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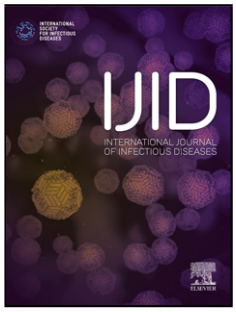

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Performance evaluation of five ELISA kits for detecting anti-SARS-COV-2 IgG antibodies

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Highlights:

- Performances of five commercial ELISA (EDI, AnshLabs, Dia.Pro, NovaTec, and Lionex) for detecting anti-SARS-CoV-2 IgG was evaluated
- Samples used in this study are from diverse national background and the negative group contains samples that are seropositive for all other HCoV
- All assays showed low sensitivity during the early stages (≤7 from symptom onset); however, it was greatly improved overtime
- Lionex showed the highest specificity (98.6%), followed by EDI and Dia.Pro (97.1%,), NovaTec (85.7%), and AnshLabs (75.7%)
- Lionex, which measures antibodies to the S1 protein, demonstrated the best performance and doesn't cross-react with any other HCoV

Abstract

Objectives: To evaluate and compare the performances of five commercial ELISA assays (EDI, AnshLabs, Dia.Pro, NovaTec, and Lionex) for detecting anti-SARS-CoV-2 IgG. **Methods**: 70 negative control samples (collected before the COVID-19 pandemic) and samples from 101 RT-PCR-confirmed SARS-CoV-2 patients (collected at different time points from symptoms onset: ≤7, 8-14, and >14 days) were used to compare the sensitivity, specificity, agreement, positive and negative predictive values of each assay with RT-PCR. A concordance assessment between the five assays was also conducted. Cross-reactivity with other HCoV, non-HCoV respiratory viruses, non-respiratory viruses, and nuclear antigens was investigated. **Results:** Lionex showed the highest specificity (98.6%, 95%CI: 92.3-99.8), followed by EDI and Dia.Pro (97.1%, 95%CI: 90.2-99.2), NovaTec (85.7%, 95%CI: 75.7-92.1), then AnshLabs (75.7%, 95%CI: 64.5-84.2). All ELISA kits cross-reacted with one anti-MERS IgG positive sample except Lionex. The sensitivity was low during the early stages of the disease but improved over time. After 14 days from symptoms onset, Lionex and NovaTec showed the highest sensitivity at 87.9% (95%CI: 72.7-95.2) and 86.4% (95%CI: 78.5-91.7), respectively. The agreement with RT-PCR results based on Cohen's kappa was as follows: Lionex (0.89)> NovaTec (0.70)> Dia.Pro (0.69)> AnshLabs (0.63)> EDI (0.55). **Conclusion:** The Lionex ELISA, which measures antibodies solely to the S1 protein, demonstrated the best performance.

Key Words: COVID-19; SARS-CoV-2; serology; IgG; ELISA; sensitivity; specificity

1. Introduction

Since the start of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak in December 2019 in Wuhan, China, the virus has rapidly spread and become a major

public health concern all over the world [1, 2]. As of July 1, 2020, the virus has caused more than 10 million confirmed infections and over 500,000 reported deaths [3]. Currently, real-time reverse-transcription polymerase chain reaction (RT-PCR) testing is the main technique used for the diagnosis of SARS-CoV-2 infection. However, false-negative RT-PCR results could occur in up to 30% of COVID-19 patients [4-6]. The reasons for this include poor sample collection techniques, sample collection too late after infection, or disease progression into the lower respiratory tract. Once an individual has been infected for at least seven days, the detection of antibodies is possibly more sensitive than RT-PCR for the diagnosis of COVID-19 [7]. Specific antibodies against SARS-CoV-2 can be detected as early as 4-7 days in approximately 40% of COVID-19 patients, with seroconversion rates reaching more than 90% by day 14 [7]. Therefore, serology could be used as a complementary test to RT-PCR to improve the diagnostic sensitivity, particularly in suspected COVID-19 individuals with negative RT-PCR results or those with no respiratory sample collected during the acute phase of illness [1, 2].

Serology testing also has other advantages: it is easy to perform and interpret results; it is cheaper and quicker than RT-PCR; it indicates the patient immune status and infection stage (sero-survey studies); it facilitates the selection of the best candidate donors (with the highest antibody titers) for plasma exchange, and it aids in assessing the efficacy of vaccines that are in development. Due to urgency and demand in the current crisis, a large number of commercial serological tests have been developed and introduced into the global market, but often with insufficient validation on clinical samples. Hence, there is a pressing need for identifying reliable immunoassays with high sensitivity and specificity for serology testing and surveillance of SARS-CoV-2 infection as there remain persistent concerns regarding the accuracy and reliability of the available SARS-CoV-2 immunoassays.

In order to address this challenge, the present study evaluated the performance of five commercial CE-marked ELISA kits for detecting anti-SARS-CoV-2 IgG antibodies in samples from RT-PCR-confirmed COVID-19 patients. The sensitivity, positive predictive value, negative predictive value, positive percent agreement and Cohen's kappa were measured for each assay using samples collected from SARS-CoV-2 RT-PCR-positive patients at different times from symptoms onset (≤7, 8-14, >14 days). The specificity and cross-reactivity were evaluated using pre-pandemic serum samples collected from healthy blood donors. A concordance assessment was conducted to compare the agreement between the kits.

