

SARS-CoV-2 IgG/IgM Rapid Test as a Diagnostic Tool in Hospitalized Patients and Healthcare Workers, at a large Teaching Hospital in northern Italy, during the 2020 COVID-19 Pandemic

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SUMMARY

We describe the outcome of a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) IgG/IgM rapid test, and discuss the potential suitability of antibody testing.

Retrospective single cohort study on patients with suspected Coronavirus Disease 2019 (COVID-19) and asymptomatic Healthcare Workers, enrolled from March to April 2020. Subjects had quantitative PCR (qPCR) test for detection of SARS-CoV-2 via nasal swab and serological testing using the COVID-19 IgG/IgM Rapid Test (PRIMA Lab SA) immunochromatographic assay. Some subjects underwent chemiluminescence immunoassay (CLIA) after rapid test. The aim of the study was to analyse the proportion of those who developed a positive IgM/IgG response for SARS-CoV-2. The correspondence between the results from rapid testing and CLIA, when available, was evaluated.

97 subjects underwent qPCR for SARS-CoV-2 through nasal swab, which resulted positive in 40/43 (93.0%) of symptomatic patients, 2/40 (5%) of asymptomatic HCW, in no subjects with suspected COVID-19 (clinical and radiological findings) then excluded by repeated nasal swabs and alternative diagnosis (COVID-19-negative patients, CNPs), and in 6/6 (100%) of patients with confirmed diagnosis and negative follow-up nasal swabs (COVID-19-recovered patients, CRPs). IgM resulted positive in 8/43 (18.6%) of symptomatic patients and in 1/6 (16.7%) of CRPs. IgG resulted positive in 36/43 (83.7%) of symptomatic patients, 2/40 (5%) of HCW, and in 1/8 (12.5%) and 6/6 (100%) of CNPs and CRPs, respectively.

A comparison between an IgG/IgM Rapid Test and a following CLIA test showed consistency in negative results in 25/28 of HCW and 8/8 of CNPs tested.

Our preliminary data support the role of IgG/IgM Rapid Test (PRIMA Lab SA) immunochromatographic assay as a point-of-care test that may complement molecular tests in the screening of SARS-CoV-2 carriers. The test may gain particular relevance in shortening the time needed to refer patients to a COVID or non-COVID Hospital area and to achieve diagnosis in patients with persistently negative nasal swabs.

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INTRODUCTION

In December 2019, a new coronavirus emerged in China and caused an acute respiratory disease now known as coronavirus disease 2019 (COVID-19) (Zhou, 2020). After the first outbreak in Wuhan, the capital city of Hubei province, the virus rapidly spread worldwide, and on 11 March 2020 the World Health Organization (WHO) declared COVID-19 a pandemic, pointing to over 118,000 cases of the coronavirus illness in over 110 countries and

territories around the world and the sustained risk of further global spread.

The virus was identified as a betacoronavirus related to severe acute respiratory syndrome coronavirus (SARS-CoV) and thus was named SARS-CoV-2 (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020). Rapid identification of the aetiology and sharing of the genetic sequence of the virus, followed by international collaborative efforts initiated because of emergence of SARS-CoV-2, has led to rapid availability of real-time PCR diagnostic assays that support case ascertainment and tracking of the outbreak.

The kinetics of antibody response and viraemia has been studied in patients with SARS-CoV infection: IgM antibodies decreased and became undetectable 11 weeks into the recovery phase, while IgG antibodies remained detectable for a period beyond 11 weeks and were found in 100%

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of patients in the early convalescent phase. SARS-CoV viraemia mainly appeared one week after the onset of illness and then decreased over a period of 30 days, becoming undetectable in the blood samples of convalescent patients (Chen, 2004). The detectability of high titres of IgG for long periods of time might indicate the role of IgG in both humoral immune response to acute SARS-CoV infection and clearance of the remaining virus sources during recovery.

More recently, the acute antibody responses to SARS-CoV-2 in patients with COVID-19 has been collated: 100% of patients tested positive for IgG within 19 days after symptom onset, seroconversion for IgG and IgM occurred simultaneously or sequentially, and both IgG and IgM titres plateaued within 6 days after seroconversion (Long, 2020).

Despite the extraordinary efforts to obtain reliable anti-SARS-Cov-2 antibody quantification tests, validated serologic assays are still lacking for routine purposes and are urgently needed.

It is thus fully understandable that during the SARS-CoV-2 pandemic stage, the lack of rapid and reliable diagnostic tools could have severe consequences, allowing infected patients to spread the infection and hampering the efforts to contain the spread of the virus.

