

Performance of Elecsys Anti-SARS CoV-2 (Roche) and VIDAS Anti-SARS CoV-2 (Biomérieux) for SARS-CoV-2 nucleocapsid and spike protein antibody detection

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ABSTRACT

Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the novel viral pathogen that causes coronavirus disease 2019 (COVID-19) in humans, has spread worldwide since its identification in late 2019. The pandemic produced an accelerated development of new serological techniques for diagnosis.

Methods

We evaluated two commercial assays for serological diagnosis of SARS-CoV-2 infection, approved by the Administración Nacional de Medicamentos, Alimen-

tos y Tecnología Médica (ANMAT) in Argentina: Elecsys Anti-SARS-CoV-2; Roche for nucleocapsid total antibody detection, and VIDAS Anti-SARS-CoV-2 bioMérieux for spike protein IgG antibody detection. Sensitivity was assessed using a panel of 92 plasma samples from recovered COVID-19 patients who were positive for RT-PCR and positive for neutralizing antibodies by plaque reduction neutralization test (PRNT) and/or positive for IgG antibodies by indirect immunofluorescence assay (IFA). Specificity was determined studying 71 plasma samples collected during year 2018 prior to the COVID-19 pandemic. Assays were evaluated as stand-alone tests.

Results

Sensitivity was 97.8% and 98.9% for the Roche and bioMérieux assays, respectively, specificity: 98.5% (Roche) and 97.1% (bioMérieux), positive predictive value (PPV): 98.9% (Roche) and 97.8% (bioMérieux), and negative predictive value: (NPV) 97.2% (Roche) and 98.5% (bioMérieux). Additionally, Cohen's kappa coefficient demonstrated high concordance ($k=0.950$) between Roche and bioMérieux.

Discussion

In conclusion, our results evidenced a very good performance for the nucleocapsid antibody assay (Roche) and the spike protein antibody assay (bioMérieux), thus both platforms are equally adequate for indirect diagnosis of SARS-CoV-2 infection through total antibodies and IgG antibody detection, respectively.



INTRODUCTION

During the year of 2020, different trademarks have developed assays with diverse antigenic configurations for clinical use in serological diagnosis of infection by severe acute respiratory

syndrome coronavirus 2 (SARS-CoV-2). Some of these commercial assays received emergency authorization from the United States Food and Drug Administration (FDA) (1) and the Health and Safety Authority of Argentina: Administración Nacional de Medicamentos, Alimentos y Tecnología Médica (ANMAT).

Despite molecular assays are the gold standard for diagnosis of infection caused by SARS-CoV-2, serology is useful as diagnostic tool to complement viral RNA detection. Thus, RNA detection by RT-Polymerase Chain Reaction (RT-PCR) is most sensitive within the first 7 days after onset of symptoms and after that point, it diminishes below 50% (2). In contrast, many reports describe that antibodies against SARS-CoV-2 are detectable in only 50% of patients one week after onset of symptoms and sensitivity for their detection is enhanced up to 90% after two weeks (3). Likewise, it has been shown that a certain ratio of close contacts of patients with confirmed coronavirus disease 2019 (COVID-19) yield negative results or they are not tested at all with molecular techniques (4). In these cases, diagnosis of infection can be achieved with serological assays. Thus, serology arises as a very important complementary resource for diagnosis and control of this viral infection.

On the other hand, evaluation of the humoral immune response against SARS-CoV-2 by serological tests is very important for epidemiological surveillance to control the COVID-19 pandemic. In this sense, serological assays are economical, fast, easy to implement, and allow effective identification of people exposed to the virus (5, 6). In addition, serology is useful to determine immune status in workers, which facilitates return-to-work decisions and other relevant public health measures in the context of the COVID-19 pandemic (7, 8).

