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Research Article

Clinical Performance of Two Methods for Detecting Anti SARS-CoV-2 Antibodies

Jacobsen D[1#], Gonzalez D[1,2#], Jamardo J[1], Ibar C[3], Pugliese L[3], Fortuna F[3], Carrizo E[4], Caro EM[5], Perazzi B[1,2], Repetto EM[1,6], Reboredo G[7] and Fabre B[1,2*]

[1]Departamento de Bioquímica Clínica, Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Catedra de Bioquímica Clínica I. Buenos Aires, Argentina

[2]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Instituto de Fisiopatología y Bioquímica Clínica (INFIBIOC). Buenos Aires, Argentina

[3]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Residencia en Bioquímica Clínica, Argentina

[4]Coordinadora de Salud Misionar, Argentina

[5]Biogenar Laboratorio, Argentina

[6]Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Facultad de Farmacia y Bioquímica. Buenos Aires, Argentina

[7]Universidad de Buenos Aires, Hospital de Clínicas José de San Martin, Área Ambulatoria del tratamiento y seguimiento del HIV-SIDA, Argentina

[#]These authors contributed equally to this paper

***Corresponding authors:** Fabre B, Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica. Departamento de Bioquímica Clínica, Junín 956 (C1113AAD) Buenos Aires, Argentina

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Abstract

Evaluating the clinical performance of available methods to detect antibodies against Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) has become a primordial issue in clinical laboratories. The aim of this study was to evaluate the clinical performance of two methods for SARS-CoV-2 antibodies detection, an automated Chemiluminescent Immunoassay (CLIA) and an immunochromatographic Lateral-Flow Assay (LFA) in patients with positive reverse transcription polymerase chain reaction (RT-PCR). Performance for CLIA method was Positive Agreement (PA) 56.6% and Negative Agreement (NA) 96,6% for IgM and PA 85.8%/NA 90,2% for IgG. Performance for LFA method was PA 56.2% and NA 100% for IgM and PA 95.5% and NA 100 % for IgG. LFA general agreement IgG was better than CLIA. In both methods, significant differences in Kappa index are observed when IgG and IgM are compared. When evaluating the data from a clinical perspective, we found that both method performance for IgM detection may not meet the expected requirements for their clinical utility and could lead to an inappropriate medical decision. The findings of this study show that both immunoassay methods might be reliable for assessing immunological response in COVID-19 patients. Our results also confirm that IgG measurement could be helpful, especially for epidemiological studies in our population. These results provide evidence to justify epidemiological studies in our population.

Keywords: Anti SARS CoV-2 antibodies; Clinical performance; Immunoassay

Introduction

In the last decades, there is a trend of laboratories incorporating automated methodology, which is partly due to the need of searching for a better analytical and clinical performances than manual or semi-quantitative methods such as immunochromatographic methods. Nowadays, in times of pandemic, evaluating the clinical performance of available methods to detect antibodies against Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) has become a primordial issue in clinical laboratories since the contribution of these evaluations has influenced the action plan followed by health authorities to define adequate health policies in different areas.

Currently, antibody response in SARS-CoV-2 infected patients continues being investigated and the clinical utility of commercially available tests for antibodies detection is under discussion. Several authors have observed heterogeneity in the sensitivity of different antibodies tests during the course of infection [1] but most of these investigations evaluated the tests performance in North American, European or Asian population. The performance is still uncertain in our environment. The aim of this study was to evaluate the clinical performance of two methods for SARS-CoV-2 antibodies detection, an automated Chemiluminescent Immunoassay (CLIA) (Maglumi™ 2019-nCov IgM/IgG) (Shenzhen New Industries Biomedical Engineering (SNIBE) Co., Ltd., Shenzhen, China) and an immunochromatographic Lateral-Flow Assay (LFA) (Lungene® SARS-CoV-2 Virus IgG/IgM rapid test) (Hangzhou Clongene Biotech Co., Ltd., Hangzhou, China), in patients with positive Reverse Transcription Polymerase Chain Reaction (RT-PCR) for SARS-CoV-2 target ORF1ab in nasopharyngeal swab samples, and in health workers from Hospital de Clinicas "José de San Martín" in the city of Buenos Aires, Argentina. All RT-PCR analysis were performed at BIOGENAR laboratories.

Materials and Methods

MAGLUMI 2019-nCoV IgM CLIA method uses magnetic microbeads coated with anti-human IgM monoclonal antibody and SARS-CoV-2 recombinant antigen labeled with N-(4-Aminobutyl)-N-ethylisoluminol (ABEI). And for IgG detection it uses magnetic microbeads coated with SARS-CoV-2 recombinant antigen and ABEI labeled anti-human IgG antibody. Claimed clinical sensitivity and specificity for IgM are 78.6% and 98.5%, and for IgG are 91.2% and 95.6%, respectively. The manufacturer report that there is no cross reactivity with antibodies generated by other respiratory diseases such as respiratory syncytial virus, adenovirus, parainfluenza, influenza A and B. Lungene SARS-CoV-2 LFA method uses the principle of immunochromatography and presents two capture zones with mouse

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Table 1: Clinical performance results.