Additional screening methods, that can detect the presence of infection despite lower viral titres, can be highly beneficial to ensure timely diagnosis of all infected patients, particularly if these methods can be administered easily and are financially affordable, so that they can be performed on a large scale (Lisboa Bastos, 2020).

A total of 103 subjects were enrolled at the Clinic of Infectious Diseases at San Raffaele Scientific Institute in Milan in order to obtain some preliminary data about the usefulness of the COVID-19 IgG/IgM Rapid Test as a diagnostic tool in measuring antibody response either in patients with ascertained COVID-19 infection or negative exposed contacts.

MATERIALS AND METHODS

This is a retrospective, single cohort study conducted at the Clinic of Infectious Diseases at San Raffaele Scientific Institute in Milan (Lombardy Region, Italy). 89 adult

subjects, including patients admitted with suspected COVID-19 diagnosis (positive SARS-CoV-2 nasal swab and/or high clinical and radiological suspicion) and asymptomatic healthcare workers (HCW), were included in this study from March to April 2020 (Figure 1). 83 subjects underwent nasal swab with real-time PCR for SARS-CoV-2 virus (cobas® 6800/8800 by Roche) and successive serological testing using the COVID-19 IgG/IgM Rapid Test (PRIMA Lab SA) immunochromatographic assay for the qualitative detection of IgG and IgM against SARS-CoV-2 using whole blood samples from fingerstick specimens. Later, some of the subjects were tested using chemiluminescence immunoassay (CLIA) (DiaSorin LIAISON® SARS-CoV-2 S1/S2 IgG test). Furthermore, to corroborate Rapid Test results, we included a group of 6 patients who had recovered from a documented SARS-CoV-2 infection with at least two negative follow-up nasal swabs (COVID-19-Recovered Patients, CRPs), and a second group including 8 patients admitted with suspected COVID-19 (suggestive symptoms and CT scan findings of interstitial pneumonia) where COVID-19 was excluded by repeated negative nasal swabs and an alternative final diagnosis (COVID-19-Negative Patients, CNPs) (Figures 2a and 2b).

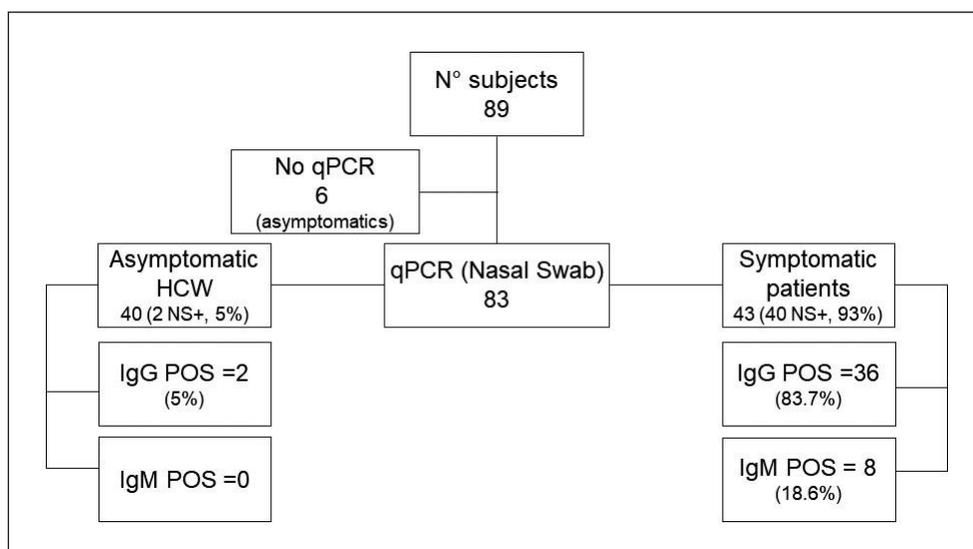
The goal of the study was to analyse the proportion of subjects who developed a positive IgM and/or IgG response for SARS-CoV-2. A positive IgG/IgM response was defined by the appearance of three coloured lines at the C (Control), IgG and IgM signs. A positive IgG response was identified by the appearance of two coloured lines at the C and IgG signs, while none at the IgM one. A positive IgM response was identified by the appearance of two coloured lines at the C and IgM signs, while none at the IgG one.

In addition, the correspondence between the results from rapid testing and CLIA was evaluated.