Previous studies regarding coronavirus SARS-CoV and MERS-CoV have revealed that the most

immunogenic antigens are the spike (S) and nucleocapsid (N) proteins; therefore, most serological techniques developed for detection of SARS-CoV-2 antibodies have focused on these viral proteins (9). In this sense, several commercial kits have been developed and evaluated (10, 11). Two of the most widely used commercial platforms to detect specific antibodies against SARS-CoV-2 are: Elecsys Anti-SARS-CoV-2 by Roche, which detects total antibodies against the viral nucleocapsid (anti-N) and VIDAS SARS-CoV-2 IgG (9-COG) by bioMérieux, which contains spike protein of the virus as antigenic conformation, allowing detection of antibodies against the S protein (anti-S) (1,12,13). These assays are available in Argentina, and they have been approved by ANMAT for diagnosis of SARS-CoV-2 infection. Hence, we evaluated their performance for detection of specific total and IgG antibodies against the virus using a panel of plasma samples from subjects recovered from infection by the SARS-CoV-2 pandemic strain (B.1 lineage).

MATERIALS AND METHODS

Sample selection

One panel of positive and one of negative plasma samples for SARS-CoV-2 antibodies were used for this study. The panel of positive samples was obtained from the sample bank of the Virology Institute “Dr. J. M. Vanella”, Facultad de Medicina, Universidad Nacional de Córdoba, Argentina, and was composed by 92 plasma samples with ethylenediaminetetraacetic acid (EDTA) as anticoagulant. They were collected during the year of 2020 from patients recovered from COVID-19 infection, 40-85 days after onset of symptoms. These patients were: i) positive by RT-PCR in nasopharyngeal swab samples and positive for both neutralizing antibodies (NTAbs) by plaque reduction neutralization test (PRNT) and IgG antibodies against SARS-CoV-2 by *in house* Indirect Immunofluorescence assay

(IFA) (n=78), and ii) positive by RT-PCR in nasopharyngeal swab samples, positive for IgG antibodies against SARS-CoV-2 by IFA, but negative for NATbs by PRNT (n=14).

The characterization of plasma samples (positive panel) for antibodies against SARS-CoV-2 by PRNT and IFA was performed at the Virology Institute within the framework of the agreement with the Cordoba Ministry of Health for characterization of convalescent plasma for therapeutic use. Assays were carried out as previously described (14) and SARS-CoV-2 strain B.1 lineage (hCoV-19/Argentina/PAIS-G0001/2020, GISAID, ID: EPI_ISL_499083) was used for both tests.

The negative panel included plasma samples (with EDTA) collected from blood donors in 2018 prior to the COVID-19 pandemic (n=71).

Methods

The Elecsys Anti-SARS CoV-2 assay was performed on a Cobas e411 analyzer (Roche Diagnostics, Mannheim, Germany) and conducted according to the manufacturer’s instructions. This sandwich assay uses a SARS-CoV-2 specific recombinant antigen representing the nucleocapsid protein. The electrochemiluminescent signal produced is compared to the cut-off signal value previously obtained with two calibrators. Results are expressed as (cut-off index, negative COI <1.0 or positive COI \geq 1.0) for anti-SARS CoV-2 total antibodies.

The VIDAS SARS CoV-2 is a two-step sandwich enzyme-linked fluorescent assay (ELFA) performed on a VIDAS analyzer (bioMérieux, Marcy-l’Étoile, France). The VIDAS SARS-CoV-2 IgG assay was conducted according to the manufacturer’s instructions. Briefly, the IgGs present in the sample are captured by a recombinant SARS-CoV-2 subdomain spike antigen coated on a solid phase, and then an anti-human IgG labelled with alkaline phosphatase is added. The intensity of the fluorescence produced by the substrate hydrolysis is

measured at 450 nm and is proportional to the antibody level. An index is calculated as the ratio between the relative fluorescence value (RFV) measured in the sample and the RFV obtained for the calibrator (humanized recombinant anti-SARS CoV-2 IgG) and interpreted as negative (index <1.0) or positive (index ≥1.0).

Table 1 shows manufacturer names, assays, methods, principles of antibody detection, recombinant antigens and types of immunoglobulins recognized by the two commercial immunoassays.

