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Comparative evaluation of six immunoassays for the detection of antibodies against SARS-CoV-2

Felipe Pérez-García a, *, Ramón Pérez-Tanoira a, María Esther Iglesias a, Juan Romanyk a,b, Teresa Arroyo a, Peña Gómez-Herruz a, Rosa González a, Sara Lapeña García a, Juan Cuadros-González a,b

a Servicio de Microbiología Clínica, Hospital Universitario Príncipe de Asturias, Madrid, Spain
b Departamento de Biomedicina y Biotecnología, Facultad de Medicina, Universidad de Alcalá de Henares, Spain

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OBJECTIVEs: Serologic techniques can serve as a complement to diagnose SARS-CoV-2 infection. The objective of our study was to compare the diagnostic performance of six immunoassays to detect antibodies against SARS-CoV-2: three lateral flow immunoassays (LFAs), one ELISA and two chemiluminescence assays (CLIA). Methods: We evaluated three LFAs (Alltest, One Step and SeroFlash), one ELISA (Dia.Pro) and two CLIA (Elecsys and COV2T). To assess the specificity, 60 pre-pandemic sera were used. To evaluate the sensitivity, we used 80 serum samples from patients with positive PCR for SARS-CoV-2. Agreement between techniques was evaluated using the kappa score (κ). Results: All immunoassays showed a specificity of 100 % except for SeroFlash (96.7 %). Overall sensitivity was 61.3 %, 73.8 %, 67.5 %, 85.9 %, 88.0 % and 92.0 % for Alltest, One Step, SeroFlash, Dia.Pro, Elecsys and COV2T, respectively. Sensitivity increased throughout the first two weeks from the onset of symptoms, reaching sensitivities over 85 % from 14 days for all LFAs, being One Step the most sensitive (97.6 %), followed by SeroFlash (95.1 %). Dia.Pro, Elecsys and COV2T showed sensitivities over 97 % from 14 days, being 100 % for COV2T. One Step showed the best agreement results among LFAs, showing excellent agreement with Dia.Pro (agreement = 94.2 %, κ = 0.884), COV2T (99.1 %, κ = 0.981) and Elecsys (97.3 %, κ = 0.943). Dia.Pro, COV2T and Elecsys also showed excellent agreement between them. Conclusions: One Step, Dia.Pro, Elecsys and COV2T obtained the best diagnostic performance results. All these techniques showed a specificity of 100 % and sensitivities over 97 % from 14 days after the onset of symptoms, as well as excellent levels of agreement.

1. Introduction

Since the beginning of the pandemic due to SARS-CoV-2 in Wuhan in December 2019, the virus has caused as of August 19, 2020, more than 21 million cases and 775,000 deaths worldwide (World Health Organization (2020a)). An early and accurate diagnosis of SARS-CoV-2 infection is essential for the adequate management of COVID-19 patients and the establishment of infection control measures in order to contain this pandemic. Polymerase chain reaction (PCR) is the reference method for COVID-19 diagnosis but its sensitivity depends on the type of sample (upper or lower respiratory tract) and the time from infection (Tang et al. 2020; Li et al., 2020; To et al., 2020). Serologic tests are useful tools for the diagnosis and management of SARS-CoV-2 infection as a complement of PCR (Krammer and Simon, 2020). They have also proved their usefulness in epidemiological surveillance studies (García-Basteiro et al., 2020; Pollán et al., 2020). The vast majority of these tests are based on the detection of IgM and IgG antibodies against SARS-CoV-2 nucleocapsid and spike (Huang et al., 2020). There are mainly three diagnostic approaches to detect these antibodies: lateral flow immunoassays (LFAs), enzyme-linked immunoassays (ELISAs) and...
chemiluminescence immunoassays (CLIA)s.

The aim of our study was to compare the diagnostic performance of six serologic tests for the detection of antibodies against SARS-CoV-2: three LFAs, one ELISA and two CLIA.s.