Patients' features were described as median (interquartile range, IQR) for continuous variables or proportional for categorical variables.

All data was anonymized and was obtained from electronic medical records, and no supplementary patient data was collected. Signed informed consent was not required due to the retrospective nature of the study and the full anonymization of patient data. The study did not generate any risk or adversely affected the rights, welfare or clinical practice of the subjects involved. Moreover, the study in-

Figure 1 - Serological survey in asymptomatic Healthcare Workers (HCW) and patients admitted with COVID-19. A group of 89 subjects were enrolled, of whom 83 (40 asymptomatic HCW and 43 symptomatic patients) were tested by qPCR (nasal swab) followed by IgG/IgM Rapid Test.



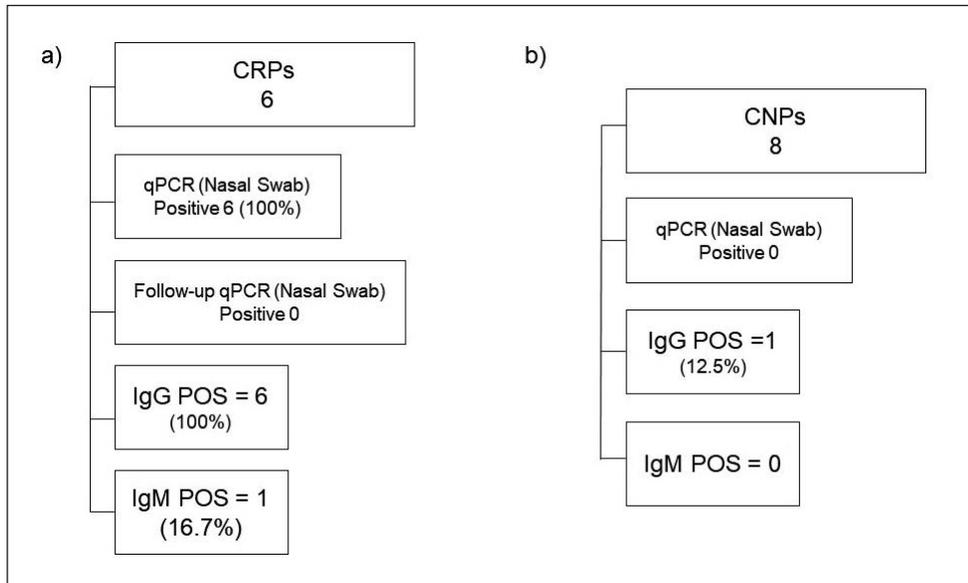


Figure 2 - Serological survey in COVID-19-recovered and COVID-19-negative patients.

a) CRPs: COVID-19-Recovered Patients, who recovered from a documented SARS-CoV-2 infection with at least two negative follow-up nasal swabs.

b) CNPs: COVID-19-Negative Patients, admitted with suspected COVID-19 (symptoms and radiological interstitial pneumonia), then excluded by negative nasal swabs and an alternative final diagnosis.

involves no prospectively collected data, so there was no access to patients or opportunity to seek informed consent.

RESULTS

A total of 103 subjects were enrolled in the study (Figure 1 and 2).

As reported in Table 1, 97 of them (94.2%) underwent quantitative PCR (qPCR) for SARS-CoV-2 through nasal swab: 40 (40/97; 41.2%) asymptomatic HCW, 43 (43/97; 44.3%) symptomatic patients, 8 (8/97; 8.3%) CNPs, and 6 (6/97; 6.2%) CRPs. Median age of the HCW was 51 years (IQR 34-58) and 31/40 (77.5%) were female. Median age of the symptomatic patients was 61 years (IQR 54-74) and 17/43 (39.5%) were female. Median age of the CNPs was 71 years (IQR 49-87) and 1/8 (12.5%) were female. Median age of the CRPs was 78.5 years (IQR 71-90) and 2/6 (33.3%) were female. Time from nasal-swab for SARS-CoV-2 to serological test was 27 days (IQR 27-28) and 21 (IQR 14-29) for the HCW and symptomatic patients, respectively. Time from nasal-swab for SARS-CoV-2 to serological test was 26 days (IQR 5-36) and 54 (IQR 24-60) for the CNPs and CRPs, respectively.

A positive qPCR through nasal swab was found in 40/43

(93%) of symptomatic patients, in 2/40 (5%) of asymptomatic HCW, 0/8 (0%) of the CNPs, and 6/6 (100%) of the CRPs.

