-PCR unchecked

Fig. 1 **a** Fold changes in the viral-specific IgM (Right) and IgG (Left) of positive individuals ($n = 54$) recorded both by the rapid test and ELISA within the virus RT-PCR unchecked (VPU) group. **b** Distribution of the viral-specific IgM fold change degrees among the positive individuals ($n = 54$) recorded both by the rapid test (Right) and ELISA (Left) within the VPU group. **c** Distribution of the viral-specific IgG fold change degrees among the positive individuals recorded both by the rapid test (Right; $n = 54$) and ELISA (Left) within the VPU group. The rapid test cut-off value was (0), i.e., any value above 0 was considered positive. The ELISA cut-off values were 0.498 for the IgM and 0.542 for IgG. The Pandas library (Version 1.1.1) was used to load data sets from excel sheets into data frame objects. All analysis was carried out in a Jupyter notebook (Version 6.0.3) with a Python 3.7.7 work environment. All plots were generated using Python Seaborn package (Version 0.10.1).

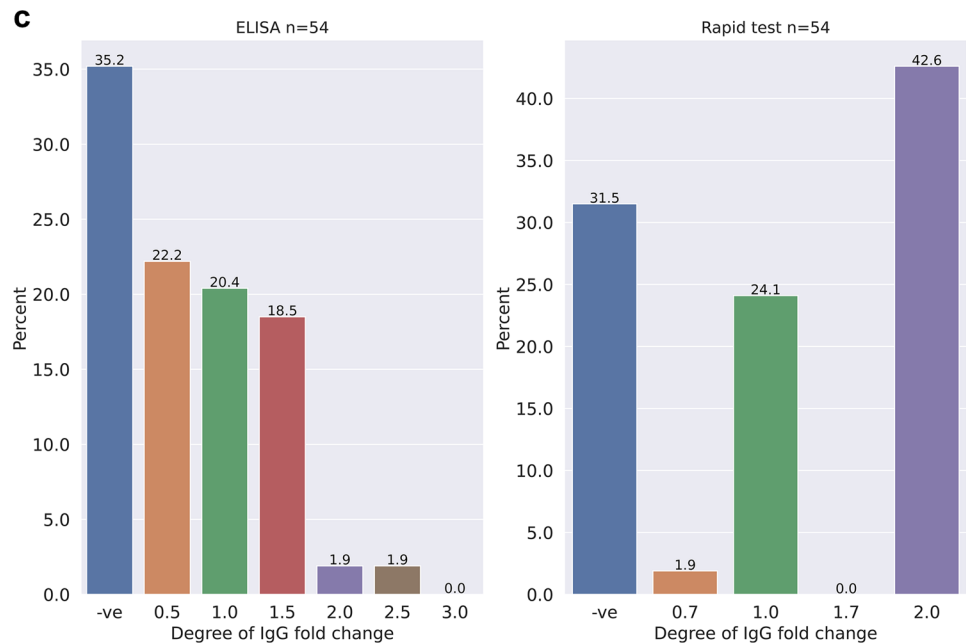


Comparing the Rt and ELISA Sensitivities for Detecting Anti-SARS-CoV-2IgM/IgG Between Genders

Using the Rt, of the 22 VPP females, 7 (31.8%) were IgM positive and 13 (59.1%) were IgG positive, whereas, of the 47 VPU females, 10 (21.3%) were IgM positive and 29 (61.7%) were IgG. In ELISA, of the 22 VPP females, 4 (18.2%) were IgM positive and 10 (45.5%) were IgG positive, whereas of the 47 VPU females, 14 (29.8%) were IgM positive and 37 (78.7%) were IgG positive (Table 2). Using the Rt, of the 23 VPP males, 21 (91.3%) were both IgM/

IgG positive, whereas, of the 43 VPU males, 7 (16.3%) were IgM positive and 22 (51.2%) were IgG positive. Using ELISA, of the 23 VPP males, 7 (30.4%) were IgM positive and 18 (78.3%) were IgG positive, whereas of the 43 VPU males, 3 (7%) were IgM positive and 22 (51.1%) were IgG positive (Table 2). From these results we can conclude that, (1) the Rt was generally highly sensitive than ELISA for detecting both IgM/IgG among the VPP females, (2) in contrast, ELISA sensitivity for detecting both IgM/IgG was generally higher than the Rt among the VPU females, (3) the Rt sensitivity for detecting both IgM/IgG was higher than ELISA among males of both the VPP and VPU groups and

Fig. 1 (continued)



the higher sensitivity of IgM/IgG detection was recorded by the Rt among the VPP males.