2. Methods

2.1 Study design, ethical compliance, and sample collection

We evaluated the performance of five CE-marked ELISA assays (EDI, Dia.Pro, AnshLabs, NovaTec, and Lionex) for detecting anti-SARS-CoV-2 IgG antibodies. The performance was assessed using anonymous samples collected from RT-PCR-confirmed SARS-CoV-2 patients admitted to Hamad Medical Corporation (HMC), the main public healthcare provider and the nationally designated provider for COVID-19 healthcare needs, with different COVID-19 clinical outcomes. For the negative control group, pre-pandemic serum samples collected from blood donors before 2019, were selected. The IRB approvals for this study were obtained from HMC (HMC-IRB# MRC-01-20-145, HMC-IRB# MRC-05-003, and HMC-IRB# MRC-05-007) and Qatar University (QU-IRB # QU-IRB 804-E/17).

2.2 Serum samples

A total of 101 case serum samples were selected from RT-PCR-confirmed COVID-19 patients, including ICU-admitted patients (n=35), hospitalized non-ICU patients (n=45), and

convalescent samples collected from COVID-19-recovered patients by the Qatar Communicable Disease Center (CDC) at HMC (n=21). Clinical records of the patients were reviewed to determine the time from symptoms onset to collection and categorized into three groups: Group: 1- less than or equal to 7 days; Group 2: 8-14 days; Group 3: more than 14 days. RNA was extracted from nasopharyngeal swab specimens using Qiagen extraction kit. The extracted RNA was tested for SARS-CoV-2 using the SuperscriptIII OneStep RT-PCR kit as recommended by the manufacture instruction (Cat No. 12594100, ThermoFisher, USA). Each sample was tested using three sets of primers targeting the E gene for screening and confirmed with two different sets of primers targeting the RdRp gene as described in [8]. CT values below 32 were considered positive. Characteristics of the 101 COVID-19 patients, including the demographic data and classification, are summarized in Table 1. The patients had a median age (IQR) of 48.0 (40.0-57.0), of which 89.1% were males and 4.9% were females. Patients in the three-time points (≤7, 8-14, and >14 days) had a median age of 50.0 (39.3-56.8), 49.0 (41.3-58.5), and 46.0 (34.3-55.5), respectively.

For the control group, we utilized samples from healthy blood donors collected before 2019 and used in previous studies [9-16]. The healthy blood donors had a median age of 36.0 (30.3-45.0) with 82.9% males and 8.6% females. Details about the collection, transport, and storage methods of the control samples were described elsewhere [9-16]. The control group included samples that are seropositive for various viruses, including all other human coronaviruses (HCoV). Further details about the control samples can be found in Table 1 and S2.

2.3 IgG ELISA kits

Commercial ELISA kits from five different companies were used for the qualitative detection of anti-SARS-CoV-2 IgG antibodies against the Spike (S) or the Nucleocapsid (N)

proteins in the sera of the COVID-19 patient group and control group. These kit are **(i)** Epitope Diagnostic (EDI™) novel coronavirus COVID-19 IgG (Ref. no. KT-1032, USA) **(ii)** AnshLabs SARS-CoV-2 IgG (Ref. no. AL-1001-I, USA), **(iii)** Diagnostic Bioprobes (Dia.Pro) COVID-19 IgG (Ref. no. COV19G.CE, Italy **(iv)** NovaTec (NovaLisa®) SARS-CoV-2 IgG (Ref. no. COVG0940, Germany), and **(v)** Lionex COVID-19 ELISA-human IgG (Ref. no. LIO-COV19-IgG, Germany). More details about the ELISA kits, including specifications, reported sensitivity, and specificity are shown in Table S2. All tests were carried out manually according to the manufacturers' instructions. A microplate reader, Epoch 2 microplate spectrophotometer (Bio-Tek, Italy) was used to read the optical density (OD) in all ELISA reactions. Borderline results were considered positive [1, 17].

2.4 Statistical analysis

The sensitivity, specificity, positive predictive value, negative predictive value, positive percent agreement and Cohen's Kappa were calculated to assess the performance of each assay with the positive SARS-CoV-2 RT-PCR patients [18, 19]. Specificity and cross-reactivity of each assay were assessed using the pre-pandemic control samples. Data were summarized by number and percentage of positive results for each assay. Borderline results were considered positive. Samples were categorized into three groups according to the time between collection and the onset of symptoms (≤7, 8-14, and >14 days), and all parameters were calculated for each group. Concordance assessment between the ELISA kits was conducted to assess the agreement between the kits. These concordance measures include overall, positive, and negative percent agreement, as well as Cohen's kappa statistic. The latter measure is a standard and robust metric that estimates the level of agreement (beyond chance) between two diagnostic tests. Ranging between 0 and 1, a kappa value ≤0.40 denotes poor agreement, a value between 0.40 and 0.75 denotes fair/good

agreement, and a value ≥0.75 denotes excellent agreement [20]. The significance level was indicated at 5%, and a 95% confidence interval (CI) was reported for each metric. All calculations were performed using Microsoft Excel 2016. Chi-square test was used to calculate the significance between the performances of ELISA kits. Significance (*) = $p < 0.05$; (**) = $p < 0.01$; (***) = $p < 0.001$. Further details about the statistical analysis and calculations can be found in Table S4.