Samples from the negative panel that yielded false-positive results were also analyzed for potentially unspecific cross-reactions: HIV antigen/antibody, hepatitis B virus (HBV) surface antigen, hepatitis C virus (HCV) total antibody, rheumatoid factor (RHF) and antinuclear antibody (ANA). Viral serology was performed by Cobas e411 analyzer (Roche Diagnostics) and RHF was performed by immunoturbidimetry with a Cobas 6000 analyzer (Roche Diagnostics). ANA was performed by indirect immunofluorescence assay and imprints with Hep-2 cell line (human laryngeal carcinoma, Biosystem) were used. Briefly, samples were diluted 1/80 with phosphate buffered saline (PBS,

pH=7) and incubated for 30 minutes at room temperature. Then, two washes with PBS were performed and anti-human IgG Abs conjugated with fluorescein isothiocyanate (Biocientífica S.A) was added to all wells, which were subsequently incubated for 30 minutes at room temperature. After two washes with PBS, Evans Blue was added to enhance the fluorescent signal. The samples were then dried, and a mounting solution was added for observation under Fluorescence microscope (Nikon Optiphot-2). The results were reported as negative or positive according to their fluorescence pattern. To guarantee the quality of the methodology internal and external controls were used and the results were interpreted and reported according to the criteria published by the Regional Committee for Laboratory Standardization based on international consensus (15).

Statistical analyses

Statistical analyses were conducted using Graph Pad Prism software version 6.0. Categorical variables were compared using Fisher’s exact test. Sensitivity, specificity, PPV and NPV values were calculated. A p-value lower than 0.05 was

Table 1 Characteristics of the commercial anti-SARS-CoV-2 serological assays from Roche and bioMérieux

Manufacturer (platform)	Assay	Method	Principle	Capture antigen	Isotype detected	Cut-off values	
						Neg.	Pos.
ROCHE	Elecsys Anti SARS-CoV-2	ECLIA	Sandwich immunoassay	Nucleocapsid	Total antibodies	<1.0	≥1.0
bioMérieux	SARS-CoV-2 IgG (9-COG)	ELFA	Sandwich immunoassay (two-step)	RBD	IgG	<1.0	≥ 1.0

Abbreviations: ECLIA: electro-chemiluminescence immunoassay; ELFA: enzyme-linked fluorescent assay; RBD: Receptor Binding Domain; IgG: immunoglobulin G. RBD is a domain within the S1 subunit of the spike protein; Neg: negative, Pos: positive.

considered statistically significant. Additionally, concordance between the two commercial assays was analyzed using Cohen's kappa coefficient (κ). The κ value was classified as slight (0.00 to 0.20), fair (0.21 to 0.40), moderate (0.41 to 0.60), substantial (0.61 to 0.80) and almost perfect (0.81 to 1.00) according to Landis and Koch criteria (16).

RESULTS

Table 2 shows the overall performance of each automated analyzer (sensitivity, specificity, PPV and NPV values). Results from the positive panel were 90 positive plasma samples for total antibodies by Elecsys Anti-SARS CoV-2 and 91 positive plasma samples for VIDAS anti SARS-CoV-2 IgG. In addition, we evaluated specificity in the negative panel and found three false positive results. We analyzed these samples containing potentially cross-reactive factors and observed that HIV, HCV and HBV were negative in all cases, while three samples were positive for antinuclear antibodies; in addition, one of these samples was also positive for RHF. The Elecsys anti-SARS-CoV-2 assay yielded one false positive result containing autoantibodies for both RHF and ANA, while VIDAS Anti-SARS CoV-2 IgG produced two false-positive results containing only ANA.

When the results obtained by Roche and bioMérieux were compared to each other, a Cohen's

kappa coefficient (κ) of 0.95 (95%CI, 0.90 to 0.99) was obtained, demonstrating high concordance between Elecsys Anti-SARS CoV-2 and VIDAS Anti-SARS CoV-2 IgG.

