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LETTER TO THE EDITOR

Anti-SARS CoV2 antibody testing in healthcare workers: comparison between rapid-cassette tests, ELISA and CLIA methods

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To the Editor,

Early diagnosis of SARS-CoV-2 infection is extremely important for effective management and treatment of patients. The gold standard for the diagnosis of infection is qRT-PCR analysis on respiratory samples. However pre-analytical variables – specimens and techniques of sample collection – and analytical variables - extraction and detection of viral nucleic acids – affect the sensitivity of the test that varies from 99 to 70% (1).

Serological analysis could be an easier, low time-consuming method to improve sensitivity and to investigate asymptomatic subjects who do not meet the appropriate criteria for RT-PCR testing. Sensitivity of serologic tests could be very high (2), however it could be affected by the timing of sampling. In particular, IgM could be detected 3 days after symptom onset and reach 95-100% of sensitivity in 12 days, while IgG could be detected as early as 4 days post-infection (2, 3), and IgM and IgG often coexist (3, 4). Specificity could be influenced by cross-reaction with other coronavirus strains. Despite all these limitations, serologic tests are easy to perform, low-cost and do not require skilled personnel. Indeed rapid-cassette tests could represent, once validated, a powerful tool to immediately obtain an indicative result when rapidity is fundamental as in an emergency. Knowledge of the analytical performance of commercial methods is of critical importance for the correct interpretation of the results. To date, a multitude of commercially available kits exist.

Here, we show the comparison of analytic performance of two different rapid-cassette tests with the ELISA method. Finally, we compared the obtained results with an FDA-approved fully automatized CLIA method to further validate the obtained data. The study was performed on a population of healthcare workers to evaluate the feasibility and the validity of these tests for application in healthcare surveillance or in large-scale screening.

Key words: Covid-19; Antibody testing; rapid-cassette tests; ELISA; CLIA

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MATERIALS AND METHODS

Study population

The study was conducted at the IRCCS Policlinico San Donato of Milan (Italy). Samples were collected from 9th March 2020 to 13th May 2020. Serum samples from healthy donors collected in April 2018 were used as negative controls, representing pre-COVID-19 background immunity. Each patient gave written informed consent. This study was performed in accordance with the International Conference on Harmonisation of Good Clinical Practice guidelines, the Declaration of Helsinki (2008) and the European Directive 2001/20/EC.

Seventy-four voluntary healthcare workers (49 females and 25 males, mean age: 45.28 years), were included in the study. The subjects had performed nasopharyngeal swabs during sanitary surveillance or because of the onset of symptoms (41 RT-PCR +, 33 RT-PCR-), and 42 serum samples were collected before May 2018 which represented negative control of background immunity. Blood samples were collected within 4-8 weeks after performing nasopharyngeal swabs (mean 28.77 days SD: 23.11 days Min-Max: 0-81 days).

Cassette rapid IgG/IgM Test

Rapid tests were performed on serum samples after separation by centrifugation at 9000 rpm 9 minutes from freshly collected blood venous specimens. Cellex rapid cassette is a lateral flow chromatographic immunoassay, formed by a coloured pad, that contains SARS-CoV-2 recombinant antigens. Briefly 10µl of freshly isolated serum were dispensed in the sample well and 2 drops of sample diluent were immediately added. The results were read after 15-20 minutes. Declared sensitivity and specificity were 93.75% and 96.40%. Cross-reactivity is denied for principal infectious agents such as influenza A virus, adenovirus, respiratory syncytial virus, HBV, HCV, chlamydia pneumonia, cytomegalovirus etc., but positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains HKU1, NL63, OC43 or 229E.

Zhejiang Orient Gene is a lateral flow chromatographic immunoassay. The cassette is analogue to Cellex Cassette. Briefly 5µl of freshly isolated serum was dispensed in the sample well and 2 drops of sample buffer were immediately added. Results were read after 10 minutes. Declared sensitivity and specificity were 87.9% and 100% for IgM and 97.2% and 100% for IgG. Interference of elevated title of rheumatoid factor is reported.

ELISA tests

EDI (Epitope Diagnostics, San Diego, CA) COVID-19 IgG ELISA kit utilizes the microplate-based enzyme immunoassay technique. Microplate was coated with COVID-19 recombinant full-length nucleocapsid protein. Cross-reactivity is denied for principal infectious agents such as influenza A and B virus, adenovirus, respiratory syncytial virus, HCV and ANA presence. Declared sensitivity and specificity was 100% and 100%, respectively.