3. Results

3.1 Diagnostic assessment of the IgG ELISA kits according to the time of sample collection after symptoms onset (n=101)

The diagnostic assessment of all ELISA kits according to each time-point after symptoms onset (≤7 days, 8-14 days, >14 days) is summarized in Table 2 and Figure 2.

In the first week of symptoms onset (≤7 days), the sensitivity (95% CI) ranged from 57.1% (39.1-73.5) to 78.6% (60.5-89.8) for EDI and AnshLabs, respectively. The highest positive and negative predictive values were estimated at 95.0% (76.4-99.1) for Lionex, and 89.8% (79.5-95.3) for AnshLabs, respectively. The best agreement with RT-PCR was observed in Lionex with 89.8% (82.2-94.4) positive percent agreement and Cohen kappa index of 0.73 (0.63-0.82). The lowest agreement was observed in AnshLabs with 76.5% (67.2-83.8) positive percent agreement and a kappa index of 0.49 (0.36-0.61).

In the second week of symptoms onset (8-14 days), all parameters increased compared to the first week, where the highest sensitivity was scored by AnshLabs at 90.0% (77.0-96.0). The highest positive and negative predictive values were estimated at 97.1% (85.1-99.5) for Lionex and 93.0% (83.3-97.2) for AnshLabs, respectively. The lowest agreement with RT-PCR was

observed in AnshLabs with 80.9% (72.6-87.2) positive percent agreement and a kappa index of 0.61 (0.51-0.72), while the highest agreement was scored Lionex with 92.7% (86.3-96.3) positive percent agreement and a kappa index of 0.84 (0.77-0.91).

The performance of the evaluated IgG ELISA kits varied after 14 days of symptoms onset. Compared to the second week, the sensitivity decreased in EDI, AnshLabs, and Dia.Pro down to 60.6% (43.7-75.3), 84.8 (69.1-93.4), and 66.7% (49.6-80.3), respectively (Figure 2). However, the sensitivity slightly increased for NovaTec and Lionex, where both assays showed the highest sensitivity at 87.9% (72.7-95.2). Also, Lionex showed the highest positive and negative predictive values at 96.7% (83.3-99.4), and 94.5% (86.7-97.9), respectively. The positive percent agreement of EDI, AnshLabs, and Dia.Pro also slightly dropped to 85.4% (77.4-91.0), 78.6% (69.8-85.5), and 87.4% (79.6-92.5), respectively. Whilst no change was observed in the positive percent agreement of NovaTec, it slightly increased in Lionex to 95.1% (89.1-97.9) with a Kappa index of 0.89 (0.82-0.95).

3.2 Assays specificity according to the negative control subgroups (n=70)

All assays showed acceptable overall specificity ranging from 85.7-98.6%, except Anshlabs which had a 75.7% (53/70; 64.5-84.2) specificity. Lionex showed the highest specificity at 98.6% (69/70; 92.3-99.8), followed by EDI and Dia.Pro at 97.1% (68/70; 90.2-99.2), and then NovaTec with 85.7% (60/70; 75.7-92.1) specificity (Table 3 and Figure 1). The specificity of each kit in relation to sample cross-reactivity with other viruses (Table 3 and S1) was also calculated. All assays cross-reacted with other human coronaviruses (HCoVs), except Lionex, which had a 100% specificity (20/20; 83.9-100) in this sub-group. AnshLabs, NovaTec, and Dia.Pro showed false-positive results with non-HCoVs respiratory viruses (RSV and influenza) with a specificity

of 60.0% (9/15; 35.8-80.2), 73.3% (11/15; 48.1-89.1), and 93.3% (14/15; 70.2-98.8), respectively. Only EDI and Lionex did not show cross-reactivity with non-HCoV respiratory viruses with 100% (15/15; 79.6-100) specificity for this subgroup. All assays showed some cross-reactivity with non-respiratory viruses except Dia.Pro, which had 100% (33/33; 93.9-100) specificity. Finally, all assays showed no cross-reactivity with antinuclear antibodies samples except AnshLabs, which cross-reacted with one control sample [50.0% (1/2; 9.5-90.6)]. However, the sample size was very small, as only two specimens were positive for antinuclear antibodies were used.

