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Usefulness of a multiplex immunodot in case of discordant results between automated COVID-19 serological assays

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Abstract

Background: At present, the only reliable test for COVID-19 diagnosis is RT-qPCR. Serological assays have been widely used to increase the detection sensitivity of infected population. Hereby, we report the performance of a new pan-IgG multiplex Enzyme Immunoassay (immunodot) method for exploration of discrepant SARS-COV-2 serological results.

Methods: A retrospective study on 38 residual serum samples from recovered COVID-19 subjects with discordant serological results on Roche and Snibe platforms, were reanalyzed on a new semi-automated pan-IgG immunodot Enzyme Immunoassay, namely COVIDOT-TEST, in order to find the source of discrepancies and to evaluate the latter method. All samples were analyzed on the BlueDiver® Instrument and all strips were read by the Blue-Scan Software.

Results: Based on our data, subject samples showed specific IgG reactions on > 2 different antigens on immunodot strips. Of these 38 samples, 97.4% of samples showed specific IgG reaction against S1 + S2 antigens, 89.5% showed against RBD antigen, 86.8% against S2 antigen reaction on the COVIDOT-TEST kit. Specific IgG-S1 antigen and IgG-N antigen reactions were detected in 73.7% and 65.8% of the samples, respectively.

Conclusion: The new semi-automated pan-IgG immunodot Enzyme Immunoassay method appeared to be a reliable assay to confirm suspicious COVID-19 serological screening results.

1. Introduction and objectives

In the last days of 2019, a cluster of pneumonia cases of unknown origin was observed in several local hospitals of the city of Wuhan (Hubei, China). Soon after, the Chinese center for disease control and prevention (CDC) informed the World Health Organization (WHO) of a new threat to the humanity due to severe acute respiratory infection (SARI) (Zhu et al., 2020; Henny et al., 2016; Wu and McGoogan, 2020; Li et al., 2020) with human-to-human transmission and in some cases with an acute respiratory distress syndrome (ARDS), or organ failures, and eventually deaths (Li et al., 2020; Chen et al., 2020; Wang et al., 2020). As of 7 January, a new virus was isolated and the genome sequence was identified by Lu, et al. showing that the virus belongs to the family of coronaviruses (Lu et al., 2020). The WHO named the new virus as the '2019 novel coronavirus' (2019-nCoV) (WHO, 2020) and studies showed animal origin with more than 90% genome sequence similarity to MERS-CoV, SARS-CoV and Bat coronaviruses (Lu et al., 2020; Zhou et al., 2020). As the new disease was spreading all around the world, the WHO declared the disease as a Public Health Emergency of International Concern on January 30, 2020 and officially named it as ‘coronavirus disease 2019’ (COVID-19) on 11 February 2020. The International Committee on Taxonomy of Viruses renamed the virus as ‘Severe Acute Respiratory Syndrome Coronavirus 2’ (SARS-CoV-2) (WHO, 2020; Guo et al., 2020).

The COVID-19 diagnosis has been challenging as the RT-qPCR is the only reliable test (Corman et al., 2020) to diagnose an ongoing infection. One other way is investigating the humoral immune response against SARS-CoV-2 which could increase the detection of infected population, particularly in asymptomatic, pauci-symptomatic or recovered/immunized subjects (Abbasi, 2020). For the latter purpose, in our laboratory we are using 2 fully automated tests, namely Roche Elecsys® Anti-SARS-CoV-2 (ani-N antibodies) as the screening test and Snibe

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R. Soleimani et al.

MAGLUMI™ 2019-nCoV (separate IgG and IgM detection) to identify a specific immune reactivity. Discordant results between these two methods, approximately 10 % of the analyzed samples, caused interpretation difficulties for patients and physicians.

To explore these discrepancies, we re-analyzed these samples with ALPHADIA S.A./N.E. COVIDOT-TEST, a semi-quantitative multiplex immunodot kit intended for the in-vitro pan-immunoglobulin G (IgG) detection against different SARS-CoV-2 antigens.

