Abstract. Background: Antibody testing is necessary to identify immune individuals in the post-initial wave of the COVID-19 pandemic. Patients and Methods: We prospectively evaluated the performance of a quantitative point-of-care test (POCT) for SARS-CoV-2 antibodies. The patient group (PG) comprised of hospitalized confirmed COVID-19 cases. Asymptomatic healthcare volunteers with negative rRT-PCR were included in the control group (CG). Measurement of IgM and IgG was obtained by dry fluorescence immunoassay. Results: Twenty-six PG (65.9±15.4 years old, male 57.7%) and 18 CG (45.6±10.1 years old, male 33.3%) were included. By manufacturer’s cut-off (≥0.04 mIU/ml), sensitivity and specificity were 73.08% and 88.89% for IgM and 88.46% and 33.33% for IgG, respectively. Estimated areas under the ROC curve were 0.907 and 0.848 for IgM and IgG, respectively. Results were improved using a cut-off of IgM ≥0.05 mIU/ml and IgG ≥0.10 mIU/ml. Conclusion: Using stringent cut-off values, SARS-CoV-2 antibody POCT detects immune people and can be used during socioeconomic normalization of communities.

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Key Words: SARS-CoV-2, novel coronavirus, COVID-19, antibodies, point-of-care testing, IgG, IgM, diagnostics.
Patients and Methods

Participants. We conducted a single-centre prospective observational study in a tertiary teaching hospital between 30th March 2020 and 6th April 2020. Data collection was planned before the index test conduction. Subjects were prospectively selected based on the results of the real-time Reverse Transcription Polymerase Chain reaction (rRT-PCR) for SARS-CoV-2 and clinical symptoms.

Two groups of eligible participants were enrolled. The patient group (PG) consisted of hospitalized symptomatic patients with rRT-PCR confirmed COVID-19 infection. The control group (CG) consisted of hospital asymptomatic volunteers, with no clinical symptoms for the past month, with negative SARS-CoV-2 rRT-PCR at the day of sampling and no reported “close contact” history (based on the ECDC definitions for confirmed cases and close contacts) (13). All participants enrolled in this study were adults (>18 years old). No additional exclusion criteria were applied. PG was further divided into three subgroups based on the days between onset of symptoms and testing with the IgG/IgM POCT: the early (<7 days), middle (7-14 days) and late COVID-19 group (>14 days), respectively. Definitions for disease severity were: 1) mild: mild symptoms, no imaging findings of pneumonia; 2) moderate: fever or respiratory symptoms; 3) severe: respiratory distress and respiratory rate >30/min or saturation <93% at rest or arterial partial pressure of oxygen to inspired fraction of oxygen ≤300 mmHg; 4) critical: respiratory failure requiring mechanical ventilation (including acute respiratory distress syndrome - ARDS) or shock or other organ failure requiring ICU.

Respiratory samples. Nasopharyngeal and/or oropharyngeal swabs were collected and transferred to the Clinical Microbiology laboratory, immersed in an appropriate virus transport medium (e.g. UTM Viral Transport, Copan Diagnostics Inc., Brescia, Italy). Flocked swabs made from synthetic material were preferred for sample collection in order to maximize viral recovery. Lower respiratory tract samples (e.g. bronchoalveolar lavage or aspirates, sputum, etc.) were also accepted.

RNA extraction and real time RT-PCR. Automated purification of viral RNA from either the viral transport medium or lower respiratory tract samples was performed using the QIAsymphony DSP virus/pathogen mini kit on the QIAsymphony SP platform (QIAGEN, Hilden, Germany). A real time, one – step reverse transcription – PCR, specific for ORF1ab gene of SARS-CoV-2 and for N gene of all, or other coronaviruses was performed on the Rotor-Gene Q MDx thermocycler (QIAGEN), using the VIASURE DSP virus/pathogen mini kit on the QIAasyphony SP platform (QIAGEN), the RNA extracted was ≥0.04 mIU/ml for both IgG and IgM antibodies.

Ethical statement. This study was approved by the Research Ethics Committee of the participating institution (protocol number: 151/30-3-2020) and was conducted according to the STARD 2015 reporting guidelines and in line with the Declaration of Helsinki, as revised in 2013 (15). Informed consent was obtained from the participants.

Results

Overall, 44 participants (57.6±16.7 years old, male 47.7%), 26 PG (male 57.7%) and 18 CG (male 33.3%) were examined. Within PG, 5, 11 and 10 patients fulfilled the definition of early, middle and late COVID-19 infection respectively. Among patients 8, 8 and 10 were characterized with mild, moderate and severe and/or critical COVID-19 disease, respectively. Demographics are presented in Table I. Differences in sex distribution between patients and controls was not statistically significant (p=0.136).