3.3 Concordance assessment between IgG ELISA kits

Table 4 shows the concordance assessment between the different IgG ELISA kits. The overall percent agreement ranged from 79.5% (72.9-84.9) for AnshLabs/EDI test combination and 97.1% (93.3-98.8) for Dia.Pro/EDI test combination. The positive percent agreement ranged from 66.0% (56.4-74.4) for EDI vs. AnshLabs and Dia.Pro vs. AnshLabs to 100% (94.7-100) for AnshLabs vs. EDI, NovaTec vs. EDI, and NovaTec vs. Dia.Pro. The negative percent agreement ranged from 65.7% (56.1-74.2) for AnshLabs vs. Dia.Pro to 100% (95.4-100) for EDI vs. NovaTec and Dia.Pro vs. NovaTec, and also 100% (94.7-100) for EDI vs. AnshLabs. Importantly, Cohen's Kappa statistic denoted fair/good to excellent agreement and ranged between 0.59 (0.51-0.68) for AnshLabs/Dia.Pro test combination and 0.94 (0.90-0.98) for Dia.Pro/EDI test combination.

4. Discussion

In this study, we evaluated the performances of five CE-marked ELISA kits using 101 samples collected from SARS-CoV-2 RT-PCR-confirmed patients and 70 pre-pandemic control samples collected from healthy blood donors. The sensitivity, specificity, agreement, positive and negative predictive values were calculated at different time points from symptoms onset (≤7, 8-

14, >14 days) for each kit (Table 2). Also, the overall agreement and Cohen's kappa were calculated to compare the assays (Table 4).

Our results showed that most of the evaluated assays demonstrated a very good performance during the first week after symptoms onset compared to other studies [21-24]. Expectedly, the agreement between the outcome of each ELISA kit and RT-PCR increased with time after symptoms onset, consistent with a time lag between the onset of infection and the development of detectable antibodies. High rates of positive results were reached after the first week of clinical illness. This increase was observed with the sensitivity, positive and negative predictive values, positive percent agreement and Cohen's Kappa (Table 2). Even though the sensitivity was lower during the early stages of the disease, it was greatly improved 8-14 days after symptoms onset. AnshLabs showed the highest sensitivity in patients tested within the first two weeks of symptoms onset (Figure 2). However, AnshLabs had the lowest specificity compared to the other kits. After 14 days of symptoms onset, the sensitivity slightly decreased in all assays, except NovaTec and Lionex. This could be because 63.6% (21/33) of the samples in this time point were collected by the CDC from recovered patients, who we do not have their clinical data, including the severity of the disease, whether they developed symptoms or not, and the exact day of sample collection. Hence, these patients might not have elicited enough antibody response to be detected by most of the assays (Table S4, group 3 sample No. 4, 11, 15, and 26). Surprisingly, one of the ICU-admitted patients did not show a detectable antibody response by all ELISA assays (Table S3, group 3 sample No.33), which needs further investigation by other highly sensitive assays. Typically, if borderline results were obtained in ELISA testing, another sample is taken from the patient 1-2 weeks later for re-testing. However, this was not possible here as we were testing sensitivity and specificity in specific time frames. Considering that these borderline

samples were collected from RT-PCR-positive patients, borderline results were considered positive, consistent with similar studies [1, 17]. Interestingly, only one pre-pandemic sample with positive anti-MERS-CoV IgG antibodies was found positive by four ELISA kits (Table 3 and S3), except Lionex, which demonstrated the highest specificity at 98.6% (Figure 1).

Another interesting finding is the heterogeneity of IgG antibody response in COVID-19 patients. Most developed serology assays target either the spike (S) or the nucleocapsid (N) protein of SARS-CoV-2. Previous studies performed on other HCoV suggested that the anti-nucleocapsid (anti-N) antibody response may appear earlier than the anti-spike (anti-S) response and may wane more rapidly [25, 26]. Here, we expect that the differences in sensitivity between ELISA kits depend on the targeted protein used in each assay. We noticed that there was a decline in the sensitivity of the ELISA kits targeting the N protein. However, the sensitivity increased in the kit that solely targets the S1 protein (Lionex). Therefore, a possible explanation for this is that the level of anti-N and anti-S antibodies may be similar during the acute phase of COVID-19 illness, but anti-N antibodies could be waning after the second week [25, 26]. Moreover, this could also explain the high specificity of Lionex compared to the other assays (Figure 1). That is, Lionex targets the S1 protein, which is smaller and less conserved across different families of viruses than the N protein. Therefore, detection of anti-N antibodies may be useful in distinguishing more recent antibody response, while anti-S antibody may be used during the early and convalescent phases. Still, this does not explain why the sensitivity of NovaTec, which targets the N protein, remained steady after the second week compared to EDI which also targets the same protein (N).

Concordance assessment between the different assays showed good to excellent agreement between the kits. EDI and Dia.Pro had the best overall agreement (97.1%) and kappa index (0.94). However, both assays demonstrated the lowest sensitivity in all time-points compared to the assays

despite having a very high specificity (97.1%). Therefore, for diagnosis and clinical relevance, these two assays are the least recommended for such purposes. NovaTec and AnshLabs also showed an excellent positive percent agreement (91.2%) and a kappa index (0.82), where both assays had comparable overall sensitivity and specificity. Lionex, however, showed a variation in the agreement with the other ELISA kits, which could be due to the fact that Lionex is the only kit that targets S1 protein.

