Antibody identification in COVID-19 pandemic: a comparison between immunochemiluminescence and immunochromatography methods

Ruggero Buonocore[1], Matteo Vidali[1], Marco Cusini[2], Paola De Corato[1], Carmine Melchionna[1], Eleonora Galbiati[1], Emilio Berti[2], Ferruccio Ceriotti[1]

[1]UOC Laboratorio Analisi, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milano

[2]UOC Dermatologia, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milano

ABSTRACT

Introduction: in the fight against the COVID-19 pandemic, the determination of the serum antibodies against SARS-CoV-2 is highly relevant, although the reliability of the results delivered is sometimes questionable. The aim of this paper is to evaluate the performances of a rapid immunochromatography test for IgG and IgM antibodies, comparing them with an immunochemiluminescence method.

Methods: we analyzed 357 sera for the presence of IgG anti-SARS-CoV-2 spike proteins S1/S2 with an automated immunochemiluminescent test (DiaSorin®) and the presence of IgG and IgM anti-SARS-CoV-2 nucleocapside protein with an immunochromatography method (LEPU®) based on lateral flow technology.

Results: with Diasorin® method, 248 subjects resulted to be negative and 109 positives, whereas LEPU® test was positive (IgM+ and/or IgG+) in 98 subjects. The overall concordance between LEPU® and DiaSorin®, was 94.1% (95% CI 91.0-96.2). Cohen's kappa was 0.86 (95% CI 0.80-0.92), indicating good agreement. 21 out of 357 (5.9%) samples had a discordant result and were re-analyzed with a third method (Roche Diagnostics Electrochemiluminescence®): 4 out of 5 DiaSorin® negative/LEPU® positive samples were confirmed as negative by Roche®; conversely, among the 16 DiaSorin® positive/LEPU® negative samples, 5 were confirmed as positive by Roche®, 6 as negative and 5 were not retested due to insufficient sample volume.

Conclusions: despite the methods were designed to detect different antibodies an overall high agreement between techniques was found. Discrepant results were found and were likely due to different antigen targets recognized by methods. The observation that only 6 out of 11 DiaSorin® positive samples were not confirmed by ROCHE®, supports the antigen-dependent hypothesis.

INTRODUCTION

With 63.5 million of cases, COrona VIrus Disease – 19 (COVID-19) represents a dramatic emergency worldwide that highlights the urgent need for solid clinical and laboratory biomarkers to help physicians for an adequate patient's management (1). 80-85 % of patients show no or just mild respiratory symptoms; 15-20% of infected patients have more severe forms of the disease and display major clinical respiratory complications that frequently evolve in multi organ disfunction, especially if comorbidities (i.e. diabetes, hypertension, coronary heart disease, etc.) are present (2–4).

Diagnosis is based upon the detection of viral RNA through Reverse transcriptase-polymerase chain reaction (RT-PCR) in samples from upper or lower respiratory tract. Although RT-PCR requires high technical skills and automated technologies, it provides the current gold standard in SARS-CoV-2 infection detection. Several preanalytical variables, such as the time of specimen collection, are pivotal for a correct viral RNA extraction and quantification (5–8). In the upper respiratory tract, viral RNA titer reaches a maximum concentration within 7-10 days after symptoms onset and then steadily declines in the following days. RNA in lower respiratory tract is still present after 3 weeks from symptoms onset (6,9–11).

Antibody anti-SARS-CoV-2 determination could be

Corrispondenza a: Ruggero Buonocore, Laboratorio Analisi, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via Francesco Sforza 28, 20122 Milano, Italy, Tel +39 3403284928, E-mail ruggero.buonocore@policlinico.mi.it

Ricevuto: 18.01.2021 Revisionato: 23.02.2021 Accettato: 26.02.2021

Pubblicato on-line: 12.04.2021 DOI: 10.19186/BC_2021.016

another valuable tool in the pandemic surveillance. SARS-CoV-2 displays a singular antigenicity. In Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS) and also SARS-CoV-2, both IgM and IgG antibody levels are detectable simultaneously after 2-3 weeks from symptoms onset (9). However, in mild and asymptomatic patients the antibody determination may give a false negative result, causing the impossibility to identify potential contagious individuals (9,12–16).

