



Comparison of various serological assays for novel SARS-CoV-2

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Abstract

Coronavirus disease-19 (COVID19), the novel respiratory illness caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2), is associated with severe morbidity and mortality. The aim of our study was to compare different immunoassays. We evaluated three immunochromatographic test (The StrongStep®SARS-CoV-2 IgG/IgM kit, AllTest COV-19 IgG/IgM kit, and Wondfo® SARS-CoV-2 Antibody) and two chemiluminescence immunoassays (CMIA) (Covid-19 VIRCLIA® IgM+IgA/IgG monostest and the Abbott SARS-CoV-2 IgG assay) in COVID-19 patients. The assays were performed using serum samples of three group patients, i.e., healthy controls, patients with SARS-CoV-2 PCR positive, and patients with SARS-CoV-2 PCR negative clinically diagnosed of COVID-19 infection. The detection percentages of IgG with the StrongStep® SARS-CoV-2 IgG/IgM kit and AllTest COV-19 IgG/IgM kit were similar in both groups (83.3% and 80.6%, respectively in group 2, $p = 0.766$) and (42.9% and 50.0%, respectively in group 3, $p = 0.706$). There were some differences on IgM detection between StrongStep® SARS-CoV-2 IgG/IgM kit and AllTest COV-19 IgG/IgM kit (11.1% and 30.6%, respectively in group 2, $p = 0.042$ and 0.0% and 28.6%, respectively in group 3, $p = 0.031$). The positive rate of IgG in group 2 is higher compared to group 3 with the two immunoassays tested. We observe the same positive rates of IgG with the two CMIA. Our study shows excellent performance of CMIA compared to immunochromatographic test and confirms its potential use in the diagnosis of the new SARS-CoV-2.

Keywords Antibodies · Chemiluminescence immunoassays · IgG · IgM · Immunochromatographic tests · SARS-CoV-2

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Introduction

Severe acute respiratory syndrome coronavirus (SARS-CoV-2) [1] was discovered in December 2019 in the city of Wuhan, China. It soon spread to other cities and countries, and on 11 March 2020 was proclaimed a pandemic by WHO. The clinical symptoms of most patients are fever, sore throat, cough, and shortness of breath [2].

RT-PCR (reverse transcription polymerase chain reaction) remained the gold standard for the diagnosis of infection due to SARS-CoV-2, which has led to 974,449 coronavirus disease-19 (COVID19) cases and 33,992 deaths by 19 October 2020 in Spain. Its sensitivity did not reach 100% but it remained better than that of methods based on the detection of antigens. However, RT-PCR takes a long time to get results and qualified personnel is necessary. In several cases, RT-PCR has shown false negatives in patients with pneumonia, showing clinical and radiographic evidence compatible with COVID19; these patients were considered as clinically diagnosed of SARS-CoV-2 according to the 5th edition of guideline on diagnosis and treatment of the novel coronavirus pneumonia.

Different studies are being developed evaluating the use of lateral flow immunoassays to help in the diagnosis of SARS-CoV-2 and its use to know the seroprevalence state of the population [3–7]. Serology based on immunoassays, ELISA, or chemiluminescence [8] are being evaluated and they show more sensitivity and specificity than the lateral flow immunoassays [9, 10]

Materials and methods

Here, we present the newly developed serological detection methods targeting the viral antibody, lateral flow method, and a chemiluminescent analytical system (CLIA) that were conducted in the Hospital Central de la Defensa Gómez Ulla (Madrid).

Study design

In our study, we evaluate two chemiluminescence assays and three lateral flow immunoassays for the detection of SARS-CoV-2 antibodies.

Patient serum samples used in this study were submitted to the routine Microbiology Laboratory at Hospital Central de la Defensa Gómez Ulla (Madrid) for diagnostic purposes.