Statistics	Maglumi™2019-nCoV IgM/IgG (CLIA)			Lungene® SARS-CoV-2 IgG/IgM		
	IgM	IgG	IgM + IgG	IgM	IgG	IgM + IgG
Positive agreement %	56.6	85.8	86.8	56.2	95.5	96.6
with RT-PCR (95% CI)	(47.1-65.6)	(78.0-91.2)	(79.0-92.0)	(45.8-66.0)	(89.0-98.2)	(90.6-98.8)
Negative agreement %	96.6	90.2	86.2	100.0	100.0	100.0
with RT-PCR (95% CI)	(88.3-99.1)	(80.2-95.4)	(75.1-92.8)	(92.6 -100.0)	(92.6-100.0)	(92.6-100.0)
Kappa index	0.454	0.737	0.713	0.473	0.937	0.953
(95% CI)	(0.341- 0.566)	(0.633-0.841)	(0.602-0.824)	(0.354-0.592)	(0.876-0.998)	(0.899-1.006)
Chi-square test		p=0.0003 *vs* IgM	NS *vs* IgG p=0.0013 *vs* IgM		p<0.0001 *vs* IgM	NS *vs* IgG p<0.0001 *vs* IgM

*NS: Non Significative.

anti-human IgM and anti-human IgG antibodies. After adding the sample, the reaction ends with the addition of recombinant envelope antigens SARS-CoV-2 conjugated with colloidal gold. The claimed clinical sensitivity and specificity for IgM are 87.0% and 98.9% respectively, and for IgG it is only claimed a clinical sensitivity of 97.4%. It is reported that it does not present cross reactivity with other antibodies produced by HIV, Hepatitis A, B and C, Syphilis, HTLV, respiratory syncytial virus, Influenza A and B.

Eighty-nine sera from patients with positive RT-PCR for SARS-CoV-2 in nasopharyngeal swab and 48 sera from negative RT-PCR individuals were analyzed in order to evaluate LFA method. On the other hand, 106 positive and 61 negative RT-PCR individuals were included for evaluating CLIA method. Procedure and analysis were performed according to EP-12 A2 CLSI guideline [2]. The differences between Kappa index, used to estimate general agreement between the analyzed methodologies and RT-PCR, were evaluated according to the Chi-square test. Obtained data from both methodologies and RT-PCR are shown in table (Table 1).

Results and Discussion

According to its clinical utility, the performance of IgM detection in both methodologies is poor, as it is shown by their concordance percentage (CLIA: 56.6% and LFA: 56.2%). Although the negative agreement has satisfactory values, the general agreement presents a moderate association strength according to the obtained Kappa index, meaning that the tests do not meet the optimal performance for the expected clinical utility. These results are similar to those reported by Deeks et al. where it was found that the mean clinical sensitivity in different publications varies between 58.4% at the onset of symptoms (8-14 days) and 75.4% between 15-21 days [1]. Different results were found when evaluating IgG, since IgG detection was good by both methodologies but, LFA general agreement was better than CLIA (kappa: 0.937 *vs.* 0.737, p = 0.0011, Chi square test). The positive agreement was 95.5% for LFA and 85.8% for CLIA, and the same trend is observed in negative agreement (100% and 90.2% respectively). These results show a high degree agreement regarding to infected patients, according to what was previously published by Deeks et al. [1] and it also presented similar performance to that obtained by other platforms, including the measurement of total antibodies [3]. In both methods, significant differences in Kappa index are observed when IgG and IgM are compared. This allowed us to hypothesize that the use of the IgG test could be useful in clinical application. We also proposed combining the IgG and IgM results in order to identify positive individuals but, we found that there were no significant differences in Kappa index between the combined tests (IgG + IgM) and IgG. This would support that the combination does not improve the tests performance.

Table 2: Agreement evaluation of antibodies detection between CLIA and LFA methods.

Statistics	IgM	IgG
Positive agreement % (95% CI)	77.5 (62.5-87.7)	*96* (88.9-98.6)
Negative agreement % (95% CI)	58.2 (45.0-70.3)	95 (76.4-99.1)
Kappa index (95% CI)	0.341 (0.116-0.520)	0.878(0.761-0.995)
Chi-square test		p<0.0001 *vs* IgM

Considering that the obtained results might be explained because both methods differ in their designs, we set out to evaluate the agreement by comparing them with each other. 45 samples that had been classified as positive for IgM by LFA and 55 classified as negative were analyzed by CLIA, the same scheme for IgG with 75 positive and 20 negative samples. The results obtained are summarized in (Table 2).

Both methods present high degree of agreement in IgG detection according to the results obtained in the evaluation of both methods separately with RT-PCR. The poor performance regarding the detection of IgM antibodies is notable, where we found 9 positive IgM results by LFA that were negative by CLIA when in all cases RT-PCR was positive. And 23 negative IgM results for LFA and positive for CLIA when 22 of them had positive RT-PCR. This erratic behavior would evidence the lack of standardization that currently exists in the design of the tests for IgM detection, mainly regarding the recombinant antigen used in the design and cross-reactivity.

When evaluating the data from a clinical perspective, we found that both method performance for IgM detection may not meet the expected requirements for their clinical utility and could lead to an inappropriate medical decision. On the contrary, IgG degree of agreement with RT-PCR and between the methods is satisfactory and could be considered a useful tool for epidemiological purposes.

Conclusion

In conclusion, the findings of this study show that both immunoassay methods might be reliable for assessing immunological response in COVID-19 patients. Our results also confirm that IgG measurement could be helpful, especially for epidemiological studies in our population. These results provide evidence to justify epidemiological studies in our population.

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