2. Material and methods

2.1. Studied subjects (recovered COVID-19 & external quality controls)

A retrospective study was conducted on sera from 38 recovered COVID-19 subjects (17 male, mean age = 37.5; 95 % CI = 33.1–41.8 year) and 4 external quality controls (EQC; 2 positives and 2 negatives samples provided by Sciensano, Belgian institute for health, Brussels, Belgium) collected from September 01–10, 2020. The inclusion criteria were recovered and non-hospitalized COVID-19 subjects with at least 1 positive RT-qPCR test on nasopharyngeal swab samples 6 months (March 2020) prior of the sample collection (c.f. Soleimani et al. (2020)), having discordant serological results (positive anti-N antibodies on Elecsys® Anti SARS-CoV-2 and negative IgM/IgG on MAGLUMI™ 2019-nCoV kits). The subjects presented, non-specific symptoms such as fever, cough and shortness of breath, at the time of RT-qPCR testing. All subjects were immunocompetent, without any comorbidity, between 18–60 years old, with normal renal function (≥ 90 mL/min). Out of 52 candidates, 14 were excluded because of low residual sample volume (n = 6), advanced age (n = 4), chronic renal failure (n = 2) and hemorrhagic or lipemic samples (n = 2). Samples were stored at +4 °C until analysis. Testing was performed with the laboratory technologist being blinded to the prior results. Our study fulfilled the ethical principles provided by the Declaration of Helsinki.

2.2. COVIDOT-TEST

Selected samples were analyzed on COVIDOT-TEST (Covid-19 profile IgG Dot™, Alphadia), an IVD (in vitro diagnosis device) multiplex immunodot, semi-quantitative and semi-automatized kit, detecting pan-IgG against SARS-CoV-2 and other human coronaviruses antigens on the BlueDiver® Instrument (BDI). The four EQC were analyzed in duplicate (in each run) to externally verify the reproducibility of the assay. The principle of the test is based on Enzyme Immunoassay (EI) on a barcoded single strip with 14 dots allowing simultaneous detection of antibodies against different antigens, including 2 internal positive and negative controls and 1 blank dot for the validation of each strip. Of 11 remaining dots, 6 correspond to SARS-CoV-1, MERS, HKU1, OC43, 229E, NL63 nucleocapsid (N) protein antigens, and 5 others are specifically related to SARS-CoV-2, including Nucleocapsid (N), Spike (S1 + S2), S1 and S2 Receptor Binding Domain (RBD) protein antigens. Briefly, each strip was automatically and sequentially incubated in the wells of ready-to-use reagent cartridges including Nucleocapsid (N), Spike (S1 + S2), MERS, HKU1, OA43, 229E, NL63 nucleocapsid (N) protein antigens, and 5 others are specifically related to SARS-CoV-2, including Nucleocapsid (N), Spike (S1 + S2), S1 and S2 Receptor Binding Domain (RBD) protein antigens. Briefly, each strip was automatically and sequentially incubated in the wells of ready-to-use reagent cartridges of the BDI as follows: 1–30 min incubation with 10.0 μL of sample for antigen-antibody complexing, 2–6 min of serially washing to remove unbound antibodies, 3- adding alkaline phosphatase-conjugated goat antibodies against human IgG, 4–10 min incubation for binding conjugate to the antigen-antibody complexes, 5-6 min of serially washing to remove unbound conjugate, 6–10 min incubation into the substrate solution, 7–2 min washing step. After a brief drying step, strips were scanned by the BlueScan® reader using DR DOT® Software to semi-quantify the dot intensities. The intensity of purple dots on the strips is directly proportional to the antibody concentration in the sample and calculated (arbitrary unit (AU); from 0 to 100) using the following formula = x 100. Results less than 5 AU were considered negative, ≥ 5 AU were interpreted as positive. Based on manufacturer’s data, the COVIDOT-TEST reaches 100 % sensitivity by the fourth week after the onset of symptoms. Anti-N, anti-S1 + S2 and anti-S2 appeared to be the earliest antibodies and specificities were estimated more than 99 % for S1 + S2, S2 and RBD antigens, at 99 % for S1 and at 96 % for N antigens.

