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Evaluation of five immunoassays and one lateral flow immunochromatography for anti-SARS-CoV-2 antibodies detection

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ARTICLE INFO

Article history:
Received 28 September 2020
Accepted 6 December 2020
Available online 20 January 2021

Keywords:
SARS-CoV-2
Serology
Immunochromatography
ELISA
CLIA
RT-PCR

ABSTRACT

Introduction: In order to deal with the current pandemic caused by the novel SARS-CoV-2 coronavirus several serological immunoassays have been recently developed with the objective of being used as a complementary diagnostic tool and to support the RT-PCR technique currently considered the “gold-standard” method. However, these new assays need to be evaluated and validated. The purpose of this study was to assess the performance of five immunoassays (two ELISA and three CLIA assays) and one rapid immunochromatographic test for the detection of anti-SARS-CoV-2 antibodies.

Methods: Five semiquantitative immunoassays (MENARINI®, PALEX®, VIRCLIA®, ROCHE® and SIEMENS®) and one lateral flow rapid test (WONDFO®) were performed. A total of 124 samples were studied. Case serum samples (n = 78) were obtained from COVID-19 patients confirmed by real-time RT-PCR/epidemiological-clinical-radiological criteria, and control non-SARS-CoV-2 samples (n = 46) belonged to healthy healthcare workers involved in a seroprevalence study.

Results: Overall, the tests showed sensitivities around 70–90% and specificities greater than 95%, including the immunochromatographic test. In addition, we observed very good agreements among them, being better for the detection of IgG than for IgM antibodies (Cohen's kappa index of 0.95 for VIRCLIA® IgG with ROCHE®), as well as good diagnostic power of the tests as determined by the ROC curves.

Conclusions: This study demonstrates the proper performance of the different immunoassays in order to be applied in the clinical practice as support in the diagnostic approach and in the development of vaccines and seroepidemiological studies of COVID-19.

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Métodos: Se utilizaron cinco inmunoensayos semicuantitativos (MENARINI®, PALEX®, VIRCLA®. ROCHE® y SIEMENS®) y un test de inmunocromatografía rápida (WONDFO®). Se estudiaron un total de 124 muestras. Las muestras de suero (n = 78) se obtuvieron de pacientes COVID-19 confirmados por RT-PCR en tiempo real/criterios epidemiológicos-clínico-radiológicos. Las muestras control negativas (n = 46) pertenecieron a personal sanitario involucrado en un estudio de seroprevalencia.

Resultados: En general, las pruebas mostraron menores sensibilidades en torno al 70-90% y especificidades superiores al 95%, incluso la prueba inmunocromatográfica. Además, observamos muy buenas concordancias entre ellas, presentando mayores sensibilidades para la detección de anticuerpos IgG que para IgM (índice kappa de Cohen de 0,95 para VIRCLA® IgG con ROCHE®), así como un buen poder diagnóstico de las técnicas determinado por las curvas ROC.

Conclusiones: Este estudio demuestra el buen rendimiento de los diferentes inmunoensayos para ser empleados en la práctica clínica como apoyo en el proceso de diagnóstico, en el desarrollo de vacunas y estudios seroepidemiológicos de COVID-19.

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in human serum and plasma using the ADVIA Centaur® XP Immunoassay System analyzer (Siemens Healthcare Diagnostics Inc., Tarrytown, USA). The test is based on a sandwich principle using acridinium ester chemiluminescent technology and its duration is about 15 min. The solid phase contains a preformed complex streptavidin-coated microparticles and biotinylated SARS-CoV-2 recombinant antigens. It employs S1 subunit of the SARS-CoV-2 spike protein and the receptor binding domain (RBD).

Rapid immunochromatographic test

WONDFO® SARS-CoV-2 Antibody Test (Lateral Flow Method) (Guangzhou Wondfo Biotech Co., Guangzhou, PR China) is an immunochromatographic assay for rapid, qualitative detection of SARS-CoV-2 IgG/IgM antibody in human whole blood, serum or plasma samples. The test was performed at real time according to the manufacturer’s instructions and with serum samples. The result was read visually after 15 min.

Statistical analysis

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for each serological test. Receiver operator characteristic (ROC) curves were performed using R packages pROC for comparisons of the area under the curve (AUC). The area under the curve (AUC) was calculated for agreement between all analyzed assays and were shown in accompany with 95% confidence interval (CI). A p-value <0.05 was considered statistically significant. Statistical analysis was performed with R Commander.