Comparing the Rt and ELISA Sensitivities for Detecting Anti-SARS-CoV-2 IgM/IgG Among Symptomatic and Asymptomatic Subjects

Using the Rt, of the 37 VPPS subjects, 25 (67.6%) were IgM positive and 29 (78.4%) were IgG positive, whereas, of the 8 VPPAS subjects, 3 (37.5%) were IgM positive and 5 (62.5%) were IgG positive. In ELISA, of the 37 VPPS subjects, 9 (24.3%) were IgM positive and 25 (67.6%) were IgG positive, whereas, of the 8 VPPAS subjects, 2 (25%) were IgM positive and 4 (50%) were IgG positive (Table 3). From these results we concluded that, (1) the Rt was more sensitive for detecting IgM/IgG among the VPPS subjects than among the VPPAS one, (2) generally, ELISA sensitivity for detecting IgM was comparable among both the VPPS and VPPAS subjects, whereas, it showed higher sensitivity for detecting IgG among the VPPS subjects compared to VPPAS ones, (3) the Rt was more sensitive than ELISA for detecting IgM/IgG among both the VPPS and VPPAS subjects. Using the Rt, of the 53 VPUS subjects, 14 (26.4%) were IgM positive and 37 (69.8%) were IgG positive, whereas, of the 37 VPUAS subjects, 2 (5.4%) were IgM positive and 12 (32.4%) were IgG positive. In ELISA, of the 53 VPUS subjects, 6 (11.3%) were IgM positive and 36 (67.9%) were IgG positive, whereas, of the 37 VPUAS subjects, 11 (29.7%) were IgM positive and 22 (59.5%) were IgG positive (Table 4). From these results we concluded that, (1) the Rt was highly sensitive than ELISA for detecting IgM/IgG among VPUS subjects compared to VPUAS one, (2) ELISA was better sensitive than the Rt for

detecting IgM among the VPUAS subjects compared to VPUS ones, whereas, it showed higher sensitivity than the Rt for detecting IgG among the VPUS subjects compared to VPUAS ones, (3) the Rt was more sensitive than ELISA for detecting IgM among the VPUS subjects compared to ELISA, and (4) the ELISA was highly sensitive than the Rt for detecting IgM/IgG among the VPUAS subjects.