Positive IgM was found in 0/40 (0%) and 8/43 (18.6%) of samples in the HCW and symptomatic patients groups, respectively, while 0/8 (0%) were found in the CNPs and 1/6 (16.7%) in the CRPs.

Positive IgG was found in 2/40 (5%) and 36/43 (83.7%) of the HCW and symptomatic patients groups, respectively, while 1/8 (12.5%) was found in the CNPs and 6/6 (100%) in the CRPs.

The three symptomatic patients with negative qPCR were characterized by a first positive IgG test, followed by an indeterminate second test, and this did not allow us to formally include those subjects among antibody-positive patients.

In the symptomatic patients subgroup, median time from positive nasal swab qPCR to positive IgM was 13 days (IQR 11-21), while for negative IgM it was 23 days (IQR 14-35) (data not shown).

In 28 HCW a further comparison was made between the results of the IgG/IgM Rapid Test and a following CLIA test. With the limit of time asynchrony, consistency in negative results was observed in 25/28 cases. One HCW

Table 1 - Characteristics and serological survey of 97 enrolled subjects who underwent quantitative PCR (qPCR) for SARS-CoV-2 through nasal swab.

	Symptomatic Patients	Asymptomatic HCW	CRPs	CNPs
Number	43	40	6	8
Sex (F/M)	17/26	31/9	2/4	1/7
Age median (IQR)	61 (54-74)	51 (34-58)	78.5 (71-90)	71 (49-87)
Nasal Swabs N positive (%)	40 (93)	2 (5)	6 (100)	0
Time NS-Ig assay median (IQR)	21 (14-29)	27 (27-28)	54 (24-60)	26 (5-36)
IgG positive N (%)	36 (83.7)	2 (5)	6 (100)	1 (12.5)
IgM positive N (%)	8 (18.6)	0	1 (16.7)	0

HCW: Healthcare Workers; CRPs: COVID-19-Recovered Patients; CNPs: COVID-19-Negative Patients; F: female; M: male; IQR: Interquartile range; NS: nasal swab; N: number.

presented a negative Rapid Test and positive CLIA (with negative nasal swab, no symptoms and a negative second Rapid Test after the positive CLIA), and in two cases (both with negative nasal swab, one positive and one negative at the Rapid Test) CLIA resulted “doubtful.” With the same CLIA test, 8/8 CNPs were confirmed IgG-negative (data not shown).

DISCUSSION

Nasal swab qPCR, while being the cornerstone of SARS-CoV-2 infection diagnostics, is limited by its dependence on well-equipped centralized laboratories, its inability to provide immediate results, and by being characterized by a significant rate of false-negative tests, especially in asymptomatic subjects and in those with advanced disease (Service, 2020).

In the last few weeks, an increasing amount of data has been shared about antibody response in subjects with COVID-19. A magnetic chemiluminescence enzyme immunoassay (MCLIA) demonstrated a median 13 day seroconversion for both IgM and IgG in 100% of symptomatic subjects 2.5 weeks after clinical onset as well as being able to identify 4.3% positive contacts missed by PCR test (7 of 148 subjects) (Zhao, 2020). Preliminary studies depicted specific immunoglobulin detection, a valid candidate to comprehend SARS-CoV-2 immunopathogenesis more accurately and to maximize diagnostic and preventive tools (Guo, 2020).

In our setting, at the beginning of the study, we lacked an authorized and reliable serological test and sought a fast, point-of-care test (POCT) needing minimum training to perform.

With the limit of a small sample and a retrospective design, we are sharing our local experience with the aim of contributing to and rapidly clarifying the role and usefulness of an IgG/IgM Rapid Test.

In our experience, 83.7% of symptomatic patients showed positive IgG, of which 8 also have associated positive IgM. IgM detection was associated with a shorter median

time from nasal swab to serological test (13 vs 23 days for IgM-positive group and IgM-negative group, respectively). Given a variable unknown time from clinical onset to nasal swab, this observation complies with data suggesting IgM decline from the 4th-5th week after symptoms onset (Xiao, 2020; Sethuraman, 2020). Interestingly, new insights about SARS-CoV-2 contagiousness are emerging, highlighting a decreased likelihood of inter-human spread eight days after symptoms onset (Bullard, 2020). This evidence may offer the opportunity to speculate about the role of the IgG/IgM profile with regard to clarifying the individual ability to spread the infection.

