Microbes and Infectious Diseases 2022; 3(3): 544-553

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Antibody responses to acute COVID-19 infection; assessment via multiplex LABScreen COVID Plus Assay

Nora M. Said [1], Mohammad Walaa [2], Dina Gamal [1], Rania M. Amer [*3], Alshymaa A. Ahmed [1]

1-Clinical Pathology Department, Faculty of Medicine, Zagazig University, Al Sharqia Governorate, Egypt.

2-Chest Department, Faculty of Medicine, Zagazig University, Al Sharqia Governorate, Egypt.

3-Medical Microbiology and Immunology Department, Faculty of Medicine, Scientific and Medical Researc Center, Zagazig University, Al Sharqia Governorate, Egypt.

ARTICLE INFO

Article history:
Received 15 May 2022
Received in revised form 11 June 2022
Accepted 15 June 2022

Keywords:
Antibodies
COVID-19
Performance
Real time PCR
Sensitivities

Background: Understanding the profile of antibody responses following acute COVID-19 infection is required. **Aim:** to describe the pattern of IgG anti-COVID-19 antibody production in patients with acute infection using the LABScreen COVID Plus assay. **Results**: The overall seropositivity was 69/73(94.5%). Anti-Spike, Spike 1 and spike S2 subunits were positive in 78.1%, while anti spike receptor binding domain (RBD) was detected in 68.4% and anti nucleocapsid protein in 61.6%. The overall positivity of the assay reached 100.0% during the second week post symptoms. The mean fluorescent intensities (MFI) of anti-Spike S1 was higher in the second week than the first week, p=0.03. MFI of anti-Spike S2 was significantly higher in PCR positive patients in comparison with the negative ones, p=0.006. When compared to the RT-PCR results; the overall antibodies positivity, anti-Spike, and anti-Spike2 antibodies had sensitivities (100% and 84.7%) and specificities (28.6% and 50.0%) and accuracies (86.3% and 78.1%). Patients' outcome correlated significantly with the time of hospital admission, p=0.001. **Conclusion:** COVID-19 IgG antibodies are detectable with considerable frequencies during the first two weeks post infection. Anti S2 antibodies correlates well with the RT-PCR results. The LABScreen COVID Plus is a sensitive assay for the detection of post-acute COVID-19 infection antibody responses.

Introduction

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is the cause of Coronavirus disease 2019 (COVID-19). Over 518 million person have been infected worldwide with nearly 6.0million deaths as of 15 may 2022 [1] . A specific treatment has not been developed till the time of writing this work, so, early diagnosis and adequate isolation of infected persons are required for disease control [2].

Understanding the profile of antibody responses either following acute infection or post vaccination is required for several concerns including; diagnostic purposes, seroepidemiology studying, testing of convalescent plasma, and post-vaccination testing of seropositivity. An additional utility for antibody testing in organ transplant patients has arisen that; an outstanding proinflammatory condition such as pathogenic viral

DOI: 10.21608/MID.2022.138557.1315
* *Corresponding author:* Rania M Amer
E-mail address: Rania_aamir80@yahoo.com

infection may alter the breadth and amplitude of anti-HLA antibodies in pre or post-transplant candidates [3].

While the exact mechanisms are still not understood, the emerging belief is that acute viral infections, such as COVID-19, may trigger an anamnestic B-cell response or induce alloreactive responses by molecular mimicry [4]. Also, vaccine administration may have the same potential to induce alterations in the HLA antibody profile of transplant patients [5, 6]. The presence of COVID-19 antibody and its titer in transplant patients is critical in assessing the risk for transplant operations. High-risk patients need higher levels of immunosuppression, an undiagnosed COVID-19 case from a mild or asymptomatic infection "particularly with false negative RT.PCR test" may have an impact on a transplant patient's HLA antibody profile. The LABScreen COVID Plus assay enables detection of COVID-19 antibodies alongside HLA antibodies to better manage transplant patients that may have been exposed to COVID-19 [7].