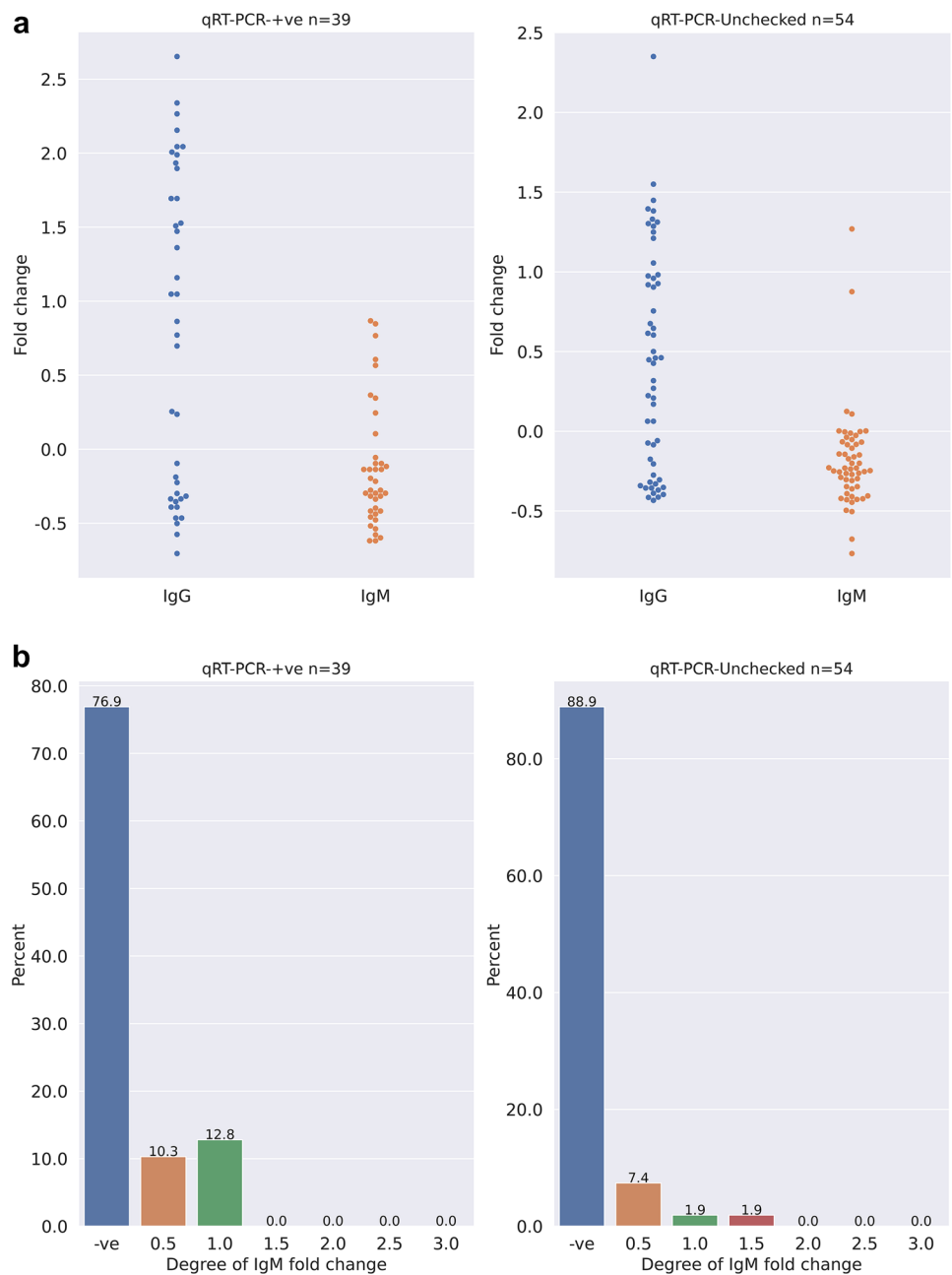
Comparing the General and the Gender-Specific Means of the Anti-SARS-CoV-2 IgM/IgG Among the VPP and VPU Subjects Measured Using the Rt and ELISA

The results revealed neither significant differences between IgM/IgG means of the two used serodiagnostics (supplementary Table 2) nor between IgM/IgG means of the two used assays among the genders (Supplementary Tables 3, 4).

Correlation Between Symptom Grades and Anti-SARS-CoV-2 IgM/IgG Levels Among the Study Cohorts

The results revealed an inverse correlation between the symptom grades and sero-prevalence of the anti-SARS-CoV-2 IgM/IgG that was significant (P value = 0.008) in case of the measured IgG by the Rt (Supplementary Table 5).

Fig. 2 **a** Fold changes in the virus-specific IgM/IgG positive individuals within the virus qRT-PCR-unchecked group (Right; $n = 54$) and of virus-specific IgM/IgG positive individuals within the virus qRT-PCR-positive group (Left; $n = 39$) recorded both by the rapid test and ELISA. **b** Distribution of the virus-specific IgM fold change degrees among the virus-specific IgM/IgG positive individuals within the virus qRT-PCR-unchecked group (Right; $n = 54$) and within the virus qRT-PCR positive individuals (Left; $n = 39$). **c** Distribution of the virus-specific IgG fold change degrees among the virus-specific IgM/IgG positive individuals within the qRT-PCR-unchecked group (Right; $n = 54$) and within the virus qRT-PCR positive individuals (Left; $n = 39$). The rapid test cut-off value was (0), i.e., any value above 0 was considered positive. The ELISA cut-off values were 0.498 for the IgM and 0.542 for IgG. The Pandas library (Version 1.1.1) was used to load data sets from excel sheets into data frame objects. All analysis was carried out in a Jupyter notebook (Version 6.0.3) with a Python 3.7.7 work environment. All plots were generated using Python Seaborn package (Version 0.10.1).