DISCUSSION

In the current study, we compared two commercial serology platforms for detection of antibodies against SARS-CoV-2 using panels of positive and negative plasma samples. We tested total antibodies against nucleocapsid protein with the assay from Roche and IgG-specific antibodies against spike protein with the bioMérieux assay; performance of the assays as stand-alone tests was also assessed. We found overall comparable sensitivity of 97.8% and 98.9% for Elecsys Anti-SARS CoV-2 and VIDAS Anti-SARS CoV-2 IgG, respectively. Results are in accordance with previous reports showing that Elecsys and VIDAS assays have better performance than other automated assays (12), reporting high rates of sensitivity, similar to what is described herein (13).

Moreover, other studies describe good levels of sensitivity for Elecsys anti-SARS-CoV-2 assay, supporting its use for detection of SARS-CoV-2 infection in areas of low prevalence (10) and evidencing a good performance as stand-alone test (1). Additionally, The National SARS-CoV-2 Serology Assay Evaluation Group from Oxford recommended the Elecsys anti-SARS-CoV-2 assay

Table 2 Clinical sensitivity, specificity, PPV and NPV of serological assays from Roche and bioMérieux in patients recovered from COVID-19

Platform	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
ROCHE	97.8 (92.3-99.7)	98.5 (92.4-99.9)	98.9 (94.0-99.9)	97.2 (90.3-99.6)
bioMérieux	98.9 (94.0-99.9)	97.1 (90.1-99.6)	97.8 (92.4-99.7)	98.5 (92.3-99.9)

Abbreviations: CI: confidence interval; PPV: positive predictive values; NPV: negative predictive values.

for serological testing due to its high sensitivity (17). In this sense, VIDAS anti-SARS-CoV-2 IgG assay probed to be a sensitive serological test, suitable for detecting specific antibody subtypes (11).

To assess specificity of the two automated assays, we analyzed a panel of pre-pandemic samples obtained two years before the first report of SARS-CoV-2 infection in the world. As a result, we found specificity rates of 98.5% and 97.1% for Elecsys Anti-SARS CoV-2 and VIDAS anti-SARS-CoV-2 IgG assays, respectively. These rates are concordant with values previously reported, when high-throughput assays for detection of antibodies against SARS-CoV-2 were analyzed (18). Similarly high rates of specificity have been described for Elecsys Anti-SARS CoV-2 (1, 10, 17) in studies of different populations. Moreover, the high rate of specificity found for VIDAS Anti-SARS CoV-2 IgG by bioMérieux was also concordant with the findings of other researchers (12, 13) and this is the reason why this assay has been previously used as a useful tool for antibody detection and epidemiological surveillance (11).

A low cross-reactivity rate due to non-specific factors when using both automated assays was observed. In this study, only 1/92 and 2/92 plasma samples containing potential cross-reacting analytes showed reactivity with Elecsys Anti-SARS CoV-2 and VIDAS Anti-SARS CoV-2 IgG, respectively. Previous reports have described similar results for these platforms (1, 10, 11, 13). Together with the evaluation of sensitivity and specificity, both assays showed similarly high rates of PPV and NPV. This finding, along with the high concordance between Roche and bioMérieux assays determined by Cohen's kappa index (0.95), proved that these two immunoassays are equally suitable for diagnosis of SARS-CoV-2 infection through antibody detection, being also adequate for sero-epidemiological surveillance in Argentina.

In conclusion, the relevance of this study was to determine the clinical usefulness of two

commercial platforms with regional samples reporting these results, which show that both platforms are highly recommended for detection of specific antibodies against SARS-CoV-2 in medium and high-complexity laboratories at Argentina. Furthermore, these results demonstrate that reliable decisions can be made based on serological results obtained with these commercial assays, whether for health policies, return-to-work decisions and/or epidemiological studies to control viral spread.



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Personal and financial conflicts of interest

The authors declare that there are not competing interest.



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