Based on this; LABScreen COVID Plus-One LAMBDA assay kit, a multiplex bead based platform, was intended to characterize the immune response to COVID-19, to fully understand a transplant patient's risk factors side by side with the anti-HLA antibody testing. The LABScreen COVID Plus assay features a comprehensive SARS-CoV-2 specific multiplex antibody detection panel including; full spike extracellular domain (ECD), Spike S1 domain, Spike S2 domain, Spike receptor binding domain (RBD), and nucleocapsid protein. In addition; the assay incorporates Spike S1 fragments from six other coronaviruses, including HCoV-HKU1, HCoV-229E, HCoV-OC43, HCoV-NL63, MERS-CoV, and SARS-CoV; to roll out cross reactivity with the SARS-CoV-2 proteins in the COVID Plus assay [8]. This multiplexed, solid-phase assay has many advantages including; the evaluation of multiple viral targets simultaneously thereby improving the assay specificity, a relatively short assay time, high-throughput and semi-quantitative evaluation of the immune response [8].

The Spike S protein is formed of two subunits: S1 and S2. The spike S1 protein mediates interaction with the angiotensin converting enzyme 2 (ACE2) receptor and is highly immunogenic. Receptor-binding domain (RBD), a part of S1 protein, is the main target for neutralizing antibodies [9] . The S2 subunit is more conserved, so it is a key player in the cross reactivity seen upon using the whole S protein as an antigen [10]. The nucleocapsid is vital for viral transcription and replication, and is proposed to be more sensitive than the S protein for early detection of infections [11, 12].

The aim of this work is to study the pattern of IgG anti-COVID-19 antibody production in confirmed or probable COVID-19 patients during the peak of pandemic and before the obligatory Egyptian vaccination regimen, and to evaluate the performance of the assay in the detection of acute COVID-19 infection in comparison with the RT-PCR results.

Subjects and Methods

Study settings and patient criteria

This cross-sectional study was conducted at the Clinical Pathology Department, in collaboration with the Scientific and Medical Research Center, Faculty of Medicine, Zagazig University, during the period from January 2021 to June 2021. Eligible participants include; consecutive patients, aged ≥ 18 years old, from both genders and the same ethnicity, referred to the COVID 19 isolation and intensive care units of the Zagazig University Hospitals, during the peak of the epidemic and had not received any COVID vaccination. Participants were selected based on the WHO case definition for COVID-19 [13]. Patients who refused to participate or who started therapy prior to blood sample collection were excluded from this work. According to the time of testing, patients were categorized into those who were tested within the first week and those who were tested in the second week since the appearance of symptoms.

Data collection

All the data including; patient's history, date of onset of symptoms, clinical examination, routine laboratory investigations results "Complete blood counts (CBC), Liver and kidney functions, CRP, LDH, Ferritin levels, Prothrombin time (PT) and international normalization ratio (INR)", and radiological chest imaging findings "X ray and computerized tomography (CT)" were collected from patients' records.

Nucleic Acid Amplification Test (NAAT)

Nasopharyngeal and oropharyngeal swabs were collected and processed based on the Centers for

Disease Control and Prevention (CDC) recommendations [14] and as described previously [15], test was performed in the Scientific & Medical Research Centre of the faculty of medicine Zagazig university. Extraction of viral RNA was done under BSL-2 using the QIAampR viral RNA mini kit (cat.no.52906, Qiagen). A one-step real-time PCR kit (Primerdesign Ltd,Ref:Z-Path-COMD-A9-CE,UK) was used for detection of SARS-COV-2 RNA in the extracts using Stratagene Mx3000P qPCR System (Agilent).

Serological testing by LABScreen COVID Plus

The LABScreen COVID Plus, a flowcytometric assay that utilizes microbeads as a platform, is intended for the semi-quantitative detection of SARS-CoV-2 IgG antibodies in human serum or plasma. Three milliliters of whole blood in plane tubes were withdrawn from patients at the time of admission. Sera were separated and preserved at -80oC till the time of analysis. The LABScreen COVID Plus-One LAMBDA assay kit and the Luminex® 100/200 flow analyzer (Thermo Fisher Scientific, USA) were used according to the manufacturer's guidelines [11].

Statistical analysis

SPSS 22.0 (IBM Corp., Chicago, IL, USA) was used for processing and analysis of data. Numerical data were expressed as mean± standard deviation, frequencies were expressed as percentages. The sensitivity and specificity of the LABScreen COVID Plus kit were calculated in comparison with the reference test "NAAT". Agreement of the different assays was assessed using Cohen's Kappa coefficient. Spearman correlation coefficient (r) was performed for numerical variables, independent sample t test and one way analysis of variance (ANOVA) with post-hoc test were used to compare numerical variables, Results were considered to be significant when $p< 0.05$ [16, 17].