SARS-CoV-2 expresses four major structural proteins named Spike (S), Envelope (E), Membrane (M) and Nucleocapsid (N) protein (2). S protein is composed by 2 subunits known as Spike 1 (S1), which binds the virus to human angiotensin-converting-enzyme-2 (ACE-2), and Spike 2 (S2) which permits the entrance of SARS-CoV-2 in the patient's cells. N protein instead is important for the viral pathogenicity, replication and RNA packing. S protein and N protein seem to represent the prevalent targets of immune response. Nowadays, the scientific community is still arguing regarding which antibody could be the most efficient in neutralizing the virus activity. The S protein might be the most representative target, since many *in vitro* assays demonstrated that IgG and IgM toward S proteins could block the viral infectivity and may be used as vaccine against SARS-CoV-2 (2,9,17).

The most used technologies for anti-SARS-CoV-2 antibodies detection are based on enzyme linked immunosorbent assay (ELISA), chemiluminescence (CLIA) and immunochromatography. Despite the more reliable performance of ELISA and CLIA in comparison to immunochromatography, this method still may represent a useful option as a Point Of Care Testing (POCT), which enables a rapid identification of anti SARS-CoV-2 antibodies. Indeed, the interest in POCT anti-SARS-CoV-2 antibody detection is growing and it is explained by the availability of immediate result compared with RT-PCR, ELISA and CLIA (6,18,19). However variable analytical performances, demonstrated by different authors in SARS-CoV-2 antibodies identification using POCT, raise doubts regarding their sensitivity and specificity to identify true positive patients (6,19,20). The aim of our study was to evaluate the agreement between an immunochromatography POCT system (LEPU®) and an automated CLIA platform.

METHODS

Patients

357 sera of health-care workers from Ospedale Maggiore Policlinico in Milan were analyzed for presence of antibody anti-SARS-CoV-2. The collection was part of a large screening program aiming to define the prevalence of anti-SARS-CoV-2 antibodies in the hospital health care workers population. All samples were anonymized before the analysis.

Sample preparation

Samples were collected using Serum Sep Clot Activator (VACUETTE-Greiner Bio-one®) and kept at room temperature for 30 minutes to allow the clot formation. All specimens were centrifuged at 3500 rpm for 8 minutes and divided in two aliquots: one was immediately tested with an automated immunochemiluminescent assay (DiaSorin® Saluggia, Italy), while the other one was stored at -20 °C and tested afterward with lateral flow immunochromatography (LEPU® Technology, Beijing Lepu Medical Technology Co., China). 21 samples, that resulted discordant with the two previous techniques, were reanalyzed in the same day of tawing with a third method using the second aliquot (Elecsys Anti-SARS-CoV-2, Roche®).

The study was carried out in accordance with the Declaration of Helsinki as amended in 2013, under the terms of all relevant local legislation; all participants subscribed an informed consent.

Analytical methods

Lateral flow immunochromatography assay

LEPU® Technology lateral flow immunochromatography (Beijing, China, lot: 20CG2505X) is a rapid lateral flow immunoassay for anti-SARS-CoV-2 antibody detection against Nucleocapsid proteins, based on the principle of antigen-antibody reaction and immunoassay technique. The test uses a nitrocellulose membrane coated with SARS-CoV-2 colloidal gold labeled recombinant protein, a mouse-anti human IgG and IgM immobilized in G and M test area respectively and a corresponding antibody in the quality control area. 10 µL of serum and 80 µL of sample dilution buffer, are added in two dedicated wells. The sample migrates upward through capillary effect. When SARS-CoV-2 IgG and/or IgM antibodies were at or above the limit of detection of the method, they bind the colloidal labeled SARS-CoV-2 recombinant protein on a gold labeled pad. Subsequently the antigen-antibody complex is captured by mouse-anti-human IgG and/or IgM antibodies immobilized in G or M area and produce a purple-red band. In samples with an antibody content below the limit of detection of the method, no purple-red band appears in IgG and/or IgM test area. Regardless of the presence or absence of antibodies in patient's sample, a purple-red band must appear in the control area to validate the test. The result must be read within 10-20 minutes as indicated by the producer.

