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Abstract

Comparative validation data and clinical performance data are essential for the reliable interpretation of SARS-CoV-2 antibody test results. This study aimed to assess the performance of six SARS-CoV-2 IgG immunoassays in different disease severity settings. Four automated chemiluminescence immunoassays Access (Beckman Coulter), Architect (Abbott), Atellica-IM (Siemens) and Elecsys (Roche) and two ELISA assays (SARS-CoV-2 IgG-S1-based and NCP IgG, Euroimmun) were evaluated in 143 patients and 50 pre-pandemic control sera. Accuracy and precision tests were performed for validation. Overall sensitivity differed between 73.38-88.65%, being higher in spike protein-based assays. Specificity was $\geq$ 98% in all immunoassays. IgG response was lower for the samples taken <20 days post-symptom onset (87.30%) than for the samples taken $\geq$20 days post-symptom onset (94.80%). Higher rate of antibody was detected in the clinically moderate disease group. In the asymptomatic and mild group more antibody positivity was detected with spike protein-based assays. Clinical performance of the immunoassays differs according to disease severity and antigen targeted; moderate disease leading to highest rate of IgG response. All the assays tested were eligible for the detection of SARS-CoV-2 IgG however, spike-based assays revealed relatively higher sensitivity than the nucleoprotein-based assays particularly in the asymptomatic and mild disease severity.

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Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) which has emerged in late 2019, has become a pandemic since 2020. The detection of viral RNA by real-time reverse transcriptase polymerase chain reaction (RT-PCR) is considered as the gold standard for diagnosis and screening (1, 2).

Owing to the nature of the pandemic, several manufacturers have rapidly designed assays to detect specific IgM, IgG or total antibodies, with limited validation procedures. The sensitivity, specificity and accuracy of the assays differ according to the test method which are categorized as lateral flow immunoassays (LFA), enzyme linked immunosorbent assays (ELISA) and chemiluminescent immunoassays (CLIA). The sensitivity and specificity data also vary according to the immunoglobulin class detected (IgM, IgG, or both), the SARS-CoV-2 antigen used in the assay (spike/S, nucleoprotein/NP) and the timing of sample collection following a positive RT-PCR and/or development of specific symptoms (3-7). There is still limited data related to the accuracy and clinical evaluation of the results of the SARS-CoV-2 immunoassays particularly in relation to disease severity and also in RT-PCR negative patients with typical symptoms and chest CT findings. Comparative assessment of different high throughput immunoassays will provide valuable validation data which will guide the interpretation of the antibody test results.

This study was conducted under the guidance of Society for the Clinical Microbiologists of Turkey to evaluate the performance characteristics of different SARS-CoV-2 antibody assays in the light of variable parameters taken into account as determinants of diagnostic accuracy, with regard to disease severity.

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Methods

Study design: This prospective study was approved by the Standing Committee on Ethics of Scientific Research of Alaaddin Keykubat University, Turkey (14/08/2020; 22-34) and permission was taken from the Scientific Research Council of Ministry of Health of Republic of Turkey (Permission no: 2020-07-28T11_45_09).

Serum samples were obtained from SARS-CoV-2 infected patients admitted to three university hospitals (Hacettepe, Ankara, Ege universities) and Ankara Bilkent City Hospital between 24 June-7 November 2020. The patient group consisted of 143 patients with positive RT-PCR and/or typical COVID-19 symptoms and/or chest CT findings. RT-PCR was performed by Bio-Speedy® SARS-CoV-2 N RT-qPCR (Bioeksen, Turkey) which is a one-step RT-PCR assay for the qualitative detection of SARS-CoV-2.

A subset of 50 serum samples obtained in 2019 from anti-CMV, anti-HSV1 and anti-EBNA IgG positive patients and stored at -80^{o}C, were used as prepandemic control samples. .

All the serum samples of the patients were aliquoted and stored at -80^{o}C until assayed.

Clinical data: For the 143 COVID-19 patients the basic demographic information, the dates of symptom onset, RT-PCR positivity, chest CT findings, date of serum collection were recorded to a standardized questionnaire by the related infectious diseases physician. Informed written consent was obtained from each patient prior to sample collection and analysis.

Instrumentation and immunoassays: All samples were analyzed by the six immunoassays, characteristics of which were given in Table 1.

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Abbott, Beckman Coulter and Euroimmun assays were performed at Hacettepe University Hospital; Roche at Ankara University Hospital and Siemens at Bilkent City Hospital according to the instructions of the manufacturers.

Validation tests: Accuracy was tested as sensitivity, specificity, positive and negative predictive value (PPV, NPV). Sensitivity was assessed by 1)Inclusion of the only RT-PCR positive individuals, 2)Inclusion of patients either with positive RT-PCR and/or positive chest CT/typical symptoms. Specificity was assessed using the pre-pandemic samples. Performance analysis was performed in terms of serum sampling time and disease severity.

Precision studies were performed by analyzing intra-assay and inter-assay reproducibility. For intra-assay reproducibility five serum samples were analyzed as three replicates on one day. Inter-assay reproducibility was evaluated in duplicate over four sequential days for each assay except for Euroimmun assays which were assessed on three days owing to the limited availability of the reagents.

Statistical analysis: All statistical analysis were performed using SPSS 23.0 version (IBM Corporation, Armonk, NY, United States). PPV and NPV with the 95% confidence intervals (CI) were calculated based on an assumed prevalence of 10%, 15% and 20%.