From an epidemiological perspective, the high sensitivity of the assay in combination with robust specificity is desirable. Here, Lionex and NovaTec ELISA kits showed the best overall performance in terms of specificity, sensitivity, agreement with RT-PCR, positive and negative predictive values compared to the other assays. The overall performance of both NovaTec and Lionex IgG manual ELISA was comparable to other detection methods, including automated tests, reported elsewhere [27, 28]. Both assays showed a diagnostic sensitivity of 87.9% after 14 days of symptoms onset compared to Abbott Architect (84.2%) and Cobas 6800 systems (95.2%). The specificity of Lionex (98.6%) was also comparable to the aforementioned automated assays (100% and 99.3%, respectively) [27, 28].

A strength of this study is the use of a diverse control group to evaluate cross reactivity with antibodies against various viruses, including MERS, SARS-CoV, endemic coronaviruses, respiratory viruses, and other viruses. One of the limitations of this study is that the clinical details of the patients were not available which are important to understand why some of them did not develop an antibody response that is detectable by the evaluated kits. It would be very beneficial to perform a new study using a large sample size collected from patient with known disease severity outcomes. For instance, critical, severe, moderate, mild and asymptomatic to have a better implication about each assay performance and clinical practice relevance.

In conclusion, we believe that two ELISA kits (NovaLisa, and Lionex) showed promising overall results which could be used in the future for clinical testing. Further, all assays showed acceptable specificity ranging from 85.7-98.6%, except for the AnshLabs ELISA. Finally, although serological assays do not replace molecular tests in diagnosing active infection, they serve as an essential tool to accurately estimate the seroprevalence of SARS-CoV-2 in the general population and to quantify the level of herd immunity [29]. This could help ease the restrictions on human mobility and interactions without provoking a significant resurgence of transmission and mortality. However, it is still not clear whether positive results by serology reflect a protective immune response against infection [30]. Further studies are essential to distinguish functional antibodies from total binding antibodies using virus neutralization assays.

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Transparency declaration

Conflict of interest

All authors have no conflict of interest to declare

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contributions

Conceptualization: GKN, HMY, LJA; Methodology: HAJ, DWA, SNY, FS, RMM, HAA, AH, ST, MA, AK; Formal Analysis: SRD, LJA; Validation: GKN, HAJ, DWA, SNY, FS; Investigation: HAJ, GKN, PT, SRD, LJA, AA, HQ; Resources: GKN, AA, HQ; Data Curation: HAJ, GKN, DWA, SY, FS; Writing – Original Draft Preparation: HAJ, GKN, PT, SRD, LJA; Writing – Review & Editing: HAJ, SRD, GKN, PT, HMY, LJA; Visualization: GKN, HAJ; Supervision: GKN, HMY; Project Administration: GKN, DWA, Funding Acquisition: GKN, PT, HMY, LJA. All authors have read and agreed to the published version of the manuscript.

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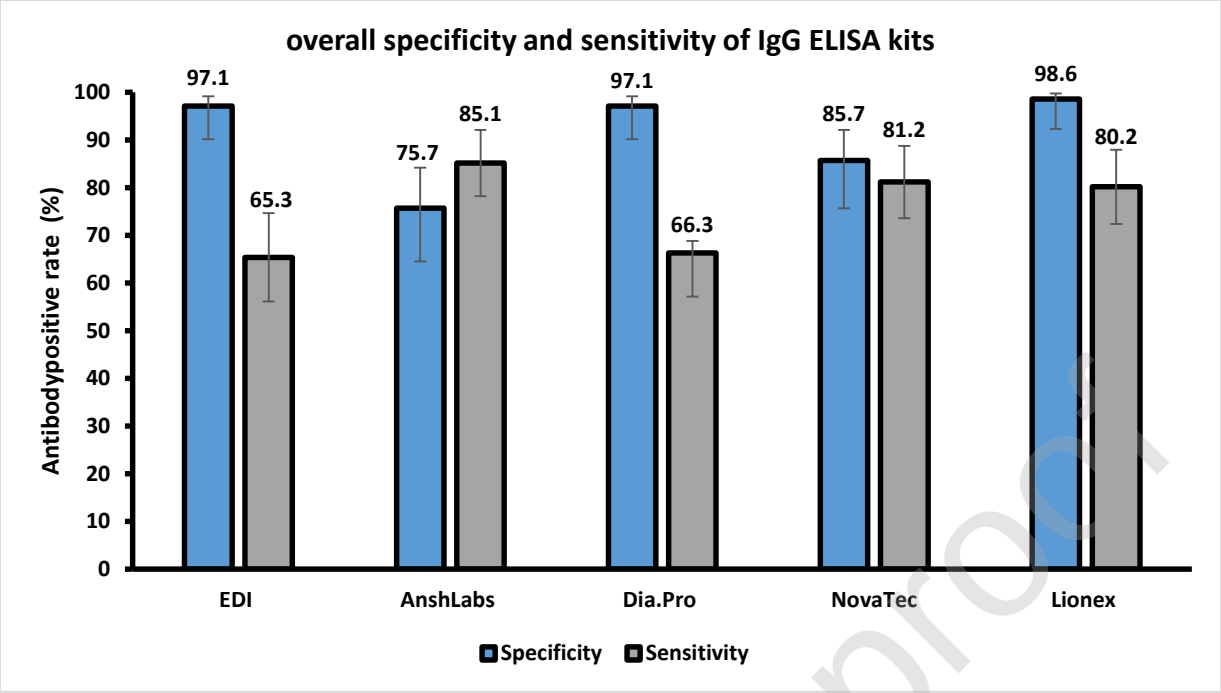

Figure 1: Comparison of overall sensitivity (n=101) and specificity (n=70) of each IgG ELISA.