Results

Sensitivities and specificities of the assays

Table 1: Analytical sensitivities, specificities and predictive parameters for SARS-CoV-2 antibody detection.

| assay                | Sensitivity (95% CI) | Specificity (95% CI) | PPV (95% CI) | NPV (95% CI) | Accuracy (95% CI) |
|----------------------|----------------------|----------------------|--------------|--------------|-------------------|
| WONDFO® IgG          | 96.1% (95.0–97.2)    | 95.7% (94.6–96.9)    | 86.1% (82.6–89.7) | 97.7% (96.3–98.7) | 96.1% (95.0–97.2) |
| PALEX® IgG           | 95.1% (94.1–96.1)    | 95.7% (94.6–96.9)    | 85.9% (82.4–89.7) | 98.8% (97.5–99.6) | 95.1% (94.1–96.1) |
| PALEX® IgM           | 95.1% (94.1–96.1)    | 95.7% (94.6–96.9)    | 85.9% (82.4–89.7) | 98.8% (97.5–99.6) | 95.1% (94.1–96.1) |
| PALEX® Combined IgG  | 95.1% (94.1–96.1)    | 95.7% (94.6–96.9)    | 85.9% (82.4–89.7) | 98.8% (97.5–99.6) | 95.1% (94.1–96.1) |
| PALEX® Combined IgM  | 95.1% (94.1–96.1)    | 95.7% (94.6–96.9)    | 85.9% (82.4–89.7) | 98.8% (97.5–99.6) | 95.1% (94.1–96.1) |
| PALEX® Combined IgG/M| 95.1% (94.1–96.1)    | 95.7% (94.6–96.9)    | 85.9% (82.4–89.7) | 98.8% (97.5–99.6) | 95.1% (94.1–96.1) |
| PALEX® Combined IgG | 95.1% (94.1–96.1)    | 95.7% (94.6–96.9)    | 85.9% (82.4–89.7) | 98.8% (97.5–99.6) | 95.1% (94.1–96.1) |
| PALEX® Combined IgM | 95.1% (94.1–96.1)    | 95.7% (94.6–96.9)    | 85.9% (82.4–89.7) | 98.8% (97.5–99.6) | 95.1% (94.1–96.1) |
| PALEX® Combined IgG/M| 95.1% (94.1–96.1)    | 95.7% (94.6–96.9)    | 85.9% (82.4–89.7) | 98.8% (97.5–99.6) | 95.1% (94.1–96.1) |

* Calculated as positive sera among total positive samples and negative sera among total negative samples.

In general, as shown in Table 1, CLIA assays had the best diagnostic odds ratio (156.35 for VIRCLIA® IgG and 113.46 for ROCHE®) compared to ELISA (best for PALEX® IgG: 89.60) and WONDFO® (79.82) immunoassays.
Table 2
Agreement between the different serological assays analyzed (s, (95% CI)).

| Assay          | IgG    | IgM    | IgG    | IgM    |
|----------------|--------|--------|--------|--------|
| PALEX® Total Ig | 0.77   | (0.64-0.89) | 0.62   | (0.34-0.71) |
| PALEX® IgG      | 0.80   | (0.70-0.91) | 0.84   | (0.74-0.93) |
| VIRCLA® IgG     | 0.95   | (0.70-0.94) | 0.59   | (0.19-0.88) |
| VIRCLA® IgM     | 0.70   | (0.57-0.83) | 0.77   | (0.45-0.97) |
| ROCHE® Total Ig | 0.77   | (0.66-0.88) | 0.72   | (0.40-0.95) |
| SIEMENS® Total Ig | 0.71  | (0.58-0.84) | 0.42   | (0.24-0.61) |

Agreement between serological assays

To determine the agreement between the different techniques, the Choen Kappa index was calculated. The results were summarized in Table 2 and divided according to IgG or IgM antibodies measurement except those for ROCHE®, SIEMENS® and WONDFO® that determine total antibodies.

For IgG, all the assays presented a good or very good concordance, being VIRCLA® the one that presented the best indexes with the other CLIA assays (0.95 with ROCHE® and 0.90 with SIEMENS®, respectively). WONDFO® had good agreements despite being a rapid and qualitative immunochromatographic test.

However, for IgM, lower correlations were observed in comparison with those for IgG. It is noteworthy to mention the very good agreement, in the case of IgM, between PALEX® (ELISA) and SIEMENS® (CLIA) with a value of 0.82. In contrast, MENARINI® showed only moderate concordances.

Comparative analysis of ROC curves

ROC curves as well as AUC for ELISA and CLIA assays are shown in Fig. 1. All the techniques presented areas higher than 0.8 indicating good or very good probability of classification. PALEX® showed the best AUC for IgG determination (0.96) followed by VIRCLA® IgG (0.92), SIEMENS® (0.91) and ROCHE® and PALEX® IgM with a value of 0.90.