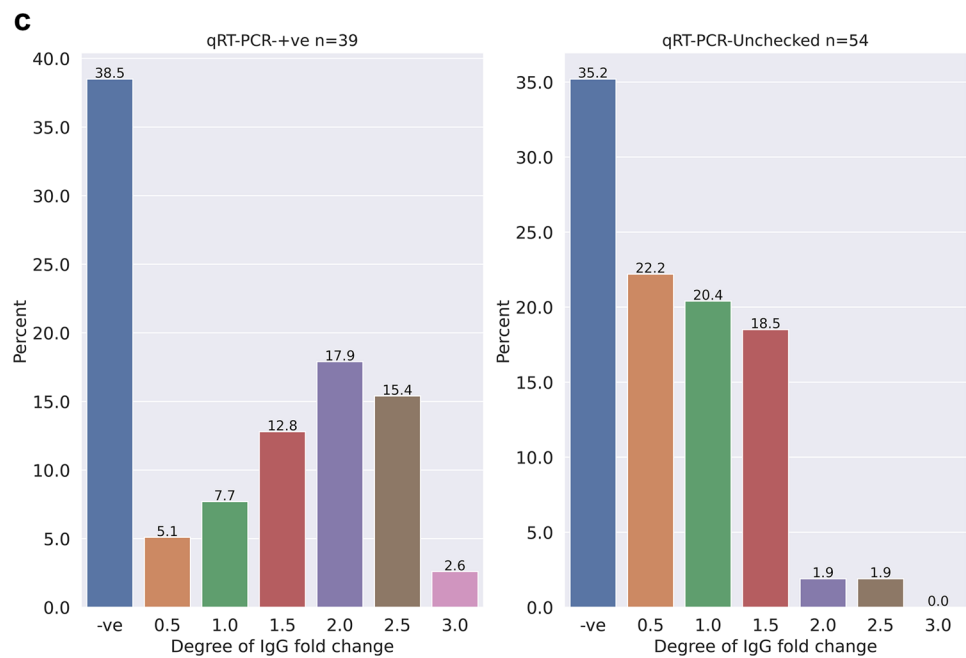


Discussion

To the best of our knowledge the current study is the first in Egypt to compare sensitivities of a rapid test and an ELISA kit for detecting anti-SARS-CoV-2 IgM/IgG in well characterized serum samples. We intentionally used the VPU group as a control for the VPP group with the aim to see how useful the used serodiagnostics are to report on infection independent of qRT-PCR results. This is of particular economic importance particularly for a developing country like Egypt where not everybody is capable to afford costs of qRT-PCR. Additionally, in an earlier report, we tested

implication of both socioeconomic level and gender on prevalence of anti-SARS-CoV-2 IgM/IgG in an Egyptian cohort and compared our findings to reports from other countries relying on the used Rt in the current study [29], accordingly, we wanted to check if the results might show differences using anti-SARS-CoV-2 IgM/IgG ELISA kit. The recorded higher sensitivity of the Rt for detecting anti-SARS-CoV-2 IgM/IgG could be due to presence of two antigens (S1 and N) on the kit cassette, while, ELISA plates of the used kit are coated with only one with (N) antigen. Previous reports indicated that serodiagnostics that have designed to detect both IgM and IgG classes were more sensitive compared to

Fig. 2 (continued)



those designed to detect only one of the Ig classes [30]. Cosgun et al. reported better sensitivity of an Rt for detecting anti-SARS-CoV-2 IgG compared to ELISA [31]. Additionally, several studies support use of serodiagnostics which contain either the S1 subunit or RBD side by side with the N antigen in order to improve both sensitivity and specificity of diagnosis [16–18]. Of note, IgM/IgG seroconversion to both the N and S antigens occurs concurrently and this partially support our observations [14, 32, 33]. Concerning the VPP subjects, the noticed higher sensitivity of the Rt for detecting both IgM/IgG than ELISA could be due to reliance of the Rt on two antigens (S1 and N) as has been mentioned above. Other explanation could be the induction of an acute immune reaction which indicated by the recorded higher IgM/IgG intensity among AS subjects. This goes along with previous reports where both the kinetic and intensity of induced immunity were a reflection for developed symptoms and levels of disease severity [30, 34]. The noticed, almost identical but poor sensitivities of the two assays for detecting IgM in the VPU subjects recommends their use in combination with virus-RNA detection assay to improve accuracy of early COVID-19 diagnosis. However, the higher sensitivity of IgG detection using the ELISA indicates its suitability to follow up the long-lasting immunity. This in part agrees with the previous reports where asymptomatic and/or mild infections induced transient responses with low to moderate antibody titers [25, 35–37]. Therefore, Chen et al. have suggested combination use of molecular-based and serology-based assays to improve recognition of asymptomatic and subclinical infections [38]. Coming to this point, we also recommend use of the herein studied Rt either alone

