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Research Notes

# Evaluation of A Rapid IgM-IgG Combined Antibody Test for SARS-CoV-2 Infection: Single Italian Center Study

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**Abstract:** The need for timely establishment of diagnostic assays of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is demanded in laboratories worldwide. We evaluated the performance of a flow immunoassay which can detect IgM an IgG antibodies simultaneously against SARS-CoV-2 virus in human blood within 15 min. Among the 132 positive novel Coronavirus Disease 19 (COVID-19) cases, 126 tests were consistent with previous quantitative Real Time PCR (qRT-PCR) assays. Among the 62 negative cases, 60 were consistent with qRT-PCR assays except for 2 cases. In this study, 2019-nCOV/COVID-19 IgG/IgM Rapid Test Device showed 95.5% sensitivity and 96.8% specificity. In conclusion, Rapid IgM-IgG Combined Antibody Test showed high detection consistency among all analysed samples. Suggesting that could be used for the rapid screening of SARS-CoV-2 carriers, either symptomatic or asymptomatic.

**Keywords:** Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), Rapid IgM-IgG Combined Antibody Test, Novel Coronavirus Disease 19 (COVID-19)

## Introduction

On December 31th, 2019 China reported first cases of atypical pneumonia in Wuhan, the capital of Hubei province. The causative virus was found to be a betacoronavirus, closely related to the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-1) from 2003 and similar to Sarbecoviruses isolated from bats (Wu *et al*., 2020; Zhou *et al*., 2020). It was therefore termed SARS-CoV-2 and the disease it causes was named Corona Virus Disease 2019 (COVID-19) (CSGICTV, 2020). Several quantitative Real-Time RT-PCR (qRT-PCT) protocols for detection of SARS-CoV-2 RNA have been developed and approved from Centers for Disease Control and Prevention Nucleic acid in US and are now widely employed to diagnose COVID-19 disease (Chu *et al*., 2020; Corman *et al*., 2020). However, qRT-PCR take at least several hours to complete and require certified laboratories, expensive equipment and trained technicians to operate. Moreover, these methods are dependent on the time-window of viral replication and they can potentially cause low predictive rate results, thereby limiting the usefulness of RT-PCR in the field. Therefore, there is an urgent need for additional tests, rapid and simple to use, to quickly identify infected patients of SARS-CoV-2 virus, especially by detecting IgM antibodies which are observed about 12 day after infection, to prevent virus transmission of infected patients (Infantino *et al*., 2020; Okba *et al*., 2020; Zhao *et al*., 2020). However, it is important to underline that the detection of SARS-CoV-2 viral nucleic acid by RT-PCR test is still the current standard diagnostic method for COVID-19. At this time a great number of different rapid assays have been proposed, but lack of analytical performance and clinical validation are a major problem in terms of reliability despite the easy access on the market of these type of test (Rashid *et al*., 2020; Cassaniti *et al*., 2020). The major type are serological assays based on colloidal gold-labeled Immunochromatography (ICT) methods that offer combination IgM and IgG detection (Rashid *et al*., 2020). All these kits are based and use capture reaction to detect SARS-CoV-2 IgM/IgG. For combination IgM and IgG kit, the cassette has two detection bands (M and G) and a quality control band (C). The M band is coated with a monoclonal anti-human IgM antibody for detecting SARS-CoV-2 antibody; the G line is fixed with a reagent for detecting SARS-CoV-2 antibody; C line is

fixed with a quality control antibody. All kits offer a one-step method with results obtained within 15 min. Samples that can be used are whole blood, serum or plasma samples (Li *et al*., 2020). Recently, has been reported that the detection accuracy of lateral flow immunoassay anti-SARS-CoV-2 IgM and IgG antibodies resulted in a sensitivity of 88.7% and a specificity of 90.6% (Li *et al*., 2020). The aim of this study was to assess the diagnostic performance of a newly developed lateral flow immunoassay anti-SARS-CoV-2 IgM and IgG antibodies test, developed by using a combination of anti-IgM-IgG Coronavirus 19 antibodies (2019-nCOV/COVID-19 IgG/IgM Rapid Test Device, Hangzhou Realy Tech Co., Ltd). Serological studies in Italy and around the world appear to be still under evaluation and reporting available laboratory data is crucial in order to understand the utility of rapid antibody detection during the course of SARS-CoV-2 infection.

## Materials and Methods

### *Sample Collection*

These samples were collected from various public healthcare center and COVID-19 accredited healthcare facilities in Italy, with oral consent from all participants and approved by the local Ethics Committee of Artemisia SPA. The 2019-nCOV/COVID-19 IgG/IgM Rapid Test Device was conducted at Altamedica Medical Centre (Rome, Italy) by clinical staffs who followed test procedure described in the product inserts (2019-nCOV/COVID-19 IgG/IgM Rapid Test Device, Hangzhou Realy Tech Co., Ltd.)

### *Sample Testing*

The IgM antibody and IgG antibody against SARS-CoV-2 in blood samples were tested using 2019-nCOV/COVID-19 IgG/IgM Rapid Test Device (Hangzhou Realy Tech Co., Ltd) according to the manufacturer's instructions. These reagents are supplied by Hangzhou Realy Tech Co., Ltd and resulted CE marked and regularly registered with the Ministry of Italian Health as an IVD Medical Device at N. 1923329. Briefly, the pouched device was opened immediately before use. Refrigerated blood samples used for the test, are warmed to room temperature. During testing, 20 uL whole blood sample are pipetted into the sample port followed by adding 2 drops (about 20 uL) of dilution buffer to drive capillary action along the strip. The entire test took about 15 min to finish.