2.3. Anti-N antibodies on Roche Elecsys® Anti SARS-CoV-2 and separate antibodies on Snibe MAGLUMI™ 2019-nCoV IgM/IgG platforms

The Roche Elecsys® Anti-SARS-CoV-2, a CE-IVD (European approved in vitro diagnosis) marked electrochemiluminescence immunoassay (ECLIA) kit, was the daily screen method intended for qualitative detection of anti-N antibodies (IgG, IgM and neutralizing antibodies) against SARS-CoV-2 in serum and plasma samples. The kit was performed on the Cobas 8000 e602® analyzer (Roche Diagnostics International Ltd, Rotkreuz, Switzerland). The ECLIA assay uses a recombinant N protein as the principal antigen for the determination of antibodies against SARS-CoV-2. A result more than 1.00 (cut-off index) was interpreted as positive. The sensitivity and specificity of the kit were estimated, 14 days after the RT-qPCR confirmation, at 99.5 % (95 % confidence interval (CI) = 97.0–100.0 %), and at 99.8 % (95 % CI = 99.7–99.9 %) respectively (Muench et al., 2020).

Samples with positive anti-N antibodies screen test were subject of second IgG/IgM assay, using Snibe MAGLUMI 2019-nCoV IgM/IgG Kit, a CE-IVD marked chemiluminescence assay (CLIA) on MAGLUMI™ 800 (Shenzhen New Industries Biomedical Engineering [Snibe] Co., Ltd., Shenzhen, China) analyzer. The CLIA assay, however, uses N and S proteins as targeted antigens for the separate determination of IgG and IgM against SARC-CoV-2. A level greater than 1.00 AU/mL was interpreted as positive for both antibodies. The sensitivity for combined IgG and IgM and the overall specificity of the 2019-nCoV IgM/IgG kit were estimated at 95.5 % (95 % CI = 84.9–99.2) 18 days after the onset of symptoms and at 100.0 % (95 % CI = 96.3–100.0), respectively (Soleimani et al., 2020).

2.4. Statistical analysis

Means and 95 % confidence intervals were determined in COVID-19 recovered subjects. The agreement between the methods was determined by the kappa index. A P < .05 was considered as statically significant. Data analysis was performed using GraphPad Prism software (Version 8.0; California, CA).

3. Results

All tested samples (selected for discrepancy between screening methods) had positive anti-N antibodies on the Elecsys® Anti-SARS-CoV-2 platform while they were negative with separate IgM/IgG MAGLUMI 2019-nCoV method. All samples (100 %, n = 38) showed a specific IgG reaction on at least 2 different SARS-CoV-2 antigens on the multiplex COVIDOT-TEST kit. 97.4 % (37/38) of the samples were positive for IgG-(S1 + S2), 89.5 % (34/38) showed an IgG-RBD antigen reaction and 86.8 % (33/38) revealed an IgG-S2 antigen reaction. IgG-S1 antigen and IgG-N antigen reactions were detected in 73.7 % and 65.8 % of the samples, respectively (Table 1). Globally, 42.1 %, 36.8 %, 13.2 %, 7.9 % showed IgG reactivity against 5, 4, 3 and 2 different SARS-CoV-2 specific antigens, respectively (Table 1).

IgG reactivity against Nucleocapsid antigens from other human coronaviruses, including SARS (55.3 %), MERS (0 %), HKU1 (63.2 %), OC43 (100 %), 229E (26.3 %) and NL63 (5.3 %) is also shown in Table 1.