Study period and serum samples

Control serum samples ($n = 50$) included archived anonymous serum obtained from healthy blood donors with no history of SARS-CoV-2 infection, between 1 September and 30 October 2019 (group 1, healthy control).

These serum samples were donated to the Microbiology Laboratory by the transfusion Center of the Armed Forces at Hospital Central de la Defensa Gómez Ulla (CTFAS).

Case serum samples were obtained from patients with SARS-CoV-2 infection ($n = 50$) between 6 March and 1 April 2020. (group 2, patients with RT-PCR—positive and group 3, patients with RT-PCR—negative, “clinically diagnosed,” that means patients with pneumonia, showing clinical and radiographic evidence compatible with COVID19 according to the 5th edition of guideline on diagnosis and treatment of the novel coronavirus pneumonia).

Real-time PCR assay

We used three types of automatic extractors to obtain viral RNA from clinical samples, i.e., MagCore HF16 (RBC bioscience, Taipei, Taiwan), Nimbus Microlab Seegene (Hamilton Company, Bonaduz, Switzerland), and m2000 system (Abbott Molecular Inc. Des Plaines, IL).

RNA amplification was made using two real-time PCR platforms, i.e., qCOVID-19 (Genomica, Madrid, Spain) and

Allplex 2019-nCoV assay (Seegene, Seoul, South Korea), and we used the CFX96™ (Bio-Rad) Real-Time Detection System. PCR did not have a human extraction control gene target. The extraction control gene target was a phage.

These kits were used according to the manufacturer’s instructions for both the handling and the interpretation of the results.

Immunochromatographic test (lateral flow method)

We evaluated 3 immunochromatographic tests (The StrongStep®SARS-CoV-2 IgG/IgM kit, AllTest COV-19 IgG/IgM kit, and Wondfo® SARS-CoV-2 Antibody).

AllTest COV-19 IgG/IgM kit was evaluated only in group 2 and group 3 because samples from group 1 were exhausted.

The tests were performed at room temperature according to the manufacturer’s instructions. For all tests, the recommended sample volume of 10 μ l serum was added to the sample well. These tests did not provide target information or suggestion of titer.

For the StrongStep® SARS-CoV-2 IgG/IgM kit and Wondfo® SARS-CoV-2 Antibody, 2 drops were transferred (approximately 80 μ l of buffer solution) to the buffer well and the result was read visually after 15 min.

In case of the AllTest COV-19 IgG/IgM kit, 1 drop (40 μ l) of buffer was added, and the result was read after 10 min.

Weak signals for IgM/IgG, together or separate, were considered positive. The samples with only C line were regarded as negative; the strips where no C line showed up should be considered as an invalid test.

The Wondfo® SARS-CoV-2 Antibody assay does not discriminate between IgM and IgG.

Chemiluminescence immunoassay

Covid-19 VIRCLIA® IgM+IgA monostest and Covid-19 VIRCLIA® IgG monostest (Vircell, S.L.)

Both were performed according to the manufacturer’s instructions. Prior to testing, samples should be inactivated at 56 °C for 30 min.

Samples have been processed in VIRCLIA® analytical system of chemiluminescent. The CLIA method is based upon the reaction of anti-nucleocapsid and anti-spike antibodies in the sample tested with the antigen adsorbed on the polystyrene surface. Unbound immunoglobulins are washed off. An enzyme-labeled anti-human globulin binds the antigen antibody complex in a second step. After a new washing step, bound conjugate is developed with the aid of a chemiluminescent substrate solution that will generate a glow-type luminescence that can be read with a luminometer.

Each sample is assayed onto two reaction wells: one coated with antigen and one processed and blocked similarly to the

reaction well except that it is not coated with antigen. The blank well is used to subtract possible unspecific backgrounds.

The Abbott SARS-CoV-2 IgG assay (Abbott Laboratories Inc., IL, USA)

This technique is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of IgG antibodies to SARS-CoV-2 in human serum or plasma on the ARCHITECT System. A minimum of 100 μ l of serum or plasma is required.