Ethical consideration

Ethical approval for this study was obtained from Zagazig University-Institutional Research Board (ZU-IRB); approval number (6501-21-3-2021). Signed informed consent was taken from patients; all procedures were performed according to the Declaration of Helsinki.

Results

General characteristics of patients

Seventy-three COVID-19 patients participated in this study including (27 females and 46 males, with mean age = 54±13.8 [range: 29–77] years. The most common symptoms were Malaise (82.2%) and dyspnea (84.9%), while the least frequent symptom was sore throat in only one patient (5.5%). According to the WHO case definition for COVID-19, 59 patients (80.8%) were confirmed by positive RT-PCR results, 14 (19.2%) patients with negative RT-PCR and positive CT chest findings were treated as probable cases. Based on the Ministry of health and population (MOH) management protocol[18], patients were classified as; mild; 42.5%, moderate; 45.2% and severe; 12.3%. Diabetes mellitus and ischemic heart diseases (IHD) were the most frequent comorbidities followed by hypertension and obesity **(Table 1)**.

Correlation of PCR and antibodies with the elapsed days post-onset symptoms (POS)

Although not significant; RT-PCR was more frequently positive in patients tested in the second week than patients tested in the first week, 32/36 versus 27/37, respectively The overall positivity of the assay reached 100.0% during the second week, the MFI of antibodies were higher in the second week than the first week particularly the anti-Spike S that was significantly higher, p=0.03 **(Table 2)**.

Antibodies positivity distribution among patients

The overall seropositivity in all patients was 69/73(94.5%). Antibodies against Spike, Spike 1 and spike S2 subunit were positive in 57/73 (78.1%), while anti spike Receptor binding domain (RBD) was detected in 50/73 (68.4%) and anti nucleocapsid protein in 45/73 (61.6%). Distributions of the detected antibodies among the RT-PCR positive and negative patients are illustrated in **table (S1)**.

The mean fluorescent intensities (MFI) of each antibody in the assay were higher in the PCR positive patients in comparison with the negative ones, however; anti COV2-Spike S2 were significantly higher p=0.006 **(Table 3)**.

A part of anti SARS-COV S1 antibodies, which were detected in 83.1% of PCR positive and 100.0% of PCR negative patients, antibodies against other community corona viruses were detected with low frequencies in both groups, **(Table S2).** Although anti SARS-S1 is frequently detected in patients there was no significant correlation with other anti SARS-COV2 antibodies or with PCR results, data not presented.

Correlation of PCR and antibodies with the severity and outcome of the disease

Patients classified as mild cases were more frequent in PCR negative patients versus PCR positive ones; 10/14 (71.4%) versus 21/59(35.5%), respectively, *p*= 0.02. Antibodies positivity and MFI did not vary among patients with different degrees of severity.

Neither PCR nor antibodies positivity were associated with the outcome of patients, interestingly; the only factor in this work that may affect the outcome was the time of hospital admission, as the frequency of patients who died among those admitted in the first week of symptoms was significantly lower than those admitted in the second week, 2/37 (5.4%) versus 14/36 (38.8%), respectively, *p*=0.001.

Correlation of PCR and antibodies with symptoms and laboratory results

In this work, RT-PCR results as well as anti-Spike, anti-Spike1, and anti-Spike2 antibodies were independent on symptoms and other laboratory results, however, patients with positive anti Spike-Receptor binding domain (RBD) and patients with positive anti nucleocapsid protein had higher hemoglobin concentrations than negative patients; mean± SD (g/dl); 13.5±2.0 versus 12.5±2.0 and 13.6±2.1 versus 12.5±1.7, *p*=0.03 and 0.02, respectively.

Assay performance in comparison to RT-PCR

The performance of LABScreen COVID Plus assay in the detection of acute COVID-19 infection was assessed against PCR results. The Spike and Spike S2 antibodies had the best performance with a sensitivity of 84.75%, specificity of 50.0%, and accuracy of 78.08%. While the anti nucleo-capsid protein antibodies had the least sensitivity 66.1% with a specificity of 57.14%, and accuracy of 64.7%. The overall positivity had 100% sensitivity, 28.57% specificity, and 86.30% accuracy. Considering the Cohen κ [19]; the overall positivity, Spike and Spike S2 antibodies had fair agreements with PCR, while the Spike S1, Receptor binding domain (RBD) and nucleocapsid protein antibodies had slight agreements, results are illustrated in **table (4**).