Non-parametric ROC curves for IgM and IgG are shown in Figure 1. The respective areas under the ROC curve (95% CI) were 0.907 (0.824-0.990) and 0.848 (0.734-0.963). The
calculated IgM sensitivity and specificity, using the manufacturer’s diagnostic cut-off of ≥0.04 mIU/ml and the corresponding results for two additional sets of cutoff values are shown in Table II. Moreover, sensitivities of both tests were calculated separately in the three subgroups of patients (Table II). More specifically, increasing the cutoff values to 0.05 mIU/ml for IgM and 0.10 mIU/ml for IgG resulted in an increased specificity of IgM (94.4%) without any loss of sensitivity (73.08%) and increased specificity of IgG (94.4%) with a mild reduction of sensitivity (73.08%). Further increase of cutoff values to 0.08 mIU/ml for IgM and 0.19 mIU/ml for IgG gave a perfect sensitivity for both (100%).

| Cut-off value ≥0.04 mIU/ml for both IgM and IgG | Sensitivity% (95%CI) | Specificity% (95%CI) |
|-----------------------------------------------|----------------------|----------------------|
| IgM test                                      | 73.08% (52.21-88.43) | 88.89% (65.29-98.62) |
| IgG test                                      | 88.46% (69.85-97.55) | 33.33% (13.34-59.01) |

| Cut-off value ≥0.05 mIU/ml for IgM and ≥0.10 mIU/ml for IgG | Sensitivity% (95%CI) | Specificity% (95%CI) |
|-------------------------------------------------------------|----------------------|----------------------|
| IgM test                                      | 73.08% (52.21-88.43) | 88.89% (65.29-98.62) |
| IgG test                                      | 88.46% (69.85-97.55) | 33.33% (13.34-59.01) |

| Cut-off value ≥0.08 mIU/ml for IgM and ≥0.19 mIU/ml for IgG | Sensitivity% (95%CI) | Specificity% (95%CI) |
|-------------------------------------------------------------|----------------------|----------------------|
| IgM test                                      | 65.38% (44.33-82.79) | 100% (81.47-100)     |
| IgG test                                      | 69.23% (48.21-85.67) | 100% (81.47-100)     |

Figure 1. *Non-parametric ROC curves for IgM and IgG antibodies.*

Table II. *Sensitivity and Specificity percentages of the quantitative antibody POC test using different cut-off values.*
but significantly reduced their sensitivities to 65.38% and 69.23%, respectively.

Since this is a quantitative test, we further analyzed the IgM and IgG levels (Figure 2). Both IgM and IgG levels differed significantly \((p<0.001)\) between patients and controls. Among patients, there was a significant trend for higher levels of IgG with increasing time of disease \((p=0.022)\) whereas the corresponding trend for IgM was marginally not significant \((p=0.083)\).

Considering the severity, neither IgM \((p=0.686)\) nor IgG \((p=0.448)\) showed significant trends. However, IgM/IgG ratio tended to be positively correlated with disease's severity with the median (IQR) levels being 0.20 (0.17-0.32), 0.32 (0.14-0.52) and 0.59 (0.53-0.75) for patients with mild, moderate and severe/critical disease, respectively \((p\text{ for trend}=0.031)\).

**Discussion**

The performance of a dry fluorescence immunoassay POCT for dual IgG and IgM antibody quantitative detection as a diagnostic method for SAR-CoV-2 immunity levels was evaluated. To our knowledge, this is the first study appraising a quantitative POCT measuring SARS-CoV-2 antibodies in an actual clinical setting. Such immunoassays will play an important role in the future for epidemiological surveillance, evaluation of immunity and the outcome of vaccination studies (16).

Currently, the World Health Organization (WHO) recommends the utilization of nucleic acid-based molecular diagnostics in respiratory samples as the mainstay of COVID-19 diagnosis (9). The FDA has recently announced the authorization of rapid molecular tests that are capable of delivering results within minutes, but which are not globally available yet (17, 18). Most importantly, although detection of SARS-CoV-2 RNA in nasopharyngeal swabs is useful for the detection of acutely infected subjects as we are moving towards the post-flattening curve era, it becomes evident that easily accessible and accurate detection of immune people is vital in order to move the economy forward without jeopardizing public health.