EDI (Epitope Diagnostics, San Diego, CA) COVID-19 IgM ELISA kit utilizes the "IgM capture method" on microplate-based enzyme immunoassay technique. A microplate was coated with an anti-human IgM specific antibody. A horseradish peroxidase (HRP) labelled recombinant COVID-19 antigen was added to each well. Cross-reactivity is denied for principal infectious agents such as influenza A and B virus, adenovirus, respiratory syncytial virus, HCV and ANA presence. Declared sensitivity and specificity was 45% and 100%, respectively. For both IgM and IgG, negative (NC) and positive (PC) controls were added. Positive cut-off was calculated on the basis of ROC curve.

Chemiluminescent method

SARS-CoV-2-IgG (Abbott, Ireland) is a chemiluminescent microparticle immune assay for qualitative assessment of IgG anti-nucleocapsid protein of SARS-CoV-2 in human serum on Architect analyser. Analyses were performed following the manufacture's instruction. Intensity of chemiluminescent reaction is measured as relative light units (RLU) that are directly correlated to the amount of SARS-CoV-2-IgG. The results are compared to RLU of the calibrator and transformed as a calculated Index (S/C): the cut-off is 1.4 Index (S/C).

Cross-reactivity for non-SARS-CoV-2 coronavirus strain was not evaluated, Cross-reactivity for principal infectious was excluded (see detailed datasheet from the manufacturer). The manufacturer declared that the positive percentage agreement was 100% following the analysis of sera after 14 days from the onset of symptoms. On the contrary, negative percentage agreement was 99.63% following the analysis of sera both from the pre-COVID19

era (before January 2019) and from subjects with other respiratory infections. Anti-human IgG do not recognize human IgM, IgA or ovine IgG. Interference of elevated title of rheumatoid factor is reported.

Statistical analysis

Sensitivity, specificity, accuracy, and ROC curve analysis were performed with Prism Graph Pad Software.

RESULTS

Rapid-cassette tests performance comparison

Seventy-four subjects (49 females and 25 males, mean age: 45.28 years) belonging to healthcare personnel of IRCCS Policlinico San Donato Hospital in Milan, Italy were screened. Both medical doctors, nurses and technical personnel were included. Firstly, the presence of simultaneous IgM or IgG responses were screened through rapid tests of two different companies, Cellex and Zhejinang Orient Gene.

Comparable results were obtained with both tests. In particular sensibility was 85.4% (95%CI 70.8-94.4), specificity was 90.91% (95%CI: 75.6-98.1) and accuracy was 87.8% for Cellex IgG, and sensitivity was 85.4% (95%CI: 70.8-94.43), specificity 87.88.6% (95%CI: 71.8-96.6) and accuracy 86.5%, respectively, for Cellex IgM. Sensitivity was 90.2% (95%CI: 76.9-97.3), specificity was 87.9% (95%CI: 71.8-96.6) and accuracy was 89.2% for Orient Gene IgG and 73.2% (95%CI: 57.1-85.8), 90.9% (95%CI: 75.7-98.1) and 81.2%, respectively, for Orient Gene IgM.

False positive subjects (3 on Cellex and 4 on Orient Gene) were observed according to IgM or IgG with both rapid tests. One hypothesis is a cross-reaction with other non-SARS-CoV-2 coronavirus strains such as HKU1, NL63, OC43 or 229E, or these subjects could be true positives turned negative to qRT-PCR, due to timing of analysis or due to low sensitivity of qRT-PCR. Deeper investigation revealed positivity to ANA and rheumatoid factor in one subject, a possible interferent.

Rapid-cassette tests showed positivity of both IgM and IgG in the majority of samples, however in some samples, IgM were undetectable while IgG were still present, possibly reflecting recovery. In fact, the time window of sampling covered 2-3 months post-infection.

Accordance between the two rapid tests was excellent (Accordance: 0.970, K Cohen: 0.936) on both IgM and IgG. All negative control samples collected before May 2018 resulted negative with both rapid tests.