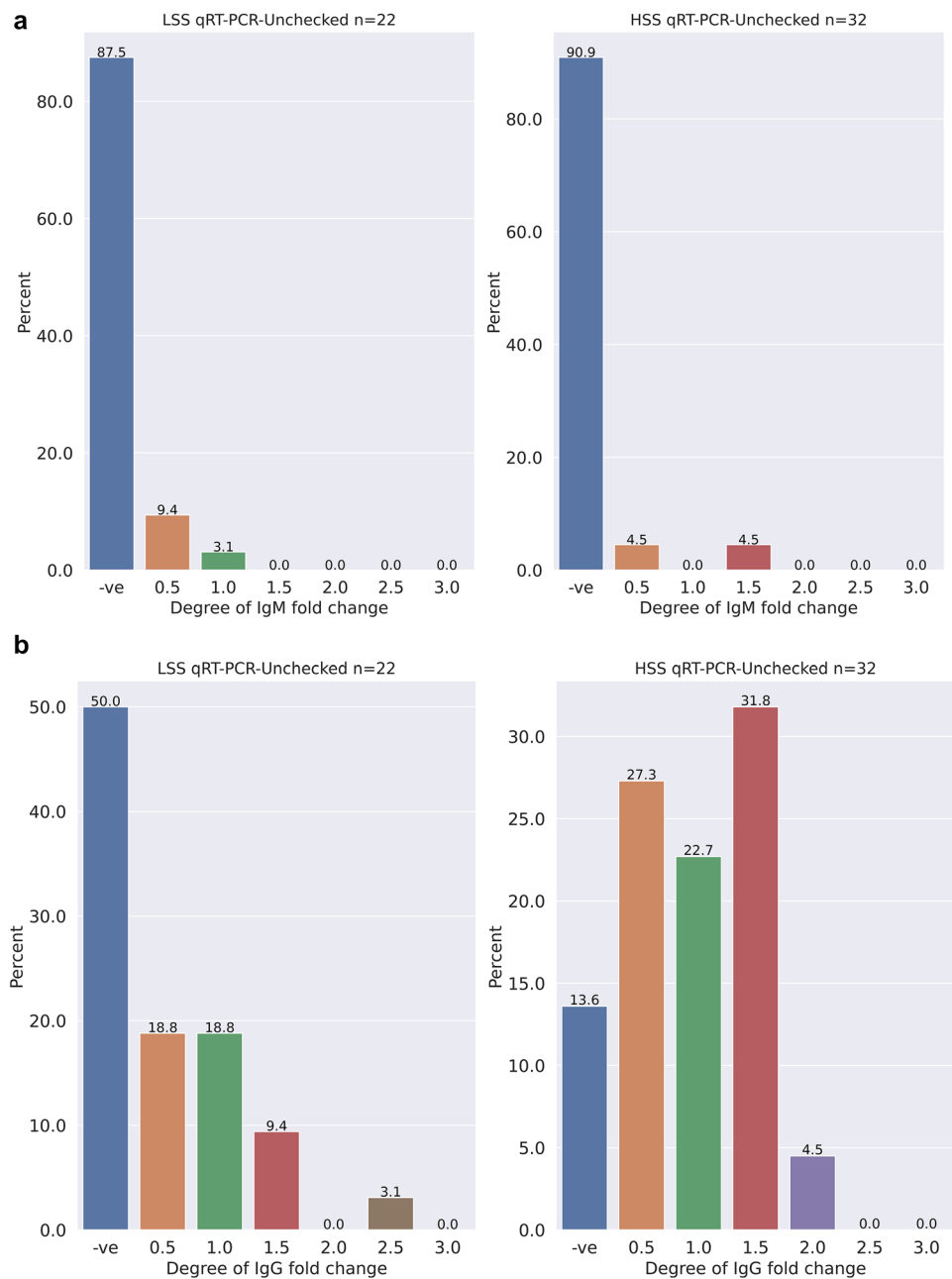
or in combination with virus-RNA detection-based assay in order to improve diagnosis sensitivity. In developing countries including Egypt, the benefits of using rapid assays for COVID-19 diagnosis are to save the cost, time, and to be feasibly used at the point-of-care unit. Of note, ELISA are more suitable to be used for detecting IgG in cross-sectional population-based studies to retrospectively record the actual sero-prevalence rates within asymptomatic populations.