Table 1. Demographic features, symptoms, PCR and CT findings (n=73).

Variables		Frequency	%
Sex	**Male**	46	63.0
	female	27	37.0
Symptoms	**Malaise**	60	82.2
	Cough	46	63.0
	Fever	41	56.2
	Dyspnea	62	84.9
	GIT symptoms	23	31.5
	Sore throat	4	5.5
Comorbidities	**Diabetes mellitus**	17	23.3
	Hypertension	16	21.9
	IHD	17	23.3
	Stroke	5	6.8
	Obesity	12	16.4
	Renal diseases	2	2.7
	None	34	46.5
PCR	**Positive**	59	80.8
	Negative	14	19.2
CT Chest	**Mild**	31	42.5
	Moderate	33	45.2
	Severe	9	12.3
Outcome	**Survived**	57	78.1
	Died	16	21.9

IHD: Ischemic heart diseases, GIT: Gastrointestinal tract

Table 2. Seropositivity and MFI of LABScreen COVID Plus assay in relation to time elapsed since symptoms onset.

	LABScreen COVID Plus Assay						PCR Positive
	Overall	**Spike Cutoff=7500**	**SpikeS1 Cutoff=4000**	**SpikeS2 Cutoff=1900**	**Spike RBD Cutoff=3500**	**Nucleo-capsid Cutoff=3500**	
Seropositivity							
First week (n=37)	33(89.1%)	27(72.9%)	28(75.7%)	27(72.9%)	25(67.5%)	20(54.0%)	27(72.9%)
Second week (n=36)	36(100.0%)	30(83.3%)	29(80.5%)	30(83.3%)	25(69.4%)	25(69.4%)	32(88.9%)
P	0.06	0.2	0.4	0.2	0.5	0.1	0.8
MFI							
First week Median Range	35389 (1461-84405)	8171 (347-22106)	6811 (382-19344)	3781 (153-15399)	5659 (116-16924)	4814 (324-16196)	
Second week Median Range	36108 (11418-71231)	10713 (167-22155)	7928 (121-19726)	4656 (220-15059)	9326 (344-17864)	7077 (0-12116)	
p	0.4	0.03	0.9	0.5	0.2	0.6	

Table 3. Mean fluorescent intensities of antibodies against SARSCoV-2 by LABScreen COVID Plus Assay in relation to RT-PCR results.

RT-PCR	**Total Median Range**	**Spike Median Range**	**SpikeS1 Median Range**	**SpikeS2 Median Range**	**Spike RBD Median Range**	**Nucleo-capsid Median Range**
Positive (n=59)	37103.00 (11290-84405)	9646.32 (167-22155)	7439.73 (121-19726)	4827.51 (205-15399)	8612.32 (344-17864)	6681.32 (0-16196)
Negative (n=14)	27305.00 (1461-70414)	7182.22 (372-19899)	5515.87 (382-16715)	1520.99 (153-8166)	3537.15 (116-16835)	2830.26 (169-12956)
p	0.08	0.2	0.2	**0.006**	0.09	0.2

Table 4. Performance of SARSCoV-2 by LABScreen COVID Plus Assay in comparison with NAAT as the gold standard.

	Overall positivity	**Spike**	**SpikeS1**	**SpikeS2**	**Spike RBD**	**Nucleo-capsid protein**
Sensitivity (95% CI)	100.0% (93.94% to 100.00%)	84.75% (73.01% to92.78%)	81.36% (69.09% to 90.31%)	84.75% (73.01% to92.78%)	72.88% (59.73% to 83.64%)	66.10% (52.61% to 77.92%)
Specificity (95% CI)	28.57% (8.39% to 58.10%)	50.00% (23.04% to76.96%)	35.71% (12.76% to 64.86%)	50.00% (23.04% to76.96%)	50.00% (23.04% to 76.96%)	57.14% (28.86% to 82.34%)
Accuracy (95% CI)	86.30% (76.25% to 93.23%)	78.08% (66.86% to 86.92%)	72.60% (60.91% to 82.39%)	78.08% (66.86% to 86.92%)	68.49% (56.56% to 78.87%)	64.38% (52.31% to 75.25%)
Cohen κ (95% CI)	0.39 (0.11-0.67)	0.33 (0.07-0.58)	0.16 (0.02-0.32)	0.33 (0.07-0.58)	0.18 (0.05-0.42)	0.18 (0.03-0.40)

Table S1. Cross tabulation of antibodies against SARSCoV-2 by LABScreen COVID Plus Assay with RT-PCR results.