Comparison of rapid-cassette tests with immune-enzymatic method

Screening was repeated on 33 of 74 samples through immune-enzymatic method. Results are shownas ROC curve in Fig. 1. According to the data,

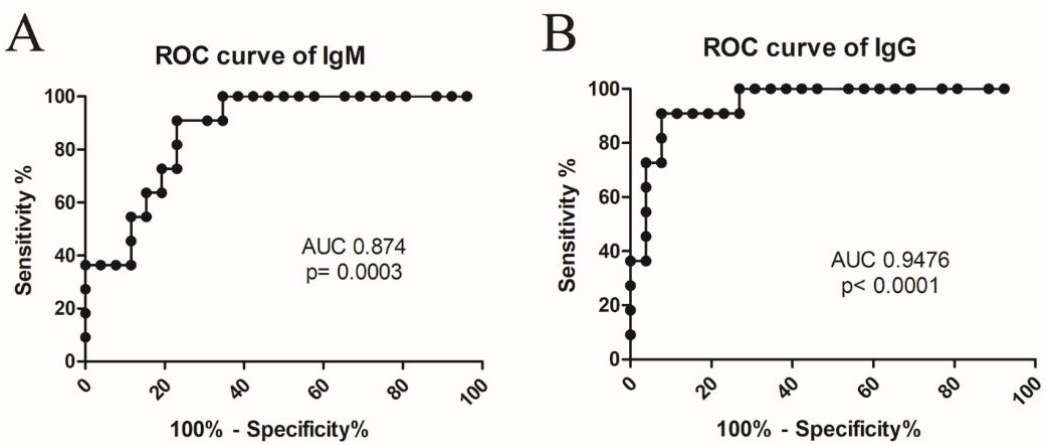

Fig. 1. *Graphs showing ROC curves of IgM (**A**) and IgG (**B**) of ELISA method.*

ROC curve of IgM showed a moderate performance (AUC=0.874, p=0.00038), optimal cut-off is 0.1870 Abs, estimated by Youden Index, it allowed a specificity of 76.92% and a sensitivity of 90.91% (Fig. 1A). IgG analysis showed a high performance according to AUC= 0.947 p=<0.0001, allowing a sensitivity of 90.91% and a specificity of 92.31% with a cut-off 0.1105 Abs (Fig. 1B).

Total agreement between ELISA and rapid test was excellent for IgG (Agreement: 0.970; k Cohen: 0.933) and, similarly to rapid-cassette tests; ELISA IgG did not avoid cross-reaction with other non-Sars-Cov-2 viruses. Total agreement between ELISA and rapid test was moderate for IgM (Agreement: 0.697; k Cohen: 0.289).

Comparison of rapid-cassette and ELISA with CLIA method

The results obtained were finally confirmed by using the CLIA method, designed to detect anti-nucleocapsid protein. Sensitivity was 85.37% (95% CI: 70.8-94.4) and specificity was 96.9% (95% CI:84.2-99.9), accuracy was 90.549%. Total agreement with ELISA was 97% (K Cohen: 0.93). Furthermore, CLIA confirmed positivity of one subject that was positive to both rapid-cassette and ELISA suggesting a false negative result of RT-PCR. Total agreement with rapid-cassette Cellex was excellent, 94.6% (K Cohen: 0.892). Total agreement with rapid-cassette Orient Gene was excellent 90.5% (K Cohen: 0.811). One negative control sample collected before May 2018 resulted positive with CLIA method.

DISCUSSION

The spreading of Covid-19 disease has represented a major challenge for healthcare systems worldwide. Furthermore, the degree of symptoms can vary, with a proportion of pauci/asymptomatic patients. In this sense, rapid recognition of new infections is of critical importance, and RT-PCR detects directly viral RNA in a brief time window, whilst serologic tests record both active and previous infections. Here, we showed that serological monitoring of antibody responses could be a reliable low-cost method to screen a large mass of individuals, such as workers in industries or travellers in airports. These methods are rapid (answer in 10 minutes), easy to perform, and do not require skilled personnel, thus allowing to screen a large number of samples (a limit of molecular analysis). The results demonstrate that the rapid tests analysed have good analytical performance, with the majority of subjects showing positive response within 3 weeks, accordingly to the literature (4). Rapid Zhejiang Orient Gene cassette has been independently evaluated by another group (5) and the results showed the same IgM sensitivity, probably due to timing of sampling, but higher sensitivity. ELISA analysis confirmed the results obtained by rapid-cassette in terms of sensitivity, but presented higher specificity. Finally, the CLIA method increased specificity up to 97%. However, a positive serological test always requires - independently from the method – to perform molecular analysis, so that specificity will be increased anyway. Conversely, to avoid false negative at first screening remains of critical importance. In synthesis, CLIA automatized methods are the best choice whenever it is possible (and easy) to collect venous blood samples and time-to-answer is not a limitation. On the other hand, rapid tests are better when non-skilled personnel have to test high numbers of people in a short time – for example to monitor passengers in airports, or workers in large industries.

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