Youden’s index was also used in conjunction with ROC analysis and provided the performance of the diagnostic test. These results are shown in Table 1. PALEX® IgG, VIRCLA® IgG and ROCHE® got a value of 0.8. A Youden’s index of 0.7 was obtained for the combined techniques (IgM or IgG), MENARINI® IgG and WONDFO®. The lowest value was for MENARINI® IgM (0.3).

Furthermore, ROC curves for paired comparisons between the serological assays were constructed and the p-values obtained are summarized in Table 3. For IgM, significant differences (p-value <0.05) were found among CLIA assays. In contrast, for IgG, MENARINI® showed no significant AUC differences only with ROCHE®, PALEX® IgG with SIEMENS® and ROCHE® also presented significant differences (p-value <0.05).

Discussion

In this study, five automated immunoassays and one rapid lateral flow immunochromatographic test were evaluated using serum samples from COVID-19 patients with PCR-confirmed diagnoses or epidemiological-clinical-radiological criteria for SARS-CoV-2, and sera from healthy hospital personnel, involved in a seroprevalence study, as non-SARS-CoV-2 control samples during the period of April–May 2020.

Regarding the sensitivities and specificities, according to our results, ELISA showed better sensitivities than CLIA (88.9% vs. 85.7%). However, regarding the specificities, CLIA presented better results than ELISA (96.7% vs. 93.4%).

WONDFO® had a sensitivity of 73.0% and 96.7% of specificity. These results are comparable with those obtained in the study carried out by The Health Institute of Carlos III in Madrid in its report of April 2020,14 with values of 77.8% and 95.0%, respectively. Despite being a qualitative test, the good results demonstrate that it could be an adequate method for antibody detection in patients who had overcome the infection, in just 15 min. So, this test has the advantage of being the most rapid and easiest detection method compared to the semiquantitative assays and can be used as point-of-care test (POCT).

In the same way, CLIA presented better PPV in comparison with ELISA.

Table 3
p-Values for the paired comparisons of ROC curves between serological assays.
In general, CLIA showed better results versus ELISA. Differences between the assays may be explained by the SARS-CoV-2 antigen targeted and the format test used. MENARINI® test employs the nucleocapsid (N) protein while PALEX® uses nucleocapsid and spike (S) recombinant antigens. VIRCLIA® employs the S1 subunit of the SARS-CoV-2 spike protein and nucleocapsid antigen, SIEMENS® detects antibodies to the S1 subunit of the SARS-CoV-2 spike protein and the receptor binding domain (RBD), however, ROCHE® uses a recombinant protein representing the nucleocapsid antigen. Okba et al.15 recently reported a study of development of serological assays in which SARS-CoV-2 specific antibody responses were evaluated according to the coronavirus structural proteins in an ELISA format, reaching the conclusion that S1 is more specific than S as an antigen for SARS-CoV-2 serological diagnosis, as well as that RBD and N ELISAs were more sensitive than S1 ELISA in detecting antibodies in mildly infected patients. Besides, they found differences between immunoglobulins, being IgG the most specific.

Another important aspect to mention is the cross-reactivity with other coronaviruses, which could generate false positive results in the serological determinations, as it was also discussed in the Okba et al.15 study. They verified the specificity of the assays in patients with SARS-CoV, MERS-CoV or HCoV-OC43, among others. Results for S1 subunit and RBD results were the best, showing none or little cross-reactivity for SARS-CoV, due to the high degree of similarity between the S1 and RBD of the SARS-CoV and SARS-CoV-2. However, further investigation is needed in order to validate SARS-CoV-2 serological assays that are currently lacking.

In addition, differences found with PCR results may be explained taking into account the viral load of the infection. In mild cases, due to the low viral load and the presence of mild symptoms, these patients may not have produced antibodies which are not detected in the immunoassays, causing discordance with PCR results (false negative results). Yongchen et al.16 reported similar results when evaluating the serological response based on the disease severity, concluding that an immediate antibody response was identified in severe cases compared to non-severe cases, and only one asymptomatic patient showed seroconversion.

Besides, false positive results regarding the PCR were low, in fact, there were 2 symptomatic patients in our study with negative PCR and positive serology. Limitations of the PCR technique have been reported and the majority of errors may occur due to preanalytical factors which interfere in the amount of viral load and/or the ARN stability giving false negative results.17 Assessment of test accuracy in terms of sensitivity and specificity of diagnostic tests are in increasing attention and being studied due to the derived implications.18

The diagnostic odds ratio is a measure of test performance and effectiveness. This statistic parameter is also useful for comparing tests.19 In this study, CLIA assays presented the best results being VIRCLIA® IgG and ROCHE® the most effective techniques (156.35 and 113.46, respectively).