RT-PCR	Overall		Spike Cutoff=7500		SpikeS1 Cutoff=4000		SpikeS2 Cutoff=1900		Spike RBD Cutoff=3500		Nucleo-capsid Cutoff=3500	
	Negative n=4(%)	Positive n=69(%)	Negative n=16(%)	Positive n=57(%)	Negative n=16(%)	Positive n=57(%)	Negative n=16(%)	Positive n=57(%)	Negative n=23(%)	Positive n=50(%)	Negative n=28(%)	Positive n=45(%)
Positive n=59	0 (0.0%)	59 (85.6%)	9 (56.2%)	50 (87.7%)	11 (68.7%)	48 (84.2%)	9 (56.2%)	50 (87.7%)	16 (69.6%)	43 (86.0%)	20 (71.4%)	39 (86.7%)
Negative n=14	4 (100.0%)	10 (14.4%)	7 (43.8%)	7 (12.3%)	5 (31.3%)	9 (15.8%)	7 (43.8%)	7 (12.3%)	7 (30.4%)	7 (14.0%)	8 (28.6%)	6 (13.3%)

Table S2. Anti COV antibodies.

RT-PCR	HCOV229E S1 n(%)	HCOVHKU1 S1 n(%)	HCOVNL63 S1 n(%)	HCOVOC43 S1 n(%)	MERSCOV S1 n(%)	SARSCOV S1 n(%)
Positive (n=59)	7(11.9%)	21(35.6%)	10(16.9%)	11(18.6%)	5(08.4%)	49(83.1%)
Negative (n=14)	3(21.4%)	5(35.7%)	2(14.3%)	4(28.6%)	2(14.3%)	14(100.0%)
p	0.3	0.6	0.6	0.3	0.4	0.1

Discussion

In this work; the pattern of IgG antibody response to acute COVID-19 infection was assessed during the first two weeks post symptoms using a multiplex antibody detection assay specific to five COVID-19 antigens in an Egyptian cohort of during the peak of the pandemic and before the introduction of the global vaccination regimen.

Orth-Höller et al. [20] showed that 4/14 (29%) of tested patients in the second week after disease onset were IgG positive. while in the third week, 15/16 (94%) developed positive IgG titers. **Zhang et al**. [21] reported that the detection of IgG can be delayed in the critical group and reached the peak at one month post symptoms. In a meta-analysis by **Borremans et al.** [22], they revealed that the mean time for the appearance of IgG was 12 days, range (1-40), and the probability of detection reached 100% at day 22 post symptoms.

In this work, all the tested antibodies were detected with considerable frequencies since the first week of symptoms and increased during the second week; while the overall positivity of the assay reached 100% during the second week. Differences in techniques used in the detection of antibodies can account for these variations, indicating that the multiplex bead based platform can be more sensitive in antibodies detection than ELISA techniques.

Although some previous literature suggested that antibody production correlates with the severity of illness and the outcome of the disease [23,24], our results go with **Wang et al.** [25] findings that, both mild and severe patient groups had comparable IgG levels tested in nine days post-onset of symptoms (POS), and **Borremans et al.** [22] that, disease severity does not affect IgG patterns. Despite The finding that, higher hemoglobin levels were detected in patients with positive anti -Receptor Binding Domain and anti-nucleocapsid, the mean of hemoglobin levels was normal in both seropositive and seronegative patients. So, this finding could not be linked to a certain clinical variation. However, extending this study on a larger scale of patients might explain this finding.

Although the (RT-PCR) test is the gold standard for identifying viral nucleic acid and the diagnosis of COVID-19, this test has some practical limitations [26], such as the difficulty and unpleasant sensation during obtaining nasopharyngeal swab [16]. In addition, the relatively high false-negative rate of viral RNA detection indicated the addition of SARS-CoV-2-specific IgM

and IgG antibody assays as an alternative diagnostic tool to suspected SARS-CoV-2 infection [27].