Antibody testing for monitoring the development of immunity in response to infection, coupled with rRT-PCR for the detection of acute infections, will be important for surveillance and may provide a tool for developing an exit strategy with selective restrictions as a reaction to the
pandemic (16). In the short term, it could also contribute to informing whether people with a demonstrated immunity could be exempt from confinement measures. However, the variability of the results between the newly developed antibody kits, due to their different detection methods and/or their individual characteristics (i.e., sensitivity, specificity, accuracy, technical issues), will definitely shape their applicability in clinical practice. Therefore, large population based comparative trials among different tests are fundamental in order to define their role, their performance and eventually their utility in daily practice. Until more data are available, the use of different tests may be prudent.

Even though a total of 101 antibody tests have been currently CE-marked, limited data about their accuracy and utility are available in a clinical setting (19). Li et al. reported 88.66% sensitivity and 90.63% specificity of a qualitative combined IgM/IgG lateral flow immunoassay POCT (20). Most recently, a commercial qualitative test using whole blood obtained by fingerstick was evaluated by testing PCR-confirmed COVID patients as well as stored serum bank samples from 2018 used as controls. The test had high specificity (>99%) for both IgM and IgG antibodies indicating potential use for detection of past immunity (21). Both studies, however, evaluated qualitative tests performed in laboratory settings, whereas the present POC was quantitative and benchmarked at bedside settings, only with fresh samples.

In this study, highest sensitivities of both IgG and IgM antibodies were observed among the late PG subgroup. This is in concordance with previously published data, supporting that most patients develop anti-SARS-CoV-2 antibodies during the second week of symptoms (10, 22-26). This could suggest that antibody POCTs may be used for the confirmation of asymptomatic or mildly symptomatic cases or close-contacts in home-quarantine that were not tested due the lack of available molecular reagents. However, it remains to be seen whether asymptomatic patients are able to mount a satisfactory antibody response. Additionally, numerical values can be important during the initial medical assessment to help limit the indeterminate cases, as well as in follow up.

Since SARS-CoV-2 antibody tests will be mainly used for the determination of the immune status against SARS-CoV-2, the diagnostic specificity is the most crucial parameter. The ROC analysis provides the potential of increasing the specificity of this test in the expense of sensitivity. Although the specificity of the test examined here was not ideal, the advantage of using quantitative immunoassays facilitates the differentiation of false positive results based on the measured antibody levels. Hence, despite the test’s observed low IgG specificity, false positive CG participants had only marginally higher levels above cut-off (0.04-0.10 mIU/ml), whereas true positive cases developed 5- to 180-fold higher titers of IgG (Figure 1). However, more data on antibody responses among asymptomatic patients is required. It is possible that false positive IgG, are possibly explained by a cross-reaction with other coronaviruses or by the exposure to a continuous ambient low virus load, since our volunteers were hospital staff (23, 27, 28).

The most important advantage of the test evaluated here was its quantitative aspect and the ease of use in a busy clinical setting or in remote areas. The system provided numerical data which can prove useful for the clinical evaluation, the confirmation and the follow up of antibody reaction during serial testing.

POC antibody testing represents a readily available portable kit and would facilitate the broad implementation of population-based testing even in areas without relevant infrastructures since such methods do not require complex laboratory equipment and expertise. Similarly, POC antibody tests, particularly serial quantitative measurements, may also be valuable for diagnostic purposes of patients with febrile respiratory illnesses in remote areas, such as small islands.

Limitations

The diagnostic specificity of antibody tests would require the availability of specimens from individuals that had never been in contact with the SARS-CoV-2 virus. We however, used hospital-based employees as negative controls; although this may have affected the specificity of the test, one may postulate that its quantitative nature allows the differentiation between false and true positive cases based on the titers detected. Considering the worldwide spread of the virus it is recommended to use specimens which were collected before November 2019 as negative controls.

Among the limitations of our study is the small number of included participants. Moreover, we did not include asymptomatic subjects and all patients were hospitalized while the number of patients with mild and early disease was small. Although in our study design we intended to include an equal number of participants from the early, middle and late groups, in order to produce more robust and balanced results during the different phases of the disease and of antibodies’ kinetic, this was not possible. In our small cohort patient with early infection were under-represented (19.2%). This is mainly due to the natural course of this disease since most patients who require hospitalization are usually admitted after the first week of symptoms, when respiratory failure develops. Evidently, larger trials are needed to establish the placement of POC testing in the diagnostic armamentarium, as well as the utility of numerical values.

