Received: November 1, 2022
Accepted: November 18, 2022

Acad Med J 2022;2(2):34-39
UDC: 616.98:578.834]-097
DOI: 10.53582/AMJ2222034s
Original article

COMPARISON OF TWO METHODS FOR SARS-COV-2 ANTIBODY TESTING

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Abstract

Serologic testing became essential for assessing seroprevalence, vaccine efficiency and public health policy since the start of the COVID-19 pandemic. Many platforms are available on the market, but studies on their performance and limitations remain scarce. This study aimed to evaluate two commercial kits: a chemiluminescent immunoassay (CLIA) and an enzyme-linked immunosorbent assay (ELISA) and show their technical requirements and result concordance in the determination of anti-SARS-CoV-2 IgG antibodies.

The study included 268 samples from PCR positive patients referred to the Institute of Immunobiology and Human Genetics for anti-SARS-CoV-2 antibody testing. Two commercially available kits were used per manufacturers' recommendations for all samples: SARS-CoV-2 RBD IgG CLIA kit from Snibe and ELISA SARS-CoV-2 IgG (RBD-S protein) from INEP. For our purposes, CLIA was chosen as the comparative method.

The positive, negative and overall percentage agreements for these two techniques were 90%, 82.7% and 87.3% respectively. Cohen's kappa was 0.72, meaning there was a moderate agreement between the two methods. Most of the discrepancies occurred in the lower concentration category with a positive percentage agreement of only 50%.

Given the highly concordant results, CLIA remains advantageous as the more efficient and convenient method. Both are reliable serological assays for antibody determination in the SARS-CoV-2 pandemic response.

Keywords: chemiluminescent immunoassay, enzyme-linked immunosorbent assay, COVID-19

Introduction

Following the outbreak of clustered cases of severe pneumonia in late 2019 in Wuhan, China, the causative agent was subsequently isolated and characterized as a novel betacoronavirus, SARS-CoV-2. Structurally, this enveloped RNA virus consists of four main proteins, among which the S protein is responsible for virus-receptor binding with the N terminal S1 subunit and virus-cell membrane fusion with the C terminal S2 subunit. S1 is further subdivided into a N-terminal domain (NTD) and a receptor binding domain (RBD) which

directly interacts with angiotensin-converting enzyme 2 (ACE-2) receptors on host cells[1]. Due to S protein's function and surface exposure, it is the main target of neutralizing antibodies upon infection and therefore the focus of designing antibody assays, vaccines and therapeutic agents[2].

Accurate and immediate diagnosis was vital for impeding the spread of the disease and enabling early supportive treatment. The gold standard is the detection of viral RNA by a RT PCR test from a nasopharyngeal swab. However, RT PCR has its fair share of limitations, and given the lack of availability and false negative results obtained due to sampling errors or inadequate sample collection timing, serological testing soon became an auxiliary diagnostic tool[3]. As the pandemic progressed, assessing seroprevalence became crucial for monitoring populations and developing public health policy. After the development of vaccines, it became even more advantageous since it provided a way for vaccine efficiency evaluation and comparison[4]. Currently there are many immunological assays available on the market based on several types of distinct platforms: ELISA (enzyme-linked immunosorbent assay), CLIA (chemiluminescence assay), LFIA (lateral flow immunoassay), eCLIA (electrochemiluminescence assay), immunofluorescence assays (IFA) and others[5]. Some of these tests were given Emergency Use Authorization (EUA) by the Food and Drugs Administration (FDA)[6]. However, more studies were needed to better understand their performance characteristics, limitations and appropriate use. Furthermore, adequate standardization of all assays is needed to facilitate extensive testing and alleviate research in this area. The aim of this study was to compare two different methods for the detection of IgG antibodies against the RBD of SARS-CoV-2 in their technical requirements, characteristics and obtained results.

Materials and methods

The study included 268 samples from patients referred to the Institute of Immunobiology and Human Genetics for anti-SARS-CoV2 antibody testing. The samples were obtained in the period from October 15th to November 15th 2021. An informed signed consent was obtained and 5 milliliters of venous blood were drawn in a vacutainer without anticoagulant. After 30 minutes at room temperature, the tubes were centrifuged for 10 minutes at 1500 rpm. The separated serum was used immediately or if needed frozen at -20°C for no more than 3 months. All measurements were performed at the Institute of Immunobiology and Human Genetics at the Faculty of Medicine in Skopje. Two commercially available kits were used: SARS-CoV-2 RBD IgG CLIA kit from Snibe (Shenzhen, China) and ELISA SARS-CoV-2 IgG (RBD-S protein) from INEP (Institute for Application of Nuclear Energy), Belgrade, Serbia. For comparison, all 268 samples were subjected to measurement using both methods, according to the manufacturers' instructions listed below.