The assay is designed to detect IgG antibodies to the nucleocapsid protein of SARS-CoV-2. Sample, SARS-CoV-2 antigen coated paramagnetic microparticles, and assay diluent are combined and incubated. The IgG antibodies to SARS-CoV-2 present in the sample bind to the SARS-CoV-2 antigen coated microparticles. The mixture is washed. Anti-human IgG acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, pre-trigger and trigger Solutions are added.

For both immunoassays evaluated, the resulting chemiluminescent reaction is measured as a relative light unit (RLU). There is a direct relationship between the amount of antibodies to SARS-CoV-2 in the sample and the RLU detected by the system optics.

Statistical analyses

Statistical analysis was carried out using the statistical package STATA/IC version 13.1 (StataCorp, TX, USA). Continuous data are expressed as median and IQR, while categorical data were expressed as frequencies and percentages. Comparisons between variables were made using two-tailed Fisher's exact test or *t* test. For these comparisons, a *p* value less than or equal to 0.05 was considered significant.

The agreement between the different serological diagnostic techniques was expressed by the Kappa Index and percentage of agreement. A Kappa value of more than 0.75 indicates good agreement between tests, while a value of less than 0.4 indicates poor agreement.

Results

The serologic results are summarized in Table 1.

Evaluation of the chemiluminescence immunoassays

Two commercial chemiluminescence immunoassays were evaluated using 50 serum samples (group 2 and group 3) and 50 control serum samples (group 1).

The positive rate of IgG in group 2 is higher compared to group 3 with the two immunoassays tested (97.2% and 50.0%, respectively, *p* = 0.001). We observe the same positive rates of IgG with the two chemiluminescence immunoassays.

With the Covid-19 VIRCLIA® Immunoassay, we also determine IgM + IgA. The positive rates of IgM + IgA in group 2 are higher compared to group 3 but there was no statistical significance (77.8% and 50.0%, respectively, *p* = 0.054).

Evaluation of the immunochromatographic test

Three commercial CE-marked immunochromatographic test (lateral flow methods) for detecting SARS-CoV-2 antibodies were evaluated in the same serum samples as describe previously.

Thirty-five cases of group 2 (97.2%) and eight cases of group 3 (57.1%) were positive for SARS-CoV-2-specific antibodies IgM or IgG by at least one of the three immunochromatographic test.

Although the nucleic acid test is the "gold standard" for microbiological diagnosis, due to certain limitations, the false negative cases are not rare. Hence, the IgM and IgG antibodies were also examined in clinically diagnosed patients (group 3).

The positive rates of IgM or IgG in group 2 (RT-PCR-positive patients) are higher in comparison to group 3 in all immunochromatographic tests. The positive percentages of IgG detected with the StrongStep® SARS-CoV-2 IgG/IgM kit and AllTest COV-19 IgG/IgM kit were similar in both groups (83.3% and 80.6%, respectively in group 2, *p* = 0.766; and 42.9% and 50.0%, respectively in group 3, *p* = 0.706). However, the positive percentages of IgM detected with the StrongStep® SARS-CoV-2 IgG/IgM kit and AllTest COV-19 IgG/IgM kit are highly different (11.1% and 30.6%, respectively in group 2, *p* = 0.042; and 0.0% and 28.6%, respectively in group 3, *p* = 0.031).

Combining the result of IgM and IgG, in example patients with either IgM or IgG positive, we do not observe a significant increase in the percentage of positive results with any of the three assays compared with the positive rate of IgG. The combined results obtained are similar with the StrongStep® SARS-CoV-2 IgG/IgM kit and AllTest COV-19 IgG/IgM kit (83.3% and 88.9% respectively in group 2, *p* = 0.766; and 42.9% and 50.0% respectively in group 3, *p* = 0.706). Wondfo® SARS-CoV-2 Antibody assay does not discriminate between IgM and IgG, and the positive rate was 88.9% and 50.0%, respectively, similar to the combined results of the other two assays tested.