Concerning genders, reliance of the Rt on two different antigens (S1 and N) increased its general sensitivity for detecting anti-SARS-CoV-2 IgM/IgG among the VPP females. Noteworthy, both of the higher prognosis and survival rates allow females to show higher IgG levels than males [24].

In contrast, the generally observed higher sensitivity of ELISA for detecting anti-SARS-CoV-2 IgM/IgG among the VPU females could reflect induction of intensive humoral immune response due to repeated exposure to subclinical infections [32].

The higher anti-SARS-CoV-2 IgM/IgG prevalence rates among females than males revealed an increase of the risk of virus transmission among households [39–42]. Likewise, here one cannot exclude the cross-reactivity between anti-N IgG of the a-CoV or b-CoV since both are known to share 25–29% and 33–47% identity with the SARS-CoV-2 N protein, respectively [43]. Noteworthy, number of AS subjects among the VPU group are higher than those among the VPP ones. Thus, anti-SARS-CoV-2 IgM/IgG detection among the VPU group is still conceivable. The noticed variations in both the reported immunity and disease severity could be attributed to an early

Fig. 3 **a** Distribution of the virus-specific IgM fold change degrees within the virus qRT-PCR-unchecked (VPU) ELISA-antibody positive individuals of low socioeconomic standards (LSS; left, $n = 22$) and of high socioeconomic standards (HSS; right, $n = 32$). **c** Distribution of the virus-specific IgG fold change degrees among VPU ELISA-antibody positive individuals of LSS (Left; $n = 22$) and of HSS (Right; $n = 32$). The ELISA cut-off values were 0.498 for the IgM and 0.542 for IgG. The Pandas library (Version 1.1.1) was used to load data sets from excel sheets into data frame objects. All analysis was carried out in a Jupyter notebook (Version 6.0.3) with a Python 3.7.7 work environment. All plots were generated using Python Seaborn package (Version 0.10.1).



infection caused by one of the seasonal human CoVs. Of interest, the Rt was highly sensitive for detecting anti-SARS-CoV-2 IgM/IgG among 28 males of the two groups. However, both assays were equally sensitive for detecting IgG among the VPU group. This also can be explained by dependency of the Rt on two viral-antigens (S1 and N) as has been indicated above. In agreement with our findings, previous reports have also documented higher susceptibility of males to SARS-CoV-2 infection with high degrees of disease severity compared to females [44]. Of note, while Ishaq et al. have reported equal prevalence of anti-SARS-CoV-2 IgG among both genders [45]. Kutsuna et al.

have reported its higher prevalence among males [46]. On the contrary, higher prevalence of anti-SARS-CoV-2 IgG among females was reported [47]. Therefore, more research is still needed to unravel gender-related cellular and molecular mechanisms behind the conflicting outcomes of these reports. In the VPU group, the higher ratio of IgM-positive subjects who show fold changes ranging from the Rt cut-off value to 1 could refer to higher sensitivity of this assay. In contrast, within the same group, the higher percentage of IgG positive subjects who show fold changes ranging from the ELISA cut-off values to 1 could point to better sensitivity of the ELISA. Therefore, we

Table 2 Comparing sensitivities of the rapid test and ELISA for detecting anti-SARS-CoV-2 IgM/IgG among both genders

Total	(n = 135)							
Gender	Females (n = 69)							
Assay	Rapid test				ELISA			
Subjects	VPP (n = 22)		VPU (n = 47)		VPP (n = 22)		VPU (n = 47)	
Anti-SARS-CoV-2 antibody	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
Prevalence (%)	7/22 (31.8)	13/22 (59.0)	10/47 (21.2)	29/47 (61.7)	4/22 (18.1)	10/22 (45.4)	14/47 (29.7)	37/47 (78.2)
Gender	Males (n = 66)							
Assay	Rapid test				ELISA			
Subjects	VPP (n = 23)		VPU (n = 43)		VPP (n = 23)		VPU (n = 43)	
Anti-SARS-CoV-2 antibody	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
Prevalence (%)	21/23 (91.3)	21/23 (91.3)	7/43 (16.2)	22/43 (51.1)	7/23 (30.4)	18/23 (78.2)	3/43 (6.9)	22/43 (51.1)

n number of samples; VPP virus-RT-PCR positive; VPU virus-RT-PCR unchecked

Table 3 Comparing sensitivities of the rapid test and ELISA for detecting anti-SARS-CoV-2 IgM/IgG within the symptomatic (S) and asymptomatic (AS) subjects of the virus-RT-PCR positive (VPP) group