Conclusion

The development of low-cost, accurate and widely available SARS-CoV-2 tests, including antibody POCTs, may
represent an essential tool in the development of de-
escalation strategies in which mobility and contact
restrictions could be removed for people with proven
immunity. There is a theoretical advantage of using
quantitative immunoassays as they facilitate the numerical
differentiation based on the measured antibody levels and
allow for follow up evaluation. Large clinical studies are
imperative in order to better understand and contextualize
each test’s intended use in real-life clinical settings.

Conflicts of Interest

The Authors declare that the research was conducted in the absence
of any commercial or financial relationships that could be construed
as a potential conflict of interest. Consumables, test strips and the
reader were provided for free by Lansion Biotech.

Authors’ Contributions

PCF, VP, AA, SAP, ST and AK contributed in the conception and
design of the study. PCF, DK, TS, CP and AK contributed in
data acquisition. PCF, VP, NP and AK drafted the article. All authors
contributed in the analysis and interpretation of data, critically
revised the manuscript for important intellectual content and
approved the final submitted version of the manuscript.

Acknowledgements

Authors would like to express their sincere gratitude to Dr
Emmanouil Karofylakis, Dr. Konstantinos Thomas, Dr. George
Tsioulos, Dr. Christina Damoulari, Dr. Maria Paneta, Dr Sotiria
Grigoropoulou and Mrs Aggeliki Perdikouli for providing us the
patients’ clinical samples and for their daily input in the care of
COVID-19 patients. Also, authors would like to thank Dr Gikias
Magiorkinis for his input in the study design.

Funding

For PCF: Supported by Doctorate scholarship by the State
Scholarships Foundation (IKY), Partnership Agreement (PA) 2014-
2020, co-financed by Greece and the European Union (European
Social Fund - ESF) through the Operational Program “Human
Resources Development, Education and Lifelong Learning 2014-
2020”.

Data Availability Statement

The datasets for this study are available upon request by the
Corresponding author.

References

1 The World Health Organization. COVID-19 situation reports.
Available at: https://www.who.int/emergencies/diseases/novel-
coronavirus-2019/situation-reports/ [Last accessed on 01 June 2020]
2 The World Health Organization. Strategic preparedness and
response plan for the novel coronavirus. Available at: https://
www.who.int/publications-detail/strategic-preparedness-and-
response-plan-for-the-new-coronavirus [Last accessed on 01 June 2020]
3 The World Health Organization. Infection prevention and control.
Available at: https://www.who.int/emergencies/diseases/novel-
coronavirus-2019/technical-guidance/infection-prevention-and-
control [Last accessed on 01 June 2020]
4 European Centre for Disease Prevention and Control. COVID-
19. Available at: https://www.ecdc.europa.eu/en/covid-19-
pandemic [Last accessed on 01 June 2020]
5 Li D, Wang D, Dong J, Wang N, Huang H, Xu H and Xia
C: False-negative results of real-time reverse-transcriptase
polymerase chain reaction for severe acute respiratory syndrome
coronavirus 2: Role of deep-learning-based CT diagnosis and
insights from two cases. Korean J Radiol 21(4): 505-508, 2020.
PMID: 32174053. DOI: 10.3348/kjr.2020.0146
6 Lippi G, Simundic A-M and Plebani M: Potential preanalytical
and analytical vulnerabilities in the laboratory diagnosis of
coronavirus disease 2019 (COVID-19). Clin Chem Lab Med, 2020.
PMID: 32172228. DOI: 10.1515/cclm-2020-0285.
7 Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, Tao Q, Sun Z and
Xia L: Correlation of chest CT and RT-PCR testing in coronavirus
disease 2019 (COVID-19) in China: A Report of 1014 Cases. Radiology
200642, 2020. PMID: 32101510. DOI: 10.1148/radiol.2020200642
8 The Food and Drug Administration (FDA). LabCorp Covid-19
RT-PCR Test EUA Summary: Accelerated Emergency Use
Authorization (Eua) Summary Covid-19 RT-PCR Test (Laboratory Corporation Of America). Available at: https://
www.fda.gov/media/136151/download [Last accessed on 25
April 2020]
9 The World Health Organization. Advice on the use of point-of-
care immunodiagnostic tests for COVID-19. Available at: https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19
[Last accessed on 25 April 2020]
10 Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, Wang X, Yuan
J, Li T, Li J, Qian S, Hong C, Wang F, Liu Y, Wang Z, He Q, Li
Z, He B, Zhang T, Fu Y, Ge S, Liu L, Zhang J, Xia N and Zhang
Z: Antibody responses to SARS-CoV-2 in patients of novel
coronavirus disease 2019. Clin Infect Dis ciaa344, 2020. PMID:
32221519. DOI: 10.1093/cid/ciaa344
11 Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, Xing F,
Liu J, Yip CC, Poon RW, Tsui HW, Lo SK, Chan KH, Poon
VK, Chan WM, Ip JD, Cai JP, Cheng VC, Chen H, Hui CK,
Yuen KY: A familial cluster of pneumonia associated with the
2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 395(10223):
514-523, 2020. PMID: 31986261. DOI: 10.1016/S0140-6736
(20)30154-9
12 Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, Yu J,
Kang M, Song Y, Xia J, Guo Q, Song T, He J, Yen HL, Peiris
M and Wu J: SARS-CoV-2 viral load in upper respiratory
specimens of infected patients. N Engl J Med 382(12): 1177-
1179, 2020. PMID: 32074444. DOI: 10.1056/NEJMc2001737.
13 The European Centre for Disease Prevention and Control. Case
definition and European surveillance for COVID-19, as of 2
March 2020. Available at: https://www.ecdc.europa.eu/en/case-
definition-and-european-surveillance-human-infection-novel-
coronavirus-2019-ncov [Last accessed on 25 April 2020]
Diagnosis of a rapid IgG/IgM combined antibody test for SARS-CoV-2 infection diagnosis. J Med Virol 10.1002/jmv.25727, 2020. PMID: 32104917. DOI: 10.1002/jmv.25727