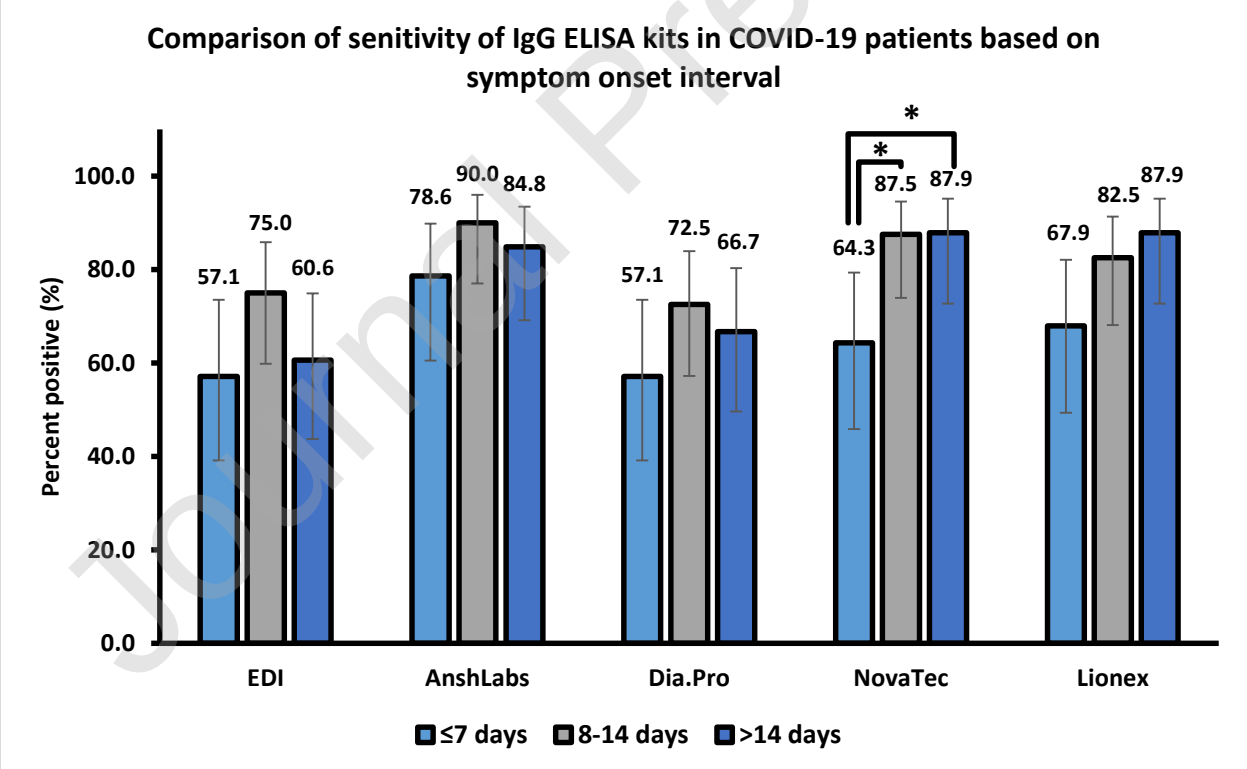

Figure 2: Proportion of samples testing positive for anti-SARS-CoV-2 IgG antibodies. Samples were stratified based on the time of collection after symptoms onset (≤7 days, n=28; 8-14 days, n=40; >14 days, n=33. (*) = $p < 0.05$.

Table 1: Demographic and clinical characteristics of COVID-19 patients.

Characteristic	All COVID-19 patients	Days between symptoms onset and sample collection			Control group
		≤7 days	8-14 days	>14 days	
N	101	28 (27.7%)	40 (39.6%)	33 (32.7%)	70
Age median (IQR)	48.0 (40.0-57.0)	50.0 (39.3-56.8)	49.0 (41.3-58.5)	46.0 (34.3-55.5)	36.0 (30.3-45.0)
Gender					
Male	76 (89.1%)	25 (89.3%)	40 (100%)	11 (33.3%)	58 (82.9%)
Female	4 (4.9%)	3 (10.7%)	-	1 (3.0%)	6 (8.6%)
N/A	21 (20.8%)	-	-	21 (63.6%)	6 (8.6%)
Sample Source					
Hospitalized, non-ICU patient	45 (44.6%)	17 (60.7%)	23 (57.5%)	5 (15.2%)	-
ICU-admitted patient	35 (34.7%)	11 (39.3%)	17 (42.5%)	7 (21.2%)	-
Recovered convalescent plasma donors	21 (20.8%)	-	-	21 (63.6%)	-

Table 2. The diagnostic assessment of the different commercial IgG ELISA according to time of sample collection after symptoms