When compared to the RT-PCR results; the overall antibodies positivity, anti-Spike, and anti-Spike2 antibodies had fair agreements with the PCR results, with high sensitivities (100% and 84.7%) and low to moderate specificities (28.6% and 50.0%) and moderate accuracies (86.3% and 78.1%). Previous literature varied; **Paradiso et al**. [16] tested positive SARS-CoV-2 sera with rapid IgM/IgG test, they revealed that assay had fair agreement with PCR, 30% sensitivity, 89% specificity, and 89% accuracy, while **Markewitz et al**. [12] reported the sensitivity of anti-spike proteins IgA and IgG antibodies detected by ELISA kit to be 81.3%. Sisay et al [17] evaluated the performance of three SARS-CoV-2 rapid IgG/IgM kits, their sensitivities, specificities, and agreements with RT-PCR were as follows; 61.18%, 96.52%, and 0.60 for kit A, 74.12%, 94.78%, and 0.71 for kit B and 83.53%, 94.78%, and 0.80 for kit C. Despite its lower specificity; the higher sensitivity of the LABScreen COVID Plus assay (100%) can be considered as a point of power in comparison to other assays.

Brochot et al. [28] observed that anti SARS-CoV-2 antibodies were detectable in patients two weeks post-symptom onset using ELISA kit, and that anti nucleocapsid and RBD were more sensitive than the anti-spike S1 or S2. Heterogeneity in results concerning antibody responses is attributed to differences in used assays or examined population characteristics.

In a study by **Bray et al**. [8], where the LABScreen COVID Plus assay was first validated on COVID-19 confirmed cases, all patients tested positive with at least three of the five SARS-CoV-2 proteins. They considered the cutoff as the mean fluorescent intensity (MFI) of the negative control plus three standard deviations (SD). When **Bray et al**. [8] tested commercial sera specific for SARS-COV-1, it showed cross-reactivity with SARS-CoV-2 Full Spike, S1, and Receptor binding domain (RBD) beads. Although antibodies against other corona viruses were detected frequently in our patients, the lack of agreements of these antibodies positivity with either RT-PCR results or anti SARS-COV-2 antibodies indicates that cross-reactivity is minimal highlighting the specificity of the assay. The most frequently detected anti COV antibody in this work was the anti-SARS spike S1.

Anti-SARS-CoV2 antibodies were detected with considerable frequencies in RT-PCR negative COVID-19 patients. In a study included 1014 patients Wuhan, China; 59% of patients had initial positive RT-PCR results while 88% of them had positive CT chest scan, and in a subgroup who underwent multiple RT-PCR testing 67% of the patients who were negative and converted to positive had initial chest CT findings [29]. Another study assumed that at least 59% of the COVID-19 cases in Wuhan went undetectable by RT-PCR test; they mostly included asymptomatic and mildly symptomatic individuals [30]. Moreover; the influence of early recognition and intervention on the outcome of patients was significant in this cohort and it goes with previous studies [31-33].

Chest CT imaging is a rapid and non-invasive modality that possesses a high accuracy in the diagnosis and evaluation of COVID-19 patients, particularly; during the epidemic episode [27]. The sensitivity of chest CT is high (74.3%–97%) in symptomatic patients [34]. In fact, a study reported that about 81% of the patients with negative RT-PCR results but positive chest CT scans were reclassified as highly probable cases of COVID-19 using combination of clinical symptoms, typical CT manifestations, and dynamic CT follow-up. Moreover, serial RT-PCR tests and CT scans have proven that 90% of these patients are confirmed COVID-19 patients [29].

Conclusion

COVID-19 IgG antibodies are detectable with considerable frequencies during the first two weeks post infection. The LABScreen COVID Plus is a sensitive assay for the detection of post-acute COVID-19 infection antibody responses, particularly; anti S2 antibodies that correlate well with the RT-PCR results.

Limitations

Beside the relatively small number of cases, this work included one sample from each patient, so this assay must be evaluated in future studies considering antibodies kinetics post infection and vaccination.

Acknowledgement

The authors would like to thank the staff members of the Zagazig Scientific and Medical Research Center, Professor Dr. Osama Gaber, Dr. Manal Abd Alhafeez, Dr. Mariem A. Soliman, Mrs. Samah Alsaeed and Mr. Peter Maher for their assistance during the laboratory work.

Conflict of interest

We declare that we have no conflict of interest.

Financial disclosures: nothing to declare.