### *Data Analysis*

The rapid SARS-CoV-2 IgG-IgM combined antibody test kits were tested on blood samples coming several hospitals and Italian COVID-19 accredited laboratories in different provinces, with a total of 132 clinical positive and 62 clinical negative patient blood samples. The test performance was calculated with the Vassarstats online calculator (http://www.vassarstats.net).

## Results and Discussion

One hundred and thirty-two patients with qRT-PCR positive SARS-CoV-2 and sixty-two qRT-PCR negative SARS-CoV-2 infection were included in the study. No clinical data were available along with the laboratory results at that moment. The available characteristics of the sample testing are reported in Table 1. All sample were tested for viral antibody with a new 2019-nCOV/COVID-19 IgG/IgM Rapid Test Device (Hangzhou Realy Tech Co., Ltd). The sensitivity and specificity of the rapid test newly developed were verified in a total of 194 cases: 132 (positive) clinically confirmed (by qRT-PCR test) SARS-CoV-2-infected patients and 62 SARS-CoV-2 qRT-PCR negative cases. In our study of the 132 SARS-CoV-2-infected patients, 126 resulted positive to the antibodies rapid test, generating a sensitivity of 95.5% ($CI_{95\%}$ 89.9-98.1), of the 62 SARS-CoV-2 negative cases 2 tested positive, generating a specificity of 95.8% ($CI_{95\%}$ 87.8-99.4) (Table 2). Moreover, the positive predictive value (PPV) of antibodies test was 98.44% (126/128) and the Negative Predictive Value (NPV) of antibodies test was 90.1% (60/66). It was also founded that 61.9% (78 out of 126) of positive patients had both IgM and IgG antibodies, while 7.9 % (10/126) where IgG positive and 30% (38/126) where IgM positive. A singular IgM response is an indication of a recent infection, while a singular IgG response meaning that the infection was encountered more than 2 months before the serological test (Matricardi *et al*., 2020) (Table 2). Thus, the antibody testing might play pivotal roles in the following settings: (1) for suspected paucisymptomatic patients, positive result of antibody increases the confidence to make a COVID-19 diagnosis; (2) for healthy subject, in this case of antibody positive result the RNA should be tested more frequently and the close contacts observed as well. Obviously, this test cannot confirm virus presence, only provide evidence of recent infection, but it provides an important immunological evidence for physicians to make the exact diagnosis along with other tests and to start treatment of patients. Moreover, rapid laboratory diagnosis is essential for commencement of infection control measures. Rapid specific antigen tests have also been widely used in the diagnosis of two other coronavirus infection disease, Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) (Lau *et al*., 2004; Chen *et al*., 2016). Testing of specific antibodies of SARS-CoV-2 in patient blood is a good choice for rapid, simple, highly sensitive diagnosis of COVID-19.

**Table 1:** Patient characteristics of the COVID-19 and control groups

| | |
|---|---|
| Characteristics COVID-19 group | |
| Age, years | 35.5 (20.5-72.4) |
| Male sex | 56 (42%) |
| Female sex | 76 (58%) |
| Characteristics Control group | |
| Age, years | 32.1 (23.5-67.3) |
| Male sex | 23 (37%) |
| Female sex | 39 (63%) |

**Table 2:** Detection sensitivity and specificity of 2019-nCOV/COVID-19 IgG/IgM rapid test device

| | qRT-PCR positive | qRT-PCR negative |
|---|---|---|
| Sample Analysed | 132 | 62 |
| IgG&IgM Positive | 78 | 0 |
| IgG Positive | 10 | 0 |
| IgM Positive | 38 | 2 |
| Sensitivity | 95.5% ($CI_{95\%}$ 89.9-98.1) | |
| Specificity | | 96.8% ($CI_{95\%}$ 87.8-99.4) |

There are some limitation to consider, like possible cross-reactivity with other coronaviruses and flu viruses. In fact, SARSCoV-2 belongs to betacoronavirus, in the same family as SARS-CoV and MERS-CoV. Thus, there is possibility of cross-reactivity with other coronaviruses occurring and other bat-related SARS coronaviruses remains to be clearly determined (Xiao *et al*., 2020). Nevertheless, combination of nucleic acid qRT-PCR and the IgM-IgG antibody test can provide more accurate SARS-CoV-2 infection diagnosis. As today, only the Cellex qSARS-CoV-2 IgG/IgM Rapid Test has been validated by the US Food and Drug Administration (FDA) in the Emergency Use Authorisation (EUA) category. The objective of this study was to evaluate the overall clinical performance and diagnostic value of a rapid serological testing the 2019-nCOV/COVID-19 IgG/IgM Rapid Test Device in detecting SARS-CoV-2-infected patients. Our study represents a private clinical experience of the IgM-IgG antibody test in an Italian laboratory for SARS-CoV-2 antibodies detections. In our hands, the performance of the 2019-nCOV/COVID-19 IgG/IgM Rapid Test Device was comparable to that of recently published clinical study, showing that viral serological testing is an effective means of SARS-CoV-2 infection (Li *et al*., 2020).

## Conclusion

A rapid 2019-nCOV/COVID-19 IgG/IgM Rapid Test Device using lateral flow immune assay techniques was evaluated. It takes less than 15 min to generate results and determine whether there is recent SARS-CoV-2 infection. It is easy to use and no additional equipment is required. Results from this study demonstrated that this test is highly sensitive and specific. In conclusion, this rapid test has great potential benefit for the fast screening of SARS-CoV-2 infections and it has already generated enormous interest in the medical community.

## Author's Contributions

**Katia Margiotti:** Provided insights on data analysis and result and drafting the manuscript.

**Marina Cupellaro and Sabrina Emili:** Did the laboratory analyses.

**Alvaro Mesoraca and Claudio Giorlandino:** Conceived the study revised the manuscript.

All authors have approved the final article.

## Ethics

The study was approved by the local Ethics Committee of Artemisia SpA..

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