Agreement between serological assays

To determine the agreement between the different assays evaluated, the proportion of case serum samples which shared the same result between two assays was calculated. The high

Table 1 Serological results from the three groups of patients

	Group 1 (No = 50)	Group 2 (No = 36)	Group 3 (No = 14)	<i>p</i> value*
StrongStep® SARS-CoV-2 IgG/IgM kit				
IgM positive no. (%)	0 (0.0)	4 (11.1)	0 (0.0)	0.194
IgG positive no. (%)	0 (0.0)	30 (83.3)	6 (42.9)	0.004**
IgM or IgG positive no. (%)	0 (0.0)	30 (83.3)	6 (42.9)	0.004**
AllTest COV-19 IgG/IgM kit				
IgM positive no. (%)	No data	11 (30.6)	4 (28.6)	0.890
IgG positive no. (%)	No data	29 (80.6)	7 (50.0)	0.030**
IgM or IgG positive no. (%)	No data	32 (88.9)	7 (50.0)	0.030**
Wondfo® SARS-CoV-2 Antibody				
Total ACS positive no. (%)	1 (2.0)	32 (88.9)	7 (50.0)	0.030**
COVID-19 VIRCLIA®				
IgM/IgA positive no. (%)	2 (4.0)	28 (77.8)	7 (50.0)	0.054
IgG positive no. (%)	0 (0.0)	35 (97.2)	7 (50.0)	0.001**
IgM or IgG positive no. (%)	2 (4.0)	36 (100.0)	8 (57.1)	0.001**
Abott				
IgG positive no. (%)	0 (0.0)	35 (97.2)	7 (50.0)	0.001**
Time between PCR result and serology (median, days)	Not applicable	11.4	4.9	0.087

*Statistical differences between group 2 and group 3

**A *p* value less than or equal to 0.05 was considered significant

concordant percentage was between Covid-19 VIRCLIA® IgG monotest and Abbott SARS-CoV-2 IgG assay (Fig. 1) that were 96.0% concordant.

Discussion

In the present study, three lateral flow immunoassays and two chemiluminescence assays for the detection of SARS-CoV-2 antibodies were evaluated using serum samples from adult patients diagnosed for SARS-CoV-2 (group 2 and group 3) and a collection of control serum samples taken before the emergence of the virus in Wuhan in December 2019.

Our study shows that immunochromatographic tests and chemiluminescence assays are very specific, with a low rate of false positive results, both in detecting IgM or IgG antibodies (< 5%, group 1). These control serum samples were taken from the Transfusion Center of the Armed Forces at Hospital Central de la Defensa Gómez Ulla, so these samples were screened for transmission of other infectious diseases, so it is improbable that there was cross-reactivity.

There is increasing evidence of the usefulness of serology in the evaluation of the course of SARS-CoV-2 infection [11, 12]; due to this, there is a high interest about the usefulness of the serologic rapid test, but there is scarce information about their utility.

The differences observed between the different assays may be in part explained by SARS-CoV-2 antigen targeted and the chemiluminescence assays format [13]

Overall, we found that the lateral flow immunoassays generally presented similar results regarding IgG, while they had greater differences in IgM detection, AllTest COV-19 IgG/IgM kit detected a higher rate of IgM than StrongStep® SARS-CoV-2 IgG/IgM kit.

Covid-19 VIRCLIA® IgG monotest Immunoassay and Abbott SARS-CoV-2 IgG assay present same rates of positivity of IgG. We observed that the detection capacity of chemiluminescence assays in group 2 is greater than 95%, while by means of lateral flow immunoassays, this value is less than 90%.