References

1-**World Health Organization.** COVID-19 weekly epidemiological update, edition 92, 18 may 2022. Available at (https://www.who.int/publicatis/m/item/weekly-epidemiological-update on-covid-19).

2-**Ozturk A, Bozok T, Simsek BT.** Evaluation of rapid antibody test and chest computed tomography results of COVID-19 patients: A retrospective study. Journal of medical virology 2021; 93(12): 6582-7.

3-**D'Orsogna L, van den Heuvel H, van Kooten C, Heidt S, Claas FHJ.** Infectious pathogens may trigger specific allo-HLA reactivity via multiple mechanisms. Immunogenetics 2017; 69(8-9): 631-41.

4-**Locke JE, Zachary AA, Warren DS, Segev DL, Houp JA, Montgomery RA, et al.** Proinflammatory events are associated with significant increases in breadth and strength of HLA-specific antibody. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2009; 9(9): 2136-9.

5-**Katerinis I, Hadaya K, Duquesnoy R, Ferrari-Lacraz S, Meier S, van Delden C, et al.** De novo anti-HLA antibody after pandemic H1N1 and seasonal influenza immunization in kidney transplant recipients. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2011; 11(8): 1727-33.

6-**Karahan GE, Claas FH, and Heidt S.** Detecting the humoral alloimmune response: we need more than serum antibody screening. Transplantation 2015; 99(5): 908-15.

7-**Cravedi P, Mothi SS, Azzi Y, Haverly M, Farouk SS, Pérez-Sáez MJ, et al.** COVID-19 and kidney transplantation: Results from the TANGO International Transplant Consortium. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2020; 20(11): 3140-8.

8-**Bray RA, Lee J-H, Brescia P, Kumar D, Nong T, Shih R, et al.** Development and Validation of a Multiplex, Bead-based Assay to Detect Antibodies Directed Against SARS-CoV-2 Proteins. Transplantation 2021; 105(1): 79-89.

9-**Premkumar L, Segovia-Chumbez B, Jadi R, Martinez DR, Raut R, Markmann A, et al.** The receptor binding domain of the viral spike protein is an immunodominant and highly specific target of antibodies in SARS-CoV-2 patients. Science immunology 2020; 5(48): 1-14.

10-**Okba NMA, Müller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM, et al.** Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody Responses in Coronavirus Disease Patients. Emerging infectious diseases 2020; 26(7): 1478-88.

11-**Mariën J, Ceulemans A, Michiels J, Heyndrickx L, Kerkhof K, Foque N, et al.** Evaluating SARS-CoV-2 spike and nucleocapsid proteins as targets for antibody detection in severe and mild COVID-19 cases using a Luminex bead-based assay. Journal of virological methods 2021; 288: 114025-32.

12-**Markewitz R, Torge A, Wandinger K-P, Pauli D, Franke A, Bujanda L, et al.** Clinical correlates of anti-SARS-CoV-2 antibody profiles in Spanish COVID-19 patients from a high incidence region. Scientific Reports 2021; 11(1):4363-75.

13-**World Health Organization (WHO). "**COVID-19: case definitions: updated in public health surveillance for COVID-19" World Health Organization: 2020. Available at https://apps.who.int/iris/handle/10665/337834.

14-**Control CfD and Prevention.** Interim guidelines for collecting, handling, and testing clinical specimens for COVID-19. 2020. Availabe at https://pesquisa.bvsalud.org/global literature-on-novel-coronavirus-2019-ncov/resource/pt/ppcovidwho-2616.

15-**Amer RM, Samir M, Gaber OA, EL-Deeb NA, Abdelmoaty AA, Ahmed AA, et al.** Diagnostic performance of rapid antigen test for COVID-19 and the effect of viral load, sampling time, subject's clinical and laboratory parameters on test accuracy. Journal of Infection and Public Health 2021; 14(10): 1446-53

16-**Paradiso AV, De Summa S, Loconsole D, Procacci V, Sallustio A, Centrone F, et al.** Rapid Serological Assays and SARS-CoV-2 Real-Time Polymerase Chain Reaction Assays for the Detection of SARS-CoV-2: Comparative Study. J Med Internet Res 2020; 22(10): e19152-61.

17-**Sisay A, Tesfaye A, Desale A, Ataro I, Woldesenbet Z, Nigusse B, et al.** Diagnostic Performance of SARS-CoV-2 IgM/IgG Rapid Test Kits for the Detection of the Novel Coronavirus in Ethiopia. Journal of multidisciplinary healthcare 2021; 14:171-80.