2. Methods

2.1. Population and study period

The study was performed between 1st March and 30th April 2020. We included two groups of patients:

2.1.1. Negative controls

60 serum samples from a randomly selected group of patients with a sample taken for other serologic studies, from September 1 to November 30, 2019. Aliquots of cryopreserved sera were recovered from the serum archive. Characteristics of patients belonging to pre-pandemic samples group are summarized in Supplementary Table 1.

2.1.2. PCR positive patients

80 patients admitted to the Emergency department between March 1 and April 28, 2020, with suspicion of COVID-19 and confirmation by PCR. All of them were symptomatic, with a median time from the onset of symptoms of 15 days (Interquartile range, 8–25 days). Residual serum samples were recovered for this evaluation.

2.2. Serological assays

2.2.1. LFAs

We evaluated one LFA which detects IgG and IgM antibodies against SARS-CoV-2 nucleocapsid (AllTest COVID-19 IgG/IgM [AllTest Biotech, Hangzhou, China]) and two LFAs which detect IgG and IgM antibodies against nucleocapsid and spike (One Step Rapid Test [Innovita Biological Technology, Hebei, China] and SeroFlash SARS-CoV-2 IgM/IgG [Epigenetek Group, New York, USA]).

2.2.2. ELISA

We evaluated one ELISA which detects IgG and IgM antibodies against nucleocapsid and spike (Dia.Pro COVID-19 [Dia.Pro Diagnostic Bioprobes, Sesto San Giovanni, Italy]).

2.2.3. CLIA

We evaluated two CLIA.s for total antibodies (IgM + IgG): Elecsys Anti-SARS-CoV-2 (Roche Diagnostics, Mannheim, Germany), which detects antibodies against nucleocapsid and SARS-CoV-2 Total COV2T (Siemens Healthineers, Erlangen, Germany), which detects antibodies against spike (S1). Sensitivity evaluation of CLIA techniques could only be performed with 50 samples due to insufficient sample volume.

2.3. Statistical analysis

We considered a positive result for samples in which IgG, IgM or both of them were detected. Specificity and sensitivity with 95 % confidence intervals (95 %CI) were calculated using the results from negative controls and positive PCR patients, respectively. Sensitivity was evaluated globally and also according to the time from the onset of symptoms. Agreement between different techniques was evaluated using the Cohen’s kappa score (McHugh, 2012). Statistical analysis was performed using Stata/IC 13.1 (StataCorp, Texas, USA).

3. Results

Overall serologic results from the different techniques are summarized in Table 1. All techniques showed a specificity of 100 %, except for SeroFlash LFA, which showed a specificity of 96.7 % due to two samples that were positive for IgM antibodies. Regarding LFAs, the overall sensitivity was 61.3 % for Alltest, 73.8 % for One Step and 67.5 % for SeroFlash. Dia.Pro ELISA showed an overall sensitivity of 85.9 % and CLIA.s showed sensitivities of 92.0 % for COV2T and 88.0 % for Elecsys.

** Statistics: values are expressed as absolute count (percentage). A positive serologic result was defined for LFA and ELISA tests for samples that resulted positive for either IgM or IgG antibodies. Abbreviations: LFA: lateral flow assay; ELISA: enzyme-linked immunoassay; CLIA: chemiluminescence; 95 %CI: 95 % confidence interval.

* Two samples presented indeterminate result for IgG or IgM and were excluded from the analysis.

** Sensitivity evaluation of CLIA techniques could only be performed with 50 samples due to insufficient sample volume.

Table 1

| Serologic test | PCR positive controls | Negative controls | Sensitivity (95 %CI) | Specificity (95 %CI) |
|----------------|-----------------------|-------------------|----------------------|----------------------|
| LFA            | Alltest (Alltest)     | 49/80             | 0/60                 | 61.3 (49.7-71.9)     | 100.0 (94.0-100.0) |
| One Step       | Innovita              | 59/80             | 0/60                 | 73.8                 | 100.0 (94.0-100.0) |
| SeroFlash      | Epigenetek            | 54/80             | 2/60                 | 67.5 (56.1-77.6)     | 96.7 (88.5-99.6)   |
| ELISA          | Dia.Pro (Dia.Pro)     | 67/78*            | 0/60                 | 85.9 (76.2-92.7)     | 100.0 (94.0-100.0) |
| CLIA           | COV2T (Siemens)       | 46/50**           | 0/60                 | 92.0 (80.8-97.8)     | 100.0 (94.0-100.0) |
|                | Elecsys (Roche)       | 44/50**           | 0/60                 | 88.0 (75.7-95.5)     | 100.0 (94.0-100.0) |