Human subject	VPP (n = 45)							
Assay	Rapid test				ELISA			
Subjects	S (n = 37; 82.2%)		AS (n = 8; 17.8%)		S (n = 37; 82.2%)		AS (n = 8; 17.8%)	
Anti-SARS-CoV-2 antibody	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
Prevalence (%)	25/37 (67.5)	29/37 (78.3)	3/8 (37.5)	5/8 (62.5)	9/37 (24.3)	25/37 (67.5)	2/8 (25.0)	4/8 (50.0)

n number of samples; VPP virus-RT-PCR positive; S symptomatic; AS asymptomatic

Table 4 Comparing sensitivities of the rapid test and ELISA for detecting anti-SARS-CoV-2 IgM/IgG within the S and AS subjects of the VPU group

Human subject	VPU (n = 90)							
Assay	Rapid test				ELISA			
Subjects	S (n = 53; 58.9%)		AS (n = 37; 41.1%)		S (n = 53; 58.9%)		AS (n = 37; 41.1%)	
Anti-SARS-CoV-2 antibody	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
Prevalence (%)	14/53 (26.4)	37/53 (69.8)	2/37 (5.4)	12/37 (32.4)	6/53 (11.3)	36/53 (67.9)	11/37 (29.7)	22/37 (59.4)

n number of samples; VPU virus-RT-PCR unchecked; S symptomatic; AS asymptomatic

recommend relying on the used ELISA for IgG detection both in diagnosis and in large-scale surveillance (Fig. 1c).

In the VPP group, the higher percentage of IgM positive subjects, who show fold changes ranging from ELISA cut-off values to 1 could reflect higher prevalence of the IgM within this group (Fig. 2b). On the other hand, in the VPU group, the higher ratio of anti-SARS-CoV-2 IgG positive

subjects, who show fold changes ranging from ELISA cut-off values to 1 could point to higher prevalence of the IgG within this group (Fig. 2c).

In the LSS subjects of the VPU group, the higher percentage of the IgM positive subjects who show fold changes ranging from the ELISA cut-off values to 1 could

refer to higher prevalence of the IgM among these individuals compared to the HSS one (Fig. 3a).

In contrast, in the HSS subjects of the same group the higher percentage of IgG positive subjects who show fold changes ranging from the ELISA cut-off values to 1 could indicate higher prevalence of the IgG among these subjects compared to the LSS one (Fig. 3b).

In conclusion, although the limitation of the sample size, this study indicates suitability of the used Rt for detecting anti-SARS-CoV-2 IgM/IgG among S subjects and sheds light on possibility of relying on the used ELISA for IgG detection among AS human subjects.

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Author Contributions MMB wrote the grant that funded the work, designed the study, discussed the experimental design and the results of the work with all coauthors and put the structure of the manuscript. MHN, RN and DNA-E supervised filling the questionnaires and interviewed the un-hospitalized virus RT-qPCR un-checked individuals. KA, WAH, FMEG and SR provided the sera from, and the virus RT-qPCR data of, the hospitalized SARS-CoV-2 patients. MHN, RN and DNA-E performed the rapid IgM/IgG detections, made the photos, transformed the raw data into numerical values and stored into excel files. MHN did the statistical analysis, AMF and KF contributed to analysis and use of the Python Seaborn package, the Pandas library to load data sets from excel sheets into data frame objects and analyzed the data in a Jupyter notebook with a Python 3.7.7 work environment to prepare the fold change figures. MHN wrote the draft of the manuscript that was extensively discussed and edited by MMB. All authors have read, discussed the final version of the manuscript and agreed on the submission.

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Data Availability All data generated or analyzed during this study are included in this published article and in the supplementary files.

Declarations

Conflict of interest The authors confirm that no competing interests are existing.

Ethical Approval and Consent to Participate The study was approved by the Medical Ethical Committee of the National Research Centre (Meeting date: 5.11.2020, Decision number: 20166).

Consent for Publication Not applicable.

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