Automated immunochemiluminescent assay

LIAISON® SARS-CoV-2 (DiaSorin®, Saluggia, Italy, Ref 311450, Lot 354029), is based on an indirect immuno-chemiluminescence full-automated technology for the quantification of IgG antibody anti-Spike1 (anti-S1) and anti-Spike2 (anti-S2) againstSARS-CoV-2. Recombinant antigens Spike1 (S1) and Spike2 (S2) of

Tabel 1
Cross-tabulation of antibody detection by DiaSorin and LEPU tests (number of samples).

		DiaSorin		
		Positive	Negative	Total
LEPU	Positive	93	5	98
	Negative	16	243	259
	Total	109	248	357

Tabel 2
Level of agreement between LEPU and DiaSorin methods.

Concordance	94.1% (95% CI 91.0-96.2)
Specific Positive Agreement	89.9% (95% CI 85.3-93.9)
Specific Negative Agreement	95.9% (95% CI 94.0-97.5)
Cohen's Kappa	0.86 (95% CI 0.80-0.92)

95% CI, 95% Confidence Interval.

SARS-CoV-2 are immobilized on dedicated magnetic beads (solid phase) while mouse monoclonal antibodies anti-human IgG are bound with isoluminol. During first incubation, the IgG anti-SARS-CoV-2 present in the samples and in controls recognize S1 and S2 antigens present on solid phase. All the unbound IgG are removed with a first washing cycle. During the second incubation the antibodies conjugated with isoluminol react with IgG anti-SARS-CoV-2 and a second wash cycle is performed to eliminate the exceeding amount of antibodies. A dedicated substrate is added to the solution and induces a chemiluminescence reaction produced with an intensity proportional to concentration of IgG anti-SARS-CoV-2 present in the patient's serum. The results are available within 30 minutes. The cut-off limit for a positive sample is ≥15 AU/mL (Arbitrary Units/mL), while the cut-off value for a negative sample is ≤12 AU/mL. Dubious samples have a concentration between 12-15 AU/mL. In these cases, the producer recommends to run the sample a second time and if the result is confirmed, a new two weeks later is recommended.

Qualitative electrochemiluminescence immune assay

Elecsys Anti-SARS-CoV-2 (Roche®, Mannheim, Germany Ref 09203079190, Lot 520916,) is a qualitative electrochemiluminescence immune assay based on the principle of sandwich antigens method, which includes recombinant proteins of SARS-CoV-2 Nucleocapsid to reveal the presence of IgG, IgA and/or IgM anti-SARS-CoV-2 antibody in the patient's serum. During the first incubation, 12 µL of serum are mixed together with a recombinant biotinylated SARS-CoV-2 antigen and with a second recombinant ruthenium labeled SARS-CoV-2 antigen to allow the sandwich complex formation. The second incubation involves dedicated microparticles coated with streptavidin, which enable the binding of the complexes to the solid phase. After a washing cycle, a specific electrode allows the chemiluminescence reaction which displays an intensity proportional to the antibody's concentration in the sample. The results are available within 18 minutes. The suggested cut-off for a positive sample is 1.0 ICO (Index of Cut Off).

Statistical analysis

Statistical analysis was performed by SPSS statistical software v.17.0 (SPSS Inc., Chicago, IL, USA) and R Language v.4.0.3 (R Foundation for Statistical Computing, Vienna, Austria). Categorical variables were reported as absolute and relative frequencies. The overall agreement was calculated as percentage of concordant pairs [(DP+DN)/total pairs], while positive or negative agreement were calculated as specific positive or negative agreement, respectively [2DP/(2DP+SP1+SP2)] or [2DN/(2DN+SP1+SP2)], where DP, DN, SP1, SP2 indicate respectively patients positive (DP) or negative (DN) to both tests, SP1 and SP2 patients positive only to a single test. 95% confidence intervals (95%CI) for the overall and specific agreements were calculated respectively by the Wilson Score or bootstrap methods. The overall agreement was also reported by Cohen's kappa with its 95 % CI.