Regarding the agreement between serological assays, for IgG, it was observed good or very good concordances especially between CLIA assays. A Chosen Kappa index of 0.95 for VIRCLIA® with ROCHE®, 0.90 for VIRCLIA® with SIEMENS® and 0.88 for ROCHE® with SIEMENS®. Moreover, for IgM, it is important to note the very good agreement between PALEX® and SIEMENS® (0.82). These results based on our data demonstrate that the different techniques are comparable.

Finally, the diagnostic power of these assays was determined by the ROC curves. The high values of AUC mean good or very good classifications. Both, the three CLIA and PALEX® IgG presented the best AUC values, all of them >0.9 (except VIRCLIA® IgM, AUC 0.84) as well as a Youden’s index of 0.8, but 0.7 for SIEMENS® and 0.6 for VIRCLIA® IgM. In addition, the paired comparisons were constructed in order to evaluate the possibility of exchanging the techniques. Based on our results, no significant differences (p>0.05) were observed except in the following cases (p<0.05): VIRCLIA® IgM with SIEMENS® and with ROCHE®; MENARINI® IgG with PALEX® IgG, with VIRCLIA® IgG and with SIEMENS® and PALEX® IgG with SIEMENS® and with ROCHE®.

It is noteworthy to mention the different results obtained for IgG and IgM. Apart from the SARS-CoV-2 antigen target used in each technique, the time that had elapsed between the moment of infection and the sample collection time is an important factor. Some authors have reported the diagnostic value of antibodies’ assays for patients at different times after onset20,21 concluding that IgM increased during the first week, reached its peak after two weeks and then its level decreased in most patients. Meanwhile, IgG was generated after 1 week and reached its peak level in 3 weeks. In this study, samples were collected on average 20 days after having positive PCR results/epidemiological-clinical-radiological criteria, this fact may explain the better results obtained for IgG than for
IgM. Thus, the use of the serological assays tested would need further studies in order to ensure their application as a diagnostic tool in the acute phase of the disease.

In general, our findings demonstrate the good performance of the different immunoassays. In particular, the usefulness of WONDO® as a rapid complementary diagnostic test; about ELISA, PALEX® IgG showed good results but the best results were found for CLIA highlighting VIRCLIA® IgG, SIEMENS® and ROCHE®. Considering the increasing demand due to the new outbreaks, those which are most profitable would be ROCHE® or SIEMENS® because the determination only takes less than 20 min, they are more economic in comparison with the rest of the assays, and are now available in many laboratories making automation possible according to the logistics organization of each laboratory. Thus, they would be suitable for supplying not only the demand in the clinical context but also the current seroprevalence studies of SARS-CoV-2 infection such as the one carried out in Spain by Prollán et al.12

Several limitations should be mentioned in this study. First, the limited number of samples that may have some impact on the statistical results, so, external validation studies would be needed in a larger number of patients. Second, this comparative study was carried out with the aim of having available as many techniques as possible in order to assist the diagnostic process and taking into account the commercial supply. It is for this reason that the comparison among assays has been carried out independently of the kind of antibodies detected (IgM, IgG or total Ig). Furthermore, false negative and false positive results of antibody detection might affect the analysis, due to different illness severities and sample collection time relative to the onset of infection. Finally, no reliable gold standard for serological tests is currently available for comparative studies, however, RT-PCR is used for this purpose. Thus, some negative PCR values may affect the comparative results due to the performance limitations of this technique.

In conclusion, this study shows the adequate performance as well as the high level of consistency of the investigated serological immunoassays for the detection of SARS-CoV-2 antibodies making them useful for application in the clinical practice as support in the diagnostic approach and in the development of seroepidemiological studies. In this way, further investigations are needed to understand the immunization against SARS-CoV-2 coronavirus and to investigate the generation not only of vaccines but also hyperimmune serum as a therapeutic approach.

Acknowledgments

We wish to thank not only the personnel of our laboratory for its technical assistance but also SIEMENS for providing the required reagent in order to carry out this study.

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Authorship contribution

Silvia Montolío: conceptualization, design of the study, analysis and interpretation of data, writing and review. Carmen Molina: design of the study, analysis and interpretation of data, revision. Frederic Gómez: analysis and interpretation of data, revision and supervision. Esther Picó: analysis and interpretation of data, revision. Núria Serrat: interpretation of data and revision. Imma Palau: data acquisition. Maria Teresa Mestre: analysis of data. Maria Teresa Sans: analysis and interpretation of data, revision, supervision and final approval.

Research funding

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Conflict of interest

Authors state no conflict of interest.

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