18-**Masoud H, Elassal G, Zaky S, Baki A, Ibrahem H, Amin W, et al.** Management Protocol for COVID-19 Patients Version 1.4/30th May 2020 Ministry of health and population (MOHP), Egypt 2020 :9-11.

19-**McHugh ML.** Interrater reliability: the kappa statistic. Biochem Med (Zagreb) 2012; 22(3): 276-82

20-**Orth-Höller D, Eigentler A, Weseslindtner L, and Möst J.** Antibody kinetics in primary- and secondary-care physicians with mild to moderate SARS-CoV-2 infection. Emerging microbes & infections 2020; 9(1):1692-4.

21-**Zhang B, Yue D, Wang Y, Wang F, Wu S, Hou H.** The dynamics of immune response in COVID-19 patients with different illness severity. Journal of medical virology 2021; 93(2): 1070-7.

22-**Borremans B, Gamble A, Prager KC, Helman SK, McClain AM, Cox C, et al.** Quantifying antibody kinetics and RNA detection during early-phase SARS-CoV-2 infection by time since symptom onset. eLife 2020; 9: 1-27.

23-**Chvatal-Medina M, Mendez-Cortina Y, Patiño PJ, Velilla PA, Rugeles MT.** Antibody Responses in COVID-19: A Review. Frontiers in Immunology 2021; 12:1-14.

24-**Yan X, Chen G, Jin Z, Zhang Z, Zhang B, He J, et al.** Anti-SARS-CoV-2 IgG levels in relation to disease severity of COVID-19. Journal of medical virology 2022; 94(1): 380-3.

25-**Wang Y, Zhang L, Sang L, Ye F, Ruan S, Zhong B, et al.** Kinetics of viral load and antibody response in relation to COVID-19 severity. The Journal of clinical investigation. 2020; 130(10): 5235-44.

26-**Livingston E , Bucher K.** Coronavirus Disease 2019 (COVID-19) in Italy. JAMA 2020; 323(14): 1335.

27-**Li Z, Yi Y, Luo X, Xiong N, Liu Y, Li S, et al.** Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. Journal of medical virology 2020; 92(9): 1518-24.

28-**Brochot E, Demey B, Touzé A, Belouzard S, Dubuisson J, Schmit J-L, et al.** Anti-spike, Anti-nucleocapsid and Neutralizing Antibodies in SARS-CoV-2 Inpatients and Asymptomatic Individuals. Frontiers in Microbiology 2020; 11(2468): 1-8.

29-**Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, et al.** Correlation of Chest CT and RT-PCR Testing for Coronavirus Disease 2019 (COVID-19) in China: A Report of 1014 Cases. Radiology 2020; 296(2): E32-e40.

30-**Mizumoto K, Kagaya K, Zarebski A, Chowell G.** Estimating the asymptomatic proportion of coronavirus disease 2019 (COVID-19) cases on board the Diamond Princess cruise ship, Yokohama, Japan, 2020. Euro Surveill 2020; 25(10): 2000180-85.

31-**Trial T, Reis G, Silva E, Silva D, Thabane L, Milagres A, et al.** Effect of early treatment with fluvoxamine on risk of emergency care and hospitalization among patients with COVID-19: the TOGETHER randomized platform clinical trial. medRxiv 2021; 10: 42-51

32-**Sun Q, Qiu H, Huang M, and Yang Y.** Lower mortality of COVID-19 by early recognition and intervention: experience from Jiangsu Province. Annals of Intensive Care 2020; 10(1): 33-7.

33-**Gupta S, Wang W, Hayek SS, Chan L, Mathews KS, Melamed ML, et al.** Association between early treatment with tocilizumab and mortality among critically ill patients with COVID-19. JAMA internal medicine 2021; 181(1):41-51

34-**Imai K, Tabata S, Ikeda M, Noguchi S, Kitagawa Y, Matuoka M, et al.** Clinical evaluation of an immunochromatographic IgM/IgG antibody assay and chest computed tomography for the diagnosis of COVID-19. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology 2020; 128:104393-408.

Said NM, Walaa M, Gamal D, Amer RM, Ahmed AA. Antibody responses to acute COVID-19 infection; assessment via multiplex LABScreen COVID Plus Assay. Microbes Infect Dis 2022; 3(3): 544-553.