The results of 4 EQC (2 positive and 2 negative controls) are shown in Table 2. One positive EQC sample showed IgG reactivity with all 5 SARS-CoV-2 specific antigens, while the other positive EQC showed IgG reactivity with RBD, S1 + S2, S2 and N antigens but not with S1 antigen on the COVIDOT TEST kit. The two negative EQC were correctly classified. All 4 EQC showed an IgG-N-OC43 antigen reaction, but only the 2 positive EQC showed antibody-antigen reaction with N-SARS (CoV-1).
Table 1
Multiplex results of discordant samples by screening methods (Roche-Electys Positive / Snibe-MAGLUMI Negative) on the COVIDOT-TEST. N = Nucleocapsid; S = Spike; RBD = Receptor Binding Domain; ND = Not Detected. Blue boxes show non-reactive dots. Positive antibodies are indicated in blue. (For interpretation of the references to colour in this Table, the reader is referred to the web version of this article).

| Samples | Roche's Index | N-SARS | N-MERS | N-HK1 | N-GC43 | N-229E | N-NL63 | N-SARS- Cov-2 | S1+S2-SARS- Cov-2 | S1-SARS- Cov-2 | S2-SARS- Cov-2 | RBD-SARS- Cov-2 |
|---------|---------------|--------|--------|--------|--------|--------|--------|-------------|--------------|--------------|--------------|----------------|
| 1       | 21            | 8      | ND     | 8      | 61     | ND     | ND     | 10          | 21           | 8            | ND           | 15             |
| 2       | 8             | ND     | ND     | ND     | 19     | ND     | ND     | ND          | 35            | 16           | 8             | 26             |
| 3       | 6             | ND     | ND     | ND     | 17     | ND     | ND     | ND          | 39            | 21           | 14           | 39             |
| 4       | 95            | 27     | ND     | 10     | 66     | ND     | ND     | 12          | 26           | ND           | 20           | ND             |
| 5       | 6             | ND     | ND     | ND     | 30     | 56     | ND     | ND          | 52            | 27           | 18           | 36             |
| 6       | 17            | 6      | ND     | ND     | ND     | 70     | ND     | ND          | 7             | 70           | 24           | 56             |
| 7       | 136           | 58     | ND     | ND     | ND     | 54     | ND     | ND          | 39            | 53           | 22           | 20             |
| 8       | 42            | ND     | ND     | ND     | ND     | 76     | ND     | ND          | 32            | ND           | 7            | 9              |
| 9       | 12            | 6      | ND     | ND     | 7      | 100    | ND     | 41          | 17           | 52           | ND           | 31             |
| 10      | 21            | 45     | ND     | 21     | 100    | 11     | 17     | 40          | 93            | 63           | 65           | 59             |
| 11      | 8             | ND     | ND     | 47     | 100    | ND     | ND     | ND          | 77            | 10           | 48           | 28             |
| 12      | 32            | ND     | ND     | ND     | ND     | ND     | 7      | 25          | ND           | 8            | ND           | 8              |
| 13      | 46            | 14     | ND     | 12     | 82     | ND     | ND     | 23          | 32           | 10           | 11           | 18             |
| 14      | 33            | 5      | ND     | 21     | 90     | ND     | ND     | 8           | 59           | 13           | 19           | 18             |
| 15      | 95            | 33     | ND     | 11     | 79     | ND     | ND     | 13          | 31           | ND           | 35           | 6              |
| 16      | 106           | ND     | ND     | ND     | 62     | 90     | ND     | ND          | 8             | 52           | ND           | 31             |
| 17      | 34            | 17     | ND     | ND     | 15     | 91     | ND     | ND          | 24            | 52           | 18           | 38             |
| 18      | 21            | 24     | ND     | ND     | ND     | 92     | ND     | ND          | 34            | 43           | 17           | 18             |
| 19      | 7             | ND     | ND     | ND     | ND     | ND     | ND     | ND          | 30            | ND           | 30           | 8              |
| 20      | 14            | ND     | ND     | ND     | ND     | 22     | ND     | ND          | 6             | 75           | 44           | 51             |
| 21      | 155           | 57     | ND     | ND     | ND     | 52     | 83     | ND          | ND            | 59           | 38           | 13             |
| 22      | 86            | ND     | ND     | ND     | ND     | 11     | 73     | ND          | ND            | 25           | ND           | ND             |
| 23      | 44            | ND     | ND     | ND     | ND     | 9      | ND     | ND          | 54            | 33           | 15           | 38             |
| 24      | 28            | 10     | ND     | ND     | ND     | 56     | 73     | ND          | ND            | 30           | 85           | 76             |
| 25      | 28            | 11     | ND     | ND     | ND     | 58     | 70     | ND          | 32            | 84           | 72           | 37             |
| 26      | 145           | 54     | ND     | ND     | ND     | 81     | ND     | ND          | 65            | 77           | 51           | 51             |
| 27      | 148           | 52     | ND     | ND     | ND     | ND     | ND     | ND          | 38            | 48           | 30           | 17             |
| 28      | 98            | 10     | ND     | ND     | ND     | 11     | 22     | ND          | 16            | 40           | 29           | ND             |
| 29      | 118           | 31     | ND     | ND     | 5      | 80     | ND     | ND          | 25            | 43           | 39           | 36             |
| 30      | 5             | ND     | ND     | ND     | ND     | ND     | ND     | ND          | 47            | 10           | 28           | 39             |
| 31      | 16            | ND     | ND     | ND     | ND     | 66     | ND     | ND          | 7             | 48           | ND           | 29             |
| 32      | 3             | ND     | ND     | ND     | ND     | ND     | ND     | ND          | 66            | 5            | 47           | 15             |
| 33      | 6             | ND     | ND     | ND     | ND     | ND     | ND     | ND          | 37            | 19           | 12           | 29             |
| 34      | 11            | ND     | ND     | ND     | ND     | 15     | 66     | ND          | ND            | 36           | ND           | 14             |
| 35      | 137           | 27     | ND     | ND     | ND     | 76     | ND     | ND          | 28            | 19           | 6            | ND             |
| 36      | 41            | 14     | ND     | ND     | ND     | 26     | 100    | ND          | ND            | 12           | 80           | 11             |
| 37      | 114           | 12     | ND     | ND     | ND     | 25     | 29     | ND          | 6             | 47           | 22           | 45             |
| 38      | 6             | ND     | ND     | ND     | ND     | 42     | ND     | ND          | ND            | ND           | 19           | ND             |