Days after symptoms onset	ELISA Kit	Sensitivity % (95% CI)	Positive Predictive Value % (95% CI)	Negative Predictive Value % (95% CI)	Positive percent Agreement % (95% CI)	Cohen's Kappa k (95% CI)
≤7 days (n=28)	EDI	57.1 (39.1-73.5)	88.9 (67.2-96.9)	85.0 (75.6-91.2)	85.7 (77.4-91.3)	0.61 (0.49-0.72)
	AnshLabs	78.6 (60.5-89.8)	56.4 (41.0-70.7)	89.8 (79.5-95.3)	76.5 (67.2-83.8)	0.49 (0.36-0.61)
	Dia.Pro	57.1 (39.1-73.5)	88.9 (67.2-96.9)	85.0 (75.6-91.2)	85.7 (77.4-91.3)	0.61 (0.49-0.72)
	NovaTec	64.3 (45.8-79.3)	64.3 (45.8-79.3)	85.7 (75.7-92.1)	79.6 (70.6-86.4)	0.50 (0.38-0.62)
	Lionex	67.9 (49.3-82.1)	95.0 (76.4-99.1)	88.5 (79.5-93.8)	89.8 (82.2-94.4)	0.73 (0.63-0.82)
8-14 days (n=40)	EDI	75.0 (59.8-85.8)	93.8 (79.9-98.3)	87.2 (78.0-92.9)	89.1 (81.9-93.7)	0.75 (0.67-0.84)
	AnshLabs	90.0 (77.0-96.0)	67.9 (54.5-78.9)	93.0 (83.3-97.2)	80.9 (72.6-87.2)	0.61 (0.51-0.72)
	Dia.Pro	72.5 (57.2-83.9)	93.5 (79.3-98.2)	86.1 (76.8-92.1)	88.2 (80.8-93.0)	0.73 (0.64-0.82)
	NovaTec	87.5 (73.9-94.5)	77.8 (63.7-87.5)	92.3 (83.2-96.7)	86.4 (78.7-91.6)	0.71 (0.62-0.81)
	Lionex	82.5 (68.1-91.3)	97.1 (85.1-99.5)	90.8 (82.2-95.5)	92.7 (86.3-96.3)	0.84 (0.77-0.91)
>14 days (n=33)	EDI	60.6 (43.7-75.3)	90.9 (72.2-97.5)	84.0 (74.5-90.4)	85.4 (77.4-91.0)	0.63 (0.53-0.74)
	AnshLabs	84.8 (69.1-93.4)	62.2 (47.6-74.9)	91.4 (81.4-96.3)	78.6 (69.8-85.5)	0.55 (0.44-0.67)
	Dia.Pro	66.7 (49.6-80.3)	91.7 (74.2-97.7)	86.1 (76.8-92.1)	87.4 (79.6-92.5)	0.69 (0.59-0.79)
	NovaTec	87.9 (72.7-95.2)	74.4 (58.9-85.4)	93.8 (85.0-97.5)	86.4 (78.5-91.7)	0.70 (0.60-0.80)
	Lionex	87.9 (72.7-95.2)	96.7 (83.3-99.4)	94.5 (86.7-97.9)	95.1 (89.1-97.9)	0.89 (0.82-0.95)

	EDI	65.3 (56.1-74.6)	97.1 (94.5-99.6)	66.0 (58.9-73.1)	78.4 (72.2-84.5)	0.58 (0.46-0.70)
	AnshLabs	85.1 (78.2-92.1)	83.5 (77.9-89.1)	77.9 (71.7-84.2)	81.3 (75.4-87.1)	0.61 (0.49-0.73)
Overall (n=101)	Dia.Pro	66.3 (57.1-75.6)	97.1 (94.6-99.6)	66.7 (59.6-73.7)	78.9 (72.8-85.1)	0.59 (0.48-0.71)
	NovaTec	81.2 (73.6-88.8)	89.1 (84.5-93.8)	75.9 (69.5-82.4)	83.0 (77.4-88.7)	0.66 (0.54-0.77)
	Lionex	80.2 (72.4-88.0)	98.8 (97.1-100)	77.5 (71.2-83.8)	87.7 (82.8-92.6)	0.76 (0.66-0.85)

Table 3: The specificity of the five evaluated IgG ELISA kits according to the negative control subgroups (n=70).

*MERS: middle east respiratory syndrome coronavirus, SARS-CoV: severe acute respiratory syndrome coronavirus, RSV: respiratory syncytial virus, HSV-1: herpes simplex virus 1, HSV-2 herpes simplex virus 2, HHV-6: human herpesvirus-6, HHV-8: human herpesvirus-8, EBV: Epstein-Barr virus, HBV: hepatitis B virus, HCV: hepatitis C virus, HEV: hepatitis E virus, HGV: hepatitis G virus, B19: Parvovirus B19, WNV: West Nile virus, SARS-CoV-2: severe acute respiratory syndrome virus-2