Table 2

| Serologic test | 0 – 7 days | 8 – 14 days | > 14 days |
|----------------|------------|-------------|-----------|
| LFA positive results |           |             |           |
| Alltest (Alltest) | 5/18       | 9/21        | 35/41     |
| One Step (Innovita) | 5/18       | 14/21       | 40/41     |
| SeroFlash (Epigenetek) | 4/18       | 11/21       | 39/41     |
| ELISA positive results |           |             |           |
| Dia.Pro (Dia.Pro) | 9/16*      | 18/21       | 40/41     |
| CLIA positive results** |           |             |           |
| COV2T (Siemens) | 2/5        | 3/4         | 41/41     |
| Elecsys (Roche) | 2/5        | 2/4         | 40/41     |

** Statistics: values are expressed as absolute count (percentage). A positive serologic result was defined for LFA and ELISA tests for samples that resulted positive for either IgM or IgG antibodies. Abbreviations: LFA: lateral flow assay; ELISA: enzyme-linked immunoassay; CLIA: chemiluminescence.

* Two samples presented indeterminate result for IgG or IgM and were excluded from the analysis.

** Sensitivity evaluation of CLIA techniques could only be performed with 50 samples due to insufficient sample volume.
agreement results among the evaluated LFAs, showing almost perfect agreement with SeroFlash (agreement = 92.1%, k = 0.838), Dia.Pro (94.2%, k = 0.884), COV2T (99.1%, k = 0.981) and Elecsys (97.3%, k = 0.943). Dia.Pro ELISA showed almost perfect agreement with CLIA (98.2%, k = 0.963 for COV2T; 96.4%, k = 0.925 for Elecsys). Finally, COV2T and Elecsys CLIA also showed almost perfect agreement between them (98.2%, k = 0.962).

4. Discussion

Our study shows that One Step, Dia.Pro, Elecsys and COV2T achieved the best diagnostic performance results. All these techniques showed a specificity of 100% and sensitivities over 97% from 14 days after the onset of symptoms, as well as excellent levels of agreement between them.

Serologic tests have emerged as complementary tools to PCR in the diagnosis of SARS-CoV-2, including subclinical presentations (Guo et al., 2020). Consequently, an increasing number of commercial tests have been developed. Table 4 contains a summary of the studies that have evaluated different commercial immunoassays. Regarding LFAs, NG Biotech (Nicol et al., 2020), LabOn Time, Avioq and QuickZen (Montesinos et al., 2020), showed similar results with One Step and SeroFlash in terms of sensitivity and specificity. Regarding ELISAs, Euroimmun has been the most frequently evaluated one, presenting overall sensitivities of 71.1–87.4% (Nicol et al., 2020; Montesinos et al., 2020; Kohmer et al., 2020; Krüttgen et al., 2020) and specificities were lower than those obtained in our study (Kohmer et al., 2020; Egger et al., 2020).

In our experience, Dia.Pro, Elecsys and COV2T obtained excellent levels of sensitivity, specificity and agreement. We also showed that those LFAs that use recombinant nucleocapsid and spike antigens (One Step and SeroFlash) could achieve practically the same diagnostic performance results than ELISA and CLIA. At the beginning of its development, the usefulness of LFAs was questioned due to a lack of official performance validations (Krammer and Simon, 2020; World Health Organization (2020b)). Nowadays these tests have demonstrated their usefulness and reliability in epidemiological studies (Pollán et al., 2020), and also in the diagnosis of pneumonia of unknown etiology with negative PCR for SARS-CoV-2 (Pérez-García et al., 2020).