Table 2
Proficiency tests evaluation. N = Nucleocapsid; S = Spike; RBD = Receptor Binding Domain; POS = Positive; NEG = Negative; ND = Not Detected. Blue boxes showing non-reactive results. Positive antibodies are indicated in blue. (For interpretation of the references to colour in this Table, the reader is referred to the web version of this article).

| Samples | Roche’s Index | N-SARS | N-MERS | N-HK1 | N-GC43 | N-229E | N-NL63 | N-SARS- Cov-2 | S1+S2-SARS- Cov-2 | S1-SARS- Cov-2 | S2-SARS- Cov-2 | RBD- SARS- Cov-2 |
|---------|---------------|--------|--------|--------|--------|--------|--------|-------------|--------------|--------------|--------------|----------------|
| Sciencano (POS1) | 140 | 91     | ND     | ND     | ND     | 96     | ND     | ND          | 20           | 96           | 57           | 57             |
| Sciencano (POS2)  | 9      | 52     | ND     | 7      | 80     | ND     | ND     | ND          | 54            | ND           | 56           | 26             |
| Sciencano (NEG1)  | <1    | ND     | ND     | ND     | ND     | 41     | ND     | ND          | ND            | ND           | ND           | ND             |
| Sciencano (NEG2)  | <1    | ND     | ND     | ND     | ND     | 45     | ND     | ND          | ND            | ND           | ND           | ND             |
Overall, a complete agreement between Roche and ALPHADIA platforms was achieved (Cohen’s Kappa = 1.00).