RESULTS

With DiaSorin® 248 (69.5%) subjects were negative and 109 (30.5%) positive, whereas LEPU® test was positive (IgM+ and/or IgG+) in 98 (27.5%) subjects. In particular, 6 patients (1.7%) were positive only for IgM, 42 (11.8%) for IgG and 50 (14.0%) displayed both IgM and IgG positivity. 21 out of 357 (5.9%) samples showed discordant results, with 16 (4.5%) subjects detected only by DiaSorin®, while 5 (1.4%) only by LEPU test (Table 1). The overall concordance between LEPU and DiaSorin®,

calculated as concordant pairs, was 94.1% (95%CI 91.0-96.2), with proportions of specific positive and negative agreement respectively of 89.9% (95%CI 85.3-93.9) and 95.9% (95%CI 94.0-97.5). Cohen's kappa was 0.86 (95% CI 0.80-0.92), indicating good agreements (Table 2). All but 5 discordant samples were re-analyzed with a third method (Total Ig anti-SARS-CoV-2 nucleocapsid protein - Roche® Diagnostics): 4 out of 5 DiaSorin® negative/LEPU positive samples were confirmed as negative by Roche; conversely, among the 16 DiaSorin® positive/LEPU® negative samples, 5 were confirmed as positive by Roche®, 6 as negative and 5 were not retested due to insufficient sample volume.

DISCUSSION

Immunodiagnostic POCT could be a particular useful tool in SARS-CoV-2 antibody determination since it requires low sample amount and it could be performed at patient's bedside or home. Moreover, the application of immunodiagnostic methods could be a pivotal strategy to spot new outbreaks thus ensuring a safer management of public health security. They give the opportunity to detect asymptomatic patients, who are not tested for viral RNA presence, to evaluate the COVID-19 spread in the population for epidemiological purposes, to plan a successful vaccination campaign and to identify subjects with post-vaccine antibody titres.

An important aspect is related to the kinetics of antibodies appearing in patients' serum. Currently, few evidences are available on the correct timing for SARS-CoV-2 antibody screening, since immunological response displays high variability both in intensity and isotype (IgM and IgG) appearance.

Guo et al. reported that the median time of IgM and IgA appearance in serum is about 3-6 days from the beginning of symptoms, while IgG show a median appearance between 10-18 days (2,19). This is in contrast with other authors who claimed that IgG and IgM in SARS-CoV-2 infection have a similar onset time (9,12–16). The difficulty to standardize the time of collection may be an explanation of antibody detection discrepancy among different methods. If the sample is collected too early, IgG and/or IgM will likely be below the method sensitivity and thus not detectable. On the opposite, if the collection is delayed, it is possible to find only IgG or low IgM levels.

In this study, an overall high agreement between methods was found, with DiaSorin® showing a slightly higher (30.5%) antibody prevalence than LEPU® POCT technology (27.5% - IgG or IgM or both). Such a high agreement was not expected, when considering that the methods employed in this study were designed to detect different targets of SARS-CoV-2 (N versus S1/S2 antigen), as well as different Ig classes (IgM/IgG versus IgG only). These results seem to suggest that SARS-CoV-2 may elicit in the same patient an antibody response towards multiple viral targets. Titers of different antibodies (anti-N or anti-S1/S2) may decrease with different kinetics, possibly becoming soon undetectable in some patients, hence explaining different prevalences, as those found also in this study. Another element which could explain the lower prevalence found with LEPU® POCT compared to DiaSorin®, is the subjective interpretation of the signal bands. Indeed, a weak positive band could be misidentified, particularly with hemolyzed samples, where the weak signal may be hidden by the pink background of sample migrating in the POCT pad.