Clia

The analyses were performed on a MAGLUMI 1000 analyzer (Snibe diagnostic, Shenzhen, China). The SARS-CoV-2 S-RBD IgG assay is an indirect chemiluminescence method. 10 µl sample, buffer, and magnetic microbeads coated with S-RBD recombinant antigen are mixed thoroughly and incubated, forming immune complexes. After precipitation in a magnetic field, the supernatant is decanted and a wash cycle is performed. The ABEI (N-(4-aminobutyl)-N-ethylisoluminol)) labeled anti-human IgG antibody is added and incubated to form immune complexes. After precipitation in a magnetic field, the supernatant is decanted, and another wash cycle is performed. Subsequently Starter 1 and 2 are added to initiate the chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light

units (RLUs) which are proportional to the concentration of SARS-CoV-2 S-RBD IgG present in the sample. The antibody levels are determined using a previously generated two-point calibration curve and a ten-point master curve provided by the kit manufacturer. Results are expressed as arbitrary units per milliliter (AU/ml). According to the manufacturer, the cut off value for this assay is 1 AU/ml. The internal validation in the everyday laboratory practice and the obtained results from the Support-e project aiming to provide high quality evaluation of COVID convalescent plasma throughout Europe showed that seronegative samples from patients who did not have any contact with the virus may be false positive with the original cut off value. Therefore, it has been raised greater than 2 AU/ml and results between 1 and 2 AU/ml are considered borderline and repeated testing is suggested. Of note, using the standard sample dilution the method is able to discriminate only concentrations less than 100 AU/ml and samples with higher concentrations are assigned for retesting using additional 1:10, and even 1:100 dilution ratios if needed. Reported sensitivity and specificity of the test are 100% and 99.6%, respectively.

Elisa

Enzyme-linked immunosorbent assays (ELISAs) were performed using a manual kit from INEP (Institute for Applied Nuclear Energy), Belgrade, Serbia. The kits included positive and negative controls. These anti-SARS-COV-2 assays provide a semiquantitative determination of IgG antibodies against the SARS-CoV-2 virus. The microplate wells are coated with the SARS-CoV-2 S-RBD protein, which is produced by a recombinant technology in HEK 293 cells. Sera are diluted 1+ 50 in Sample buffer (10 µl serum with 50 µl buffer), added to the wells and covered to avoid evaporation. The immune complexes are formed between the recombinant antigen and anti-SARS-CoV-2 IgG antibodies present in the sample during an incubation period of 30 minutes at 37°C**.** Following this, contents from all wells are aspirated and five wash cycles are performed with 300 µl working strength wash buffer. Next, 100 µl of working dilution of conjugate (anti-human IgG bound to horseradish peroxidase) is added. Following a 30-minute incubation of the covered plate at 37°C and another wash cycle, 50 µl of both substrate TMB (tetramehtylbenzidine) solution A and B are added. Again, the plate is covered and left for a final incubation of 30 minutes at 37°C. Finally, 50 µl of stop solution is added to each well to stop the enzymatic reaction. Optical density (OD) is measured on Wallac 1420 Victor 2 ELISA plate reader from Perkin Elmer (Waltham, Massachusetts, United States) at 450 nm. The results are expressed as an index calculated by the ratio of extinction of samples over the extinction of the positive control which serves as a calibrator. The cut off value for the test is 15, with a borderline zone from 15 to 20 and values above 21 are considered positive. The cut off value is defined according to the ROC curve, which was based on testing at least 70 sera positive for the presence of IgG antibodies to SARS-CoV-2 virus with various levels of positivity. Reported manufacturer's specificity and sensitivity of the test are 98% and 99%, respectively.

Statistical analysis

In order to compare the methods, positive percentage agreement (PPA), negative percentage agreement (NPA), and overall percentage agreement (OPA) were calculated. Additionally, the quality of agreement of the two methods was expressed by Cohen's kappa[7]. Further analysis was done by separating the positive samples into groups according to their dilution factors and antibody concentration and determining the mean values and standard deviation of each group.

Results and discussion

The results from both methods were compared according to their discrimination of a sample as positive or negative and the obtained results are shown in Table 1.