Our study presents some limitations. First, it is a retrospective study that has been conducted in a single institution and we did not evaluate sequential samples, which could have been interesting in order to establish the dynamic of these antibodies. Second, sensitivity evaluation of CLIA was performed only over 50 sera, due to insufficient sample volume. However, the samples that could not be analyzed belonged to the first two weeks after the onset of symptoms. As a consequence, this limitation did not affect the results about sensitivity from 14 days, when the vast majority of patients seroconvert according to different studies (Guo et al., 2020; Montesinos et al., 2020; Pérez-García et al., 2020). Third, specificity evaluation was done over 60 samples including a wide variety of physical conditions (see Supplementary Table 1). It would have been interesting to include more patients with autoimmune diseases or with other viral infections in order to evaluate the specificity of the tests in these subpopulations in which the number of false positives of the serological tests could be much higher than in the general population. Finally, we have analyzed the results of six among all commercialized immunoassays. Consequently, our results should not be extrapolated to other available immunoassays and more comparative prospective multicenter studies and meta-analysis are needed to establish the usefulness of other serologic tests and reinforce our conclusions.
Table 4

Studies that have evaluated commercial immunoassays to detect antibodies against SARS-CoV-2.

| Authors          | Immunoassay | Type of assay | Evaluated antibodies | Analyzed samples                                                                 | Overall Sensitivity | Sensitivity 14 days | Specificity |
|------------------|-------------|---------------|-----------------------|-----------------------------------------------------------------------------------|---------------------|---------------------|-------------|
| Nicol et al.     | Euroimmun   | ELISA         | IgG/IgA S protein (S1) | 141 samples from PCR positive patients                                             | 87.4 %              | 100.0 %             | 82.0 %      |
|                  | Abbott      | CLIA          | IgG N protein         | 57 samples from PCR negative patients                                              | 81.8 %              | 100.0 %             | 99.3 %      |
|                  | NG Biotech  | LFA           | IgG/IgM S protein (S1) | 50 pre-pandemic samples                                                           | 81.8 %              | 100.0 %             | 95.3 %      |
|                  | Euroimmun   | ELISA         | IgG/IgA S protein (S1) | 84.4 %                                                                         | 93.9 %              | 87.5 %              |            |
| Montesinos et al. | Maglumi     | CLIA          | IgG/IgM –            | 128 samples from PCR positive patients                                             | 64.3 %              | 93.8 %              | 100.0 %     |
|                  | LabOn Time  | LFA           | IgG/IgM –            | 72 pre-pandemic samples and healthy volunteers                                     | 71.9 %              | 93.9 %              | 100.0 %     |
|                  | Avioq       | LFA           | IgG/IgM –            | 68.8 %                                                                         | 93.9 %              | 95.8 %              |            |
|                  | QuickZen    | LFA           | IgG/IgM –            | 71.1 %                                                                         | 90.9 %              | 100.0 %             |            |
|                  | Abbott      | CLIA          | IgG N protein         | 77.8 %                                                                         | –                   | 94.6 %              |            |
|                  | Elecsys     | (Roche) CLIA | Total Abs N protein   | 75.6 %                                                                         | –                   | 91.7 %              |            |
|                  | Liaison XL  | CLIA          | IgG S protein (S1, S2) | 45 samples from PCR positive patients                                             | 75.6 %              | –                   | 94.6 %      |
|                  | VirClia     | CLIA          | IgG N protein and S protein (S1) | 37 samples including pre-pandemic samples and patients infected with other coronaviruses (including SARS-CoV and MERS-CoV) and other viruses (EBV, CMV) | 89.0 %              | –                   | 93.9 %      |
| Kohmer et al.    | Euroimmun   | ELISA         | IgG S protein (S1)    | 71.1 %                                                                         | –                   | 100.0 %             |            |
|                  | Virotech    | ELISA         | IgG N protein         | 66.7 %                                                                         | –                   | 94.6 %              |            |
|                  | Euroimmun   | ELISA         | IgG S protein (S1)    | 86.4 %                                                                         | –                   | 96.2 %              |            |
|                  | EDI         | ELISA         | IgG/IgM N protein     | 100.0 %                                                                        | –                   | 88.7 %              |            |
|                  | recomWell   | ELISA         | IgG N protein         | 86.4 %                                                                         | –                   | 100.0 %             |            |
|                  | Virachip    | Immunoblot    | IgG                   | 77.3 %                                                                         | –                   | 100.0 %             |            |
| Chew et al.      | Abbott      | CLIA          | IgG N protein         | 177 samples from COVID-19 patients                                                | 40.7 %              | 84.2 %              | 100.0 %     |
|                  | Elecsys     | (Roche) CLIA | Total Abs N protein   | 163 samples from non-COVID-19 patients                                            | –                   | 100.0 %             |            |
|                  | EDI         | ELISA         | IgG/IgM N protein     | 104 samples from PCR positive patients                                            | 48.1 %              | 88.6 %              | 99.8 %      |
|                  | Alltest     | LFA           | IgG/IgM N protein     | 456 pre-pandemic samples                                                          | 50.0 %              | 91.4 %              | 97.8 %      |
|                  | One Step    | LFA           | IgG/IgM N and S protein | 61.3 %                                                                         | 85.4 %              | 100.0 %             |            |
|                  | SeroFlash   | LFA           | IgG/IgM N and S protein | 73.8 %                                                                         | 97.6 %              | 100.0 %             |            |
|                  | Dia.Pro     | ELISA         | IgG/IgM N and S protein | 68.7 %                                                                         | 95.1 %              | 96.7 %              |            |
|                  | Elecsys     | (Roche) CLIA | Total Abs N protein   | 80 samples from PCR positive patients                                            | 67.5 %              | 95.1 %              | 96.7 %      |
|                  | COV2T (Siemens) | CLIA         | Total Abs S protein   | 85.9 %                                                                         | 97.6 %              | 100.0 %             |            |
|                  |             |               |                       | 92.0 %                                                                         | 99.0 %              | 100.0 %             |            |