When comparing results between group 3 and group 2, many differences regarding the detection of antibodies are found. The percentage of antibodies detected in group 3 is lower with any of the techniques used (immunochromatographic test or chemiluminescence assays). This could be caused either by an error in the diagnosis (the pneumonia was caused by another microorganism) or there is not enough time between the RT-PCR and the antibody development. We have no data of serial samples of group 3 patients; diagnostic performance could potentially be better if serological assay was repeated 2 weeks later. Even if a late positive sample does not help physician for acute care, it is always interesting to have a definite confirmation of diagnosis in case series or studies.

We know this study has a few limitations. First, we selected a small number of serum samples and all of them belong to patients of the same hospital. Anti-nucleocapsid CMIA platforms have limitations; some studies show N protein is not suitable to detect virus-specific antibodies due to very high-

Fig. 1 Index Kappa correlation and agreement between serological results

AllTest COV-19 IgG / IgM kit®							
IgM	0.174 (68.0)						
IgG			0.714 (88.0)				
COVID-19 VIRCLIA®							
IgM/IgA	0.06 (32.0)		0.242 (52.0)				
IgG			0.658 (88.0)		0.556 (84.0)		
Abbott®							
IgG			0.658 (88.0)		0.667 (88.0)		0.851 (96.0)
WondfoR SARS-CoV-2 Antibody							
IgM + IgG	0.048 (30.0)	0.522 (82.0)	0.168 (48.0)	0.436 (78.0)	0.352 (74.0)	0.677 (90.0)	0.548 (86.0)
	IgM	IgG	IgM	IgG	IgM	IgG	IgG
	StrongStep [®] SARS-CoV-2 IgG / IgM kit®		AllTest COV-19 IgG / IgM kit®		COVID-19 VIRCLIA®		Abbott®

level of cross-reactivity[14]; without demonstration of subsequent IgG seroconversion, it is difficult to assume that IgM positive cases are specific. Secondly, group 3 presents a shorter time between performing the RT-PCR and serology than for patients in group 2 (4.9 and 11.4 days, respectively, $p = 0.087$). This could explain the higher rate of antibody detection in group 2 with all the assays tested. The objective of the serology in group 2 is to assess the course and the host immunity of SARS-CoV-2 infection; the objective in group 3 was to increase the evidence of SARS-CoV-2 infection in patients with RT-PCR result negative for this novel coronavirus. However, this may be the consequence of a third limitation since we ignore the exact time elapsed since the beginning of the symptoms. Most studies instigate a minimum of 14 days or in some cases 21 days since symptom onset prior to serology testing in order to avoid false negatives, reporting poor performance in the first 10 days[6].

And finally, we do not have results for group 1 (healthy control) using AllTest COV-19 IgG/IgM kit because the samples were exhausted.

To the best of our knowledge, this is one of the first studies on anti-SARS-CoV-2 IgM and IgG antibodies that compare chemiluminescence methods and immunochromatographic test (lateral flow methods). More multicenter studies with a large number of serum samples from different areas would be necessary to evaluate these techniques.

Overall, our data demonstrate excellent results with the chemiluminescence immunoassays, Abbott Architect

SARS-CoV-2 IgG Assay and Covid-19 VIRCLIA® assay, compared to immunochromatographic tests and thus its use in the diagnosis of the new SARS-CoV-2. Chemiluminescence assays could be an important component in the diagnostic approach to SARS-CoV-2 infection

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Clinical and microbiological data acquisition: MSS, ACB, MZC, and CYV

Sample processing: MSS and ASG
Statistical analysis and interpretation of data: ACB
Writing of the manuscript: MSS, ACB, and MZC
Critical revision of the manuscript: MMM
All authors read and approved the final manuscript.

Data availability Not applicable

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval The study was conducted according to the ethical requirements established by the Declaration of Helsinki. The Ethics Committee of Hospital Central de la Defensa Gómez Ulla (Madrid) approved the study.

Consent to participate Not applicable

Consent for publication Not applicable

Code availability Not applicable

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