Few discrepant results (n=21) between methods were found and were likely due to the causes reported above. In particular, the observation that 6 out of 11 DiaSorin® positive samples were not confirmed by Roche®, which uses the same antigen target as LEPU® Technology, supports the hypothesis that method discrepancies were related to different antigen targets.

High concordance between qualitative tests often relies on samples with a huge difference between frequencies of positive and negative cases (few positive cases and many negative cases). This was not the case for the high agreement between LEPU® and DiaSorin® (94.1%), as also suggested by both elevated positive (89.9%) and negative (95.9%) specific agreement.

All these results suggest that LEPU® Technology can be considered a valid POCT screening test.

A possible limitation of this study is that LEPU® technology has been tested with serum samples and not with capillary blood samples as in drive-in, home or bedside settings. In these situations, a lower agreement could be assumed, due to different factors (e.g. less trained operators, more subjective band interpretation).

In conclusion, rapid tests are an interesting diagnostic solution and could play an essential role for the whole community in the fight against SARS-CoV-2.

CONFLICT OF INTEREST

None.

REFERENCES

1. Henry BM, De Oliveira MHS, Benoit S, et al. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): A meta-analysis. Clin Chem Lab Med 2020;58:1021–8.
2. Lippi G, Mattiuzzi C, Bovo C,et al. Current laboratory diagnostics of coronavirus disease 2019 (COVID-19). Acta Biomed 2020;91:137–45.
3. Wu Z, McGoogan JM. Characteristics of and important lessons from the Coronavirus Disease 2019 (COVID-19) outbreak in China: Summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention. JAMA 2020;323:1239–42.
4. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020;395:1054–62.
5. Stowell SR, Guarner J. Role of serology in the coronavirus disease 2019 pandemic. Clin Infect Dis 2020;71:1935–6.

6. Russo A, Minichini C, Starace M, et al. Current status of laboratory diagnosis for covid-19: A narrative review. Infect Drug Resist 2020;13:2657–65.
7. Technical guidance publications n.d. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance-publications (last accessed December 2020).
8. Lippi G, Simundic AM, Plebani M. Potential preanalytical and analytical vulnerabilities in the laboratory diagnosis of coronavirus disease 2019 (COVID-19). Clin Chem Lab Med 2020;58:1070–6.
9. Van Caeseele P, Bailey D, Forgie SE, et al. SARS-CoV-2 (COVID-19) serology: Implications for clinical practice, laboratory medicine and public health. CMAJ 2020;192:E973–9.
10. Woloshin S, Patel N, Kesselheim AS. False negative tests for SARS-CoV-2 infection - Challenges and Implications. N Engl J Med 2020;383:e38.
11. To KKW, Tsang OTY, Leung WS, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis 2020;20:565–74.
12. Sun B, Feng Y, Mo X, et al. Kinetics of SARS-CoV-2 specific IgM and IgG responses in COVID-19 patients. Emerg Microbes Infect 2020;9:940–8.
13. Zhang G, Nie S, Zhang Z, et al. Longitudinal change of severe acute respiratory syndrome coronavirus 2 antibodies in patients with coronavirus disease 2019. J Infect Dis 2020;222:183–8.
14. Interim Guidelines for COVID-19 Antibody Testing | CDC n.d. https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html (last accessed December 2020).
15. Yongchen Z, Shen H, Wang X, et al. Different longitudinal patterns of nucleic acid and serology testing results based on disease severity of COVID-19 patients. Emerg Microbes Infect 2020;9:833–6.
16. Long QX, Liu BZ, Deng HJ, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med 2020;26:845–8.
17. Lu H, Stratton CW, Tang YW. An evolving approach to the laboratory assessment of COVID-19. J Med Virol 2020;92:1812–7.
18. Vashist SK. In vitro diagnostic assays for COVID-19: Recent advances and emerging trends. Diagnostics 2020 doi:.10.3390/diagnostics10040202.
19. Guo L, Ren L, Yang S, et al. Profiling early humoral response to diagnose novel coronavirus disease (COVID-19). Clin Infect Dis 2020;71:778–85.