		Specificity (%, 95% confidence interval)				
Subgroup with IgG/IgM antibodies against	**No. of samples**	**EDI**	**AnshLabs**	**Dia.Pro**	**NovaTec**	**Lionex**
Other coronaviruses (SARS-CoV, MERS-CoV, HCoV-229E, NL63, OC43, and HKU1)*	20	19/20 (95.0%; 76.4-99.1%)	17/20 (85.0%; 64.0-94.8%)	19/20 (95.0%; 76.4-99.1%)	17/20 (85.0%; 64.0-94.8%)	20/20 (100%; 83.9-100%)
Non-CoV respiratory viruses (Influenza and RSV) *	15	15/15 (100%; 79.6-100%)	9/15 (60.0%; 35.8-80.2%)	14/15 (93.3%; 70.2-98.8%)	11/15 (73.3%; 48.1-89.1%)	15/15 (100%; 79.6-100%)
Non-respiratory viruses (HEV, HGV, HCV, HBV, DENV, WNV, CHIKV, B19, HSV-1, HSV-2, EBV, HHV-6, and HHV-8) *	33	32/33 (97.0%; 91.1-100%)	25/33 (75.8%; 61.1-90.4%)	33/33 (100%; 93.9-100%)	30/33 (90.9%; 81.1-100%)	32/33 (97.0%; 91.1-100%)
Nuclear antigens (ANAs)	2	2/2 (100%; 34.2-100%)	1/2 (50.0%; 9.5-90.6%)	2/2 (100%; 34.2-100%)	2/2 (100%; 34.2-100%)	2/2 (100%; 34.2-100%)

Overall specificity	70	68/70 (97.1%; 90.2-99.2)	53/70 (75.7%; 64.5-84.2)	68/70 (97.1%; 90.2-99.2)	60/70 (85.7%; 75.7-92.1)	69/70 98.6%; 92.3-99.8)

Table 4. Concordance assessment between each commercial IgG ELISA kit.

Reference Test	Compared to	Overall Percent Agreement	Positive Percent Agreement	Negative Percent Agreement	Cohen's Kappa
		% (95% CI)	% (95% CI)	% (95% CI)	k (95% CI)
EDI	**AnshLabs**	79.5 (72.9-84.9)	100 (94.7-100)	66.0 (56.4-74.4)	0.60 (0.52-0.69)
	Dia.Pro	97.1 (93.3-98.8)	97.1 (89.9-99.2)	97.1 (91.8-99.0)	0.94 (0.90-0.98)
	NovaTec	86.0 (80.0-90.4)	100 (94.7-100)	76.7 (67.7-83.8)	0.72 (0.65-0.80)
	Lionex	86.0 (80.0-90.4)	92.6 (83.9-96.8)	81.6 (73.0-87.9)	0.72 (0.64-0.79)
AnshLabs	**EDI**	79.5 (72.9-84.9)	66.0 (56.4-74.4)	100 (94.7-100)	0.60 (0.52-0.69)
	Dia.Pro	78.9 (72.2-84.4)	66.0 (56.4-74.4)	98.5 (92.1-99.7)	0.59 (0.51-0.68)
	NovaTec	91.2 (86.0-94.6)	87.4 (79.6-92.5)	97.1 (89.9-99.2)	0.82 (0.76-0.88)
	Lionex	80.7 (74.1-85.9)	73.8 (64.6-81.3)	91.2 (82.1-95.9)	0.62 (0.53-0.70)
Dia.Pro	**EDI**	97.1 (93.3-98.8)	95.7 (88.0-98.5)	98.0 (93.1-99.5)	0.94 (0.90-0.98)
	AnshLabs	78.9 (72.2-84.4)	98.6 (92.2-99.7)	65.7 (56.1-74.2)	0.59 (0.51-0.68)
	NovaTec	86.5 (80.6-90.9)	100 (94.7-100)	100 (95.4-100)	0.73 (0.66-0.81)
	Lionex	85.4 (79.3-89.9)	91.2 (82.3-96.0)	77.5 (68.4-84.5)	0.71 (0.63-0.78)
NovaTec	**EDI**	86.0 (80.0-90.4)	73.9 (64.1-81.8)	100 (95.4-100)	0.72 (0.65-0.80)
	AnshLabs	91.2 (86.0-94.6)	97.8 (92.4-99.4)	83.5 (73.9-90.1)	0.82 (0.76-0.88)
	Dia.Pro	86.5 (80.6-90.9)	75.0 (65.3-82.7)	93.3 (86.1-96.9)	0.73 (0.66-0.81)
	Lionex	83.6 (77.4-88.4)	79.3 (70.0-86.4)	81.4 (72.7-87.7)	0.67 (0.60-0.75)

Lionex	**EDI**	86.0 (80.0-90.4)	76.8 (66.6-84.6)	94.4 (87.5-97.6)	0.72 (0.64-0.79)
	AnshLabs	80.7 (74.1-85.9)	92.7 (84.9-96.6)	69.7 (59.5-78.2	0.62 (0.53-0.70)
	Dia.Pro	85.4 (79.3-89.9)	76.8 (66.6-84.6)	78.7 (69.1-85.9)	0.71 (0.63-0.78)
	NovaTec	83.6 (77.4-88.4)	89.0 (80.4-94.1)	88.6 (79.8-93.9)	0.67 (0.60-0.75)