4. Discussion

Here, we showed that a reactive humoral immune response against different SARS-CoV-2 specific antigens could be determined in COVID-19 recovered subject using a new semi-quantitative pan-IgG multiplex immunodot kit. In our study, RBD and S (S1 + S2) proteins appeared to be the most often recognized antibody targets, while anti-S1 IgG showed the lowest sensitivity. Other human coronaviruses proteins, particularly N proteins from SARS, MERS, HKU1, OC43, 229E, NL63 are known to have amino acid sequence similarities with SARS-CoV-2 N protein (Chia et al., 2020), which is likely to be subject of cross-reactivity (Chia et al., 2020). Based on our data, it appears that an IgG reactivity against N-SARS-CoV-2 antigen might be the cause of N-SARS cross-reactivity, as we observed a high correlation in positive results for these 2 antigens (N-SARS vs N-SARS-CoV-2). This is not surprising since these 2 nucleocapsid proteins share the highest amino acid sequence homology among human coronaviruses. Interestingly, even though there is a significant N proteins similarity between MERS and SARS-CoV-2, all samples were negative for N-MERS antigen. N-OC43 dots were positive in all samples and N-HKU1 in 24 samples. These immune reactivities could be explained, on the one side, by the high prevalence (more than 50% (Zhang et al., 2018; Nickbakhsh et al., 2020)) of antibodies against common coronaviruses in the general population, and on the other side by the probable cross-reactivity of SARS-CoV-2 antibodies with antigens of coronaviruses from the same genus (beta-coronaviruses). The other two N protein antigens, N-229E and N-NL63, showed less than 10% positive reactions, which is in line with the fact that these coronaviruses have a lower prevalence in the general population. Moreover, they belong to a different virus genus (alpha-coronaviruses) and thus are less likely to share cross-reactive epitopes. All results were in agreement with the data provided by the manufacturer. The Elecsys® Anti-SARS-CoV-2 assay appeared to be more sensitive than IgG/IgM MAGLUMI 2019-nCoV method. Such discrepancies between methods have been previously reported (Naaber et al., 2020; Harritshoj et al., 2021). The later variation in the test performance could partially be explained by assay design and targeted N protein epitope by each manufacturer (Tang et al., 2020). As the patients were not immuno-compromised and the period between RT-qPCR and serology was about 6 months, we assumed all patients had enough time to develop antibodies against SARS-CoV-2. Therefore, negative results by MAGLUMI are probably false negatives.

Even though we could not analytically verify the COVIDOT-TEST, the SARS-CoV-2 serological results for the EQC provided by the national Belgian institute for health, Sciensano, were in agreement with the true results. A complete agreement was also observed between results of the COVIDOT-TEST and the Roche platform, suggesting the new pan-IgG immunodot kit is at least as sensitive as Elecsys® anti-N antibodies kit. The new semi-automated COVIDOT-TEST appeared to be a reliable assay to confirm suspicious COVID-19 serological screening results and may be of interest in the vaccine response evaluation.

Our study was preliminary conducted on a limited number of samples and has limitations. First, we could not determine the exact date of symptom onset because of the heterogeneity in clinical data. Hence, the delay between symptom onset and serology sampling could not be exactly determined but was at least 6 months before sample collection for serology. Secondly, in our cohort we did not observe any inverse discrepancies as all selected samples appeared to be positive on the Elecsys® Anti-SARS-CoV-2 platform while they were negative with MAGLUMI 2019-nCoV method. At last, we did not verify analytical performance of the new Pan-IgG COVIDOT-TEST assay. Further studies are needed to confirm such discrepancies and our observations.

Author contributions

All the authors have approved the entire content of the submitted manuscript and any subsequent revised version and have accepted responsibility for the entire work. RS (clinical pathology resident, Medical Microbiology Service): Conceived the project, wrote the manuscript, reviewed the literature, and responded to the reviewers. MM & AA (clinical pathology residents, Medical Microbiology Service): Gathered all data and reviewed the manuscript. HRV & AS (Clinical pathologists, Medical Microbiology Service): Reviewed the manuscript. BK (Clinical pathologist, Medical Microbiology Service): Supervised the whole project, methodology, medical validation, manuscript preparation, responses to reviewers, and covered costs of the work/publication.

Data availability

The data that supports the findings of this study are available in the main manuscript of this article. The detailed clinical/biological data of COVID-19 recovered patients are not publicly available due to privacy or ethical restrictions.

Ethical aspect

Our study fulfilled the Ethical principles provided by the Declaration of Helsinki and was previously approved by the local Medical Ethics Committee (ref: 2020/06AVR/203).

Declaration of Competing Interest

The authors have nothing to disclose and there was no conflict of interest.

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