21 Hoffman T, Nissen K, Krambrich J, Rönnberg B, Akaber D, Esmailzadeh M, Salaneck E, Lindahl J and Lundkvist Å: Evaluation of a COVID-19 IgM and IgG rapid test; an efficient tool for assessment of past exposure to SARS-CoV-2. Infect Ecol Epidemiol 10(1): 1754538, 2020. PMID: 32363011. DOI: 10.1080/20008686.2020.1754538

22 Liu Y, Liu Y, Diao B, Ren F, Wang Y, Ding J and Huang Q: Diagnostic Indexes of a rapid IgG/IgM combined antibody test for SARS-CoV-2. medRxiv 2020.03.26.20044883, 2020. DOI: 10.1101/2020.03.26.20044883

23 Okba NMA, Müller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM, Lamers MM, Sikkema RS, de Bruin E, Chandler FD, Yazdanpanah Y, Le Hingrat Q, Descamps D, Houhou-Fidouh N, Reusken CBEM, Bosch BJ, Drosten C, Koopmans MPG and Haagmans BL: Severe acute respiratory syndrome coronavirus 2-specific antibody responses in coronavirus disease 2019 patients. Emerg Infect Dis 26(7), 2020. PMID: 32267220. DOI: 10.3201/eid2607.200841

24 Wölfler R, Corman VM, Guggemos W, Seilmaier M, Sange Z, Müller MA, Niemeyer D, Jones TC, Vollmar P, Rothe C, Hoelscher M, Bleicker T, Brünink S, Schneider J, Ehmann R, Zwigmalr K, Drosten C and Wendtner C: Virological assessment of hospitalized patients with COVID-19. Nature 581(7809): 465-469, 2020. PMID: 32235945. DOI: 10.1038/s41586-020-2196-x

25 Zhang W, Du RH, Li B, Zheng XS, Yang XL, Hu B, Wang YY, Xiao GF, Yan B, Shi ZL and Zhou P: Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerg Microbes Infect 9(1): 386-389, 2020. PMID: 32065057. DOI: 10.1080/22221751.2020.1729071

26 Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF and Shi ZL: A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579(7798): 270-273, 2020. PMID: 32015507. DOI: 10.1038/s41586-020-2012-7

27 Wang N, Li SY, Yang XL, Huang HM, Zhang YJ, Guo H, Luo CM, Miller M, Zhu G, Chmura AA, Hagan E, Zhou JH, Zhang YZ, Wang LF, Daszek P and Shi ZL: Serological evidence of bat SARS-related coronavirus infection in humans. China. Virol Sin 33(1): 104-107, 2018. PMID: 29500691. DOI: 10.1007/s12225-018-0012-7

28 Che XY, Qiu LW, Liao ZY, Wang YD, Wen K, Pan YX, Hao W, Mei YB, Cheng VC and Yuen KY: Antigenic cross-reactivity between severe acute respiratory syndrome-associated coronavirus and human coronaviruses 229E and OC43. J Infect Dis 191(12): 2033-2037, 2005. PMID: 15897988. DOI: 10.1086/430355

Received June 2, 2020
Revised June 15, 2020
Accepted June 16, 2020