Results on asymptomatic HCW highlight a low prevalence of IgM/IgG (0 and 5%), consistent with the 5% positive nasal swab. A nasal swab was realized approximately after the first 10 days of the epidemic, the IgG/IgM Rapid Test after a median time of 26 (17-26) days, and then those results were compared with a CLIA test realized at a variable later time (one-three weeks), confirming a high rate of negative IgM/IgG. Our results seem to support previously published data about the maximum rate of transmission among HCW in the early stage of the epidemic, before protective measures were introduced (Kluytmans-van den Bergh, 2020; Lai, 2020).

To understand Rapid Test performance better, we tested two other groups of patients, where COVID-19 diagnosis and subsequent healing were confirmed (COVID-19-Recovered Patients, CRPs) and where suspected COVID-19 diagnosis was excluded (COVID-19-Negative Patients, CNPs). Rapid Test may have relevant usefulness in scaling down clinical and radiological suspicion of COVID-19 in patients with repeatedly negative nasal swabs associated with interstitial pneumonia after CT scan, allowing the investigation of alternative diagnoses and more appropriate patients being referred to COVID or non-COVID Hospital areas.

The Rapid Test in use proved positive in 6/6 of recovered subjects (CRPs), suggesting its utility when diagnosis occurs during healing, after the diagnostic window of a positive nasal swab.

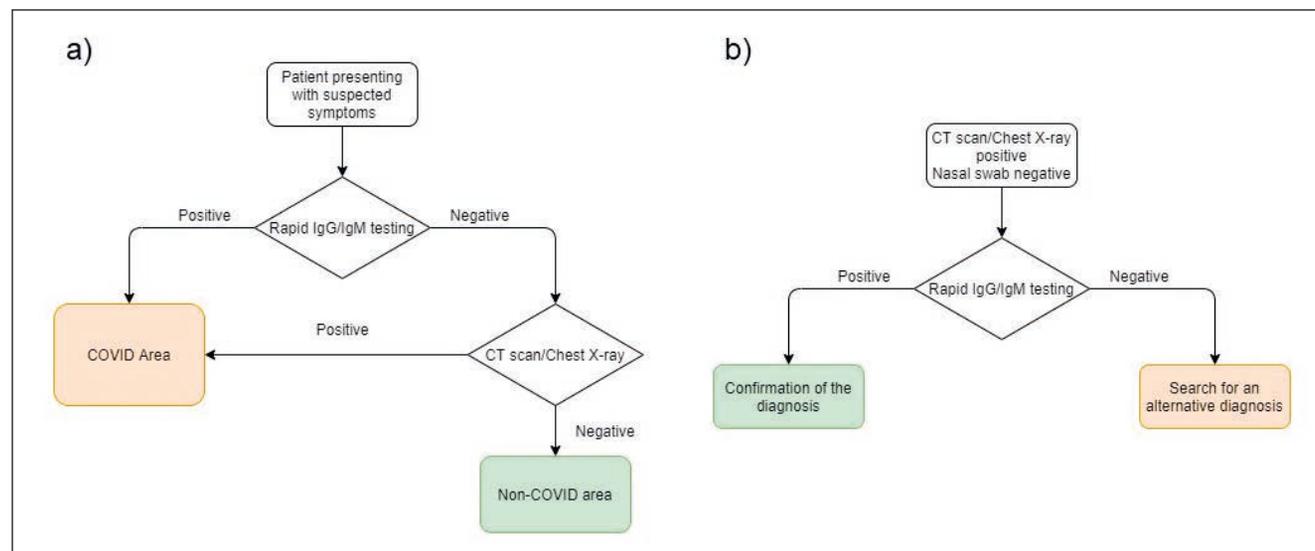


Figure 3 - Potential roles of IgG/IgM Rapid Test. a) Patients presenting with suspected symptoms, to quicken their addressing to a COVID or non-COVID area. b) Patients with COVID-19 clinical and radiological suspicion despite more than two negative nasal swabs, to support differential diagnostics.

With the above-mentioned limits and with the implied need for further randomized controlled studies, our preliminary data support the role of the IgG/IgM Rapid Test (PRIMA Lab SA) immunochromatographic assay (as well as comparable tests) as an easy-to-use POCT that may complement molecular tests. As outlined in *Figure 3*, it may be of particular relevance in patients presenting with suspected symptoms, to shorten the time needed to refer the patient to a COVID or non-COVID area (*Figure 3a*) and in subjects with lasting COVID-19 clinical and radiological suspicion, despite more than two negative nasal swabs, to hasten differential diagnosis (*Figure 3b*).

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Conflict of interest

All Authors declare no conflict of interest.

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