Statistics: values are expressed as percentage. Abbreviations: -: not reported data; sensitivity 14 days: sensitivity from 14 days after the onset of symptoms; N: Nucleocapsid; S: Spike; Ab: antibodies; EBV: Epstein-Barr Virus; CMV: Cytomegalovirus; ELISA: enzyme-linked immunoassay; LFA: lateral flow immunoassay; CLIA: chemiluminescence immunoassay.

In conclusion, One Step LFA, Dia.Pro ELISA and Elecsys and COV2T CLAs present the best diagnostic performance results. All these techniques showed a specificity of 100 % and sensitivities over 97 % from 14 days after the onset of symptoms, as well as excellent levels of agreement between them. To our knowledge, this study constitutes the first comparative evaluation of these six immunoassays. These findings indicate that these tests could be reliable tools for the diagnosis of COVID-19 and the performance of epidemiological studies.

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Informed consent

Since the present study is retrospective, informed consent was not required.

Ethical approval

The study was conducted according to the ethical requirements established by the Declaration of Helsinki. The Ethics Committee of Hospital Universitario Príncipe de Asturias (Madrid) approved the study (protocol code: Comparativa Sero-COVID).

CRediT authorship contribution statement

Felipe Pérez-García: Conceptualization, Data curation, Formal analysis, Writing - original draft. Ramón Pérez-Tanoira: Data curation, Investigation. María Esther Iglesias: Data curation, Investigation. Juan Romanyk: Data curation, Investigation. Teresa Arroyo: Data curation, Investigation. Peña Gómez-Herruz: Data curation, Investigation. Rosa González: Data curation, Investigation. Sara Lapeña Garcia: Data curation. Juan Cuadros-González: Supervision, Visualization, Writing - original draft, Writing - review & editing.
Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi: https://doi.org/10.1016/j.jviromet.2020.114047.

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