Table 1. Number of positive and negative samples according to both methods

	CLIA +		CLIA - *		Total	
	No	**%**	**No**	**%**	**No**	**%**
ELISA +	153	90%	17	17.3%	170	63.4%
ELISA - *	17	10%	81	82.7%	98	36.6%
Total	170	100%	98	100%	268	100%

* Including borderline results

CLIA testing evaluated 170 from 268 samples as positive, while ELISA found 153 positive. Therefore, the PPA for these two techniques was 90%. Analogously, the NPA and OPA were 82.7% and 87.3%, respectively. Cohen's kappa was 0.72, meaning there was a moderate agreement between the two methods[7]. Other studies similarly showed that Maglumi CLIA assays and Euroimmun ELISA assays were concordant in regards to IgG class antibodies against SARS-CoV-2[8,9].

Since Snibe CLIA as a method involves diluting the samples to precisely define the antibody concentration, further analysis could be done if we stratify the positive samples in several categories according to the dilution ratio and antibody concentration: low and medium (did not require additional dilution steps), high (diluted in a 1:10 ratio) and very high concentration (diluted in a 1:100 ratio). The number of samples in each group is shown in Table 2.

Table 2. CLIA Snibe categories according to dilution ratios on positive samples

	No of CLIA positive samples	**No of samples negative on ELISA**	**CLIA Snibe mean value (AU/ml) and SD (AU/ml)**	**ELISA INEP mean value and SD**
Undiluted low conc. (2-10 AU/ml)	32	16	4.65±2.32	27.12±17.28
Undiluted medium conc. (10-100 AU/ml)	51	0	53±28.13	59.28±17.55
Diluted 1:10 high conc. (100-1000 AU/ml)	46	0	512.6±262.2	84.94±11.70
Diluted 1:100 very high conc. (>1000 AU/ml)	41	1	5828.0±3439.5	87.53±16.20

It is clear from the analysis shown in Table 2 that most of the discrepancies occurred in the lower concentration category with a positive percentage agreement of only 50%. This supports the findings of some reviews [10] that CLIA-based assays showed better sensitivity and

specificity than ELISA or LFIA tests for anti-SARS-CoV-2 antibodies. However, a different meta-analysis[11] states that in the 38 studies they analyzed, CLIA assays showed comparable sensitivity to ELISA, but a somewhat lower specificity. When comparing samples with higher antibody titers, the two methods were completely concordant in their determination of a positive result. There was one flagrant discrepancy in one sample which was evaluated as negative with ELISA INEP, while the value obtained with CLIA SNIBE was not only positive, but also in the very high concentration category. Given the limited resources, a repeated analysis using an ELISA plate was impossible and therefore we are left with a possible explanation in a manual error or a prozone phenomenon occurring due to the oversaturation of the sample. This example clearly demonstrates a downside of the ELISA INEP technique in that repeated runs for single samples are inefficient. Also, the lack of repeated runs with accessory dilution in ELISA and the oversaturation in samples with higher concentrations led to mean concentration not rising substantially (89.54 *vs* 84.84) even though CLIA showed a tenfold increase in the mean concentration between the groups.

The authors of this article would also like to address the possibility of differences in the produced RBD antigen used as a target in both tests. We should also note that Maglumi Snibe CLIA was used as a benchmark for comparison, even though it cannot be considered a gold standard for anti-SARS-CoV-2 antibody testing. Unavailable biosafety measures and limited resources also led to a lack of a reference marker such as a virus neutralization test (VNT) to verify the sensitivity and specificity of both methods[4].

Conclusion

Our findings suggest that while both methods can be used to discriminate reactive samples, CLIA is superior in accurately distinguishing both very low and very high antibody concentrations. Given the highly concordant results, the automatic CLIA method may have an advantage in terms of low technical expertise required and relative efficiency when working with single samples. Nevertheless, the new ELISA-based assay by INEP shows satisfactory agreement and presents as a new tool in the SARS-CoV-2 pandemic response. Development of reliable serological assays and understanding their advantages and limitations continues to play a key role in the advancement of COVID-related academic research and public health improvement.

Funding: This work was supported in part by the project “Implementation of protocols for reliable application of antibody testing in management of COVID-19 crisis”, co-financed by UNDP Serbia through ODA Challenge Call: Application of innovative solutions from Serbia in response to the COVID-19 pandemic in North Macedonia and Albania.

Conflict of interest statement. None declared.

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