Concordance between the assays was evaluated using Cohen's Kappa and percent agreement. Differences between groups were calculated by Mann-Whitney U, Fisher's Exact and Chi-square test, as appropriate.

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Compliance of numerical data to normal distribution was evaluated by histogram and Kolmogorov-Smirnov test. To evaluate the precision, coefficient of variation (CV) was calculated for intra-assay and inter-assay reproducibility test results.

Results

Patient characteristics: Patients were grouped according to their RT-PCR results and clinical symptoms. Typical symptoms like anosmia and/or fever and/or dyspnea in addition to typical chest CT findings were considered for clinical suspicion. Characteristics of the patients and distribution of the antibody results according to the groups were presented in Figure 1. The majority of the cases were female (%52.4; n:75) and the median age was 43 years (range: 20-87 years; interquartile range (IQR):19).

Patients were further classified as asymptomatic (n:11), mild (n:97), moderate (n:24) or severe (n:11) disease groups according to the WHO classification (1).

Accuracy: The total rate of antibody positivity in the study group was 131/143 (91.60%) when at least one positive immunoassay result was taken into account. Twelve patients were antibody negative in all assays. Abbott assay was tested in 139, Beckman Coulter in 135, Euroimmun S and NCP in 138, Roche in 142 and Siemens in 141 patients.

The median age of the antibody positive patients at least with one test (median 42 years, IQR: 20) was significantly lower than the patients (median 52 years, IQR: 11) with negative antibody (p=0.034).

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Sensitivity ranged between 73.64 to 90.08 for the 133 RT-PCR positive patients, (Table 2). Rates of antibody positivity were 95/129 for Abbott, 112/125 for Beckman, 108/128 for Euroimmun S-Ag, 98/128 for Euroimmun NCP-Ag, 105/132 for Roche and 118/131 for Siemens. The low range of variation in sensitivity between the six immunoassays was accompanied by an overall agreement of 67.17%. The overall percentage agreement between the S-based assays was 83.93 and N-based assays was 75.13 (95% CI) (Table 3).

When the data of RT-PCR negative cases were included, no change was detected for the sensitivity ranking of the assays. Sensitivity (95%CI) was 88.15% for Beckman, 83.33% for Euroimmun S, 88.65% for Siemens; 73.38% for Abbott, 76.09% for Euroimmun NCP, 78.87% for Roche.

Ten RT-PCR negative cases had typical COVID-19 symptoms and all except one who had no chest CT, had typical chest CT findings. Seven of the RT-PCR negative cases were antibody positive by all assays (Table 4). Among the 12 patients who were antibody negative by all six immunoassays, serum samples were obtained on the 15. post-symptomatic day and three were also RT-PCR negative with symptoms and positive chest CT findings. The remaining nine antibody negative patients had mild disease and the median serum sampling time was 21.75 days (range: 15-49 days) post-symptom. Among the 12 patients who were antibody negative by all six immunoassays, three were also RT-PCR negative with symptoms and positive chest CT findings and their serum samples were obtained on the 15. post-symptomatic day. The remaining nine antibody negative, RT-PCR positive patients had mild disease and the median serum sampling time was 21.75 days (range: 15-49 days) post-symptom.

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The overall specificity of the six immunoassays was 98.00-100.00%. Both Euroimmun assays detected one CMV IgG positive serum as false positive.

Analysis according to the sampling time: Overall evaluation revealed that antibody positivity was lower (87.30%) for the samples obtained in <20 days post-symptom onset than for those in ≥20 days post-symptom onset (94.80%) (p=0.1137). No significant difference was obtained for antibody positivity (81% in <20 days versus 88.3% in ≥20 days) between NP antigen-based assays and S antigen-based assays (84.1% in <20 days versus 93.5% in ≥20 days) when sampling time was considered (p=0.225 and p=0.100, respectively), although the difference was more pronounced for S antigen-based assays.

Analysis according to the disease severity: The highest rate of antibody positivity was found in the moderate group, followed by mild, severe and asymptomatic group, orderly. In the asymptomatic and mild group more antibody positivity was detected with S antigen-based assays. Higher rate of antibody was detected in the moderate group than the other clinical groups by any of the assays or S or N antigen-based assays (95.8%) (p values 0.002, 0.004, and 0.018, respectively) (Fig.2).

Precision study: Intra-assay %CV differed between 0.48 to 5.20 and inter-assay %CV between 0.76 to 10.24 for the set of positive samples. The categorical agreement of the qualitative results was 100%.

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Discussion

Immunoassays targeting the detection of specific antibodies to SARS-CoV-2, aid to evaluate the immune response, besides supplementing diagnosis in asymptomatic patients and also in RT-PCR negative cases. Each country and diagnostic laboratory have their own prerequisites and risks of shortages for the implementation of antibody tests during COVID-19 pandemic. This study was conducted under the guidance of *Society for the Clinical Microbiologists of Turkey*, to meet the information needs on clinical performance and validation data for SARS-CoV-2 immunoassays available in our country.

In the present study we evaluated the performance of four automated CLIA and two ELISA SARS-CoV-2 immunoassays. Mean sensitivity ranged between 73.64 to 90.08%. The manufacturers of the included assays declared sensitivity ranging from 90 to 100% for the samples taken at least 14 days post-symptom onset and specificity ranging from 99.6 to 100% (3). Sensitivity of the assays differed in a wide range according to the clinical status of the patients, the sampling time and the target antigen used. All the samples collected in this prospective study were taken at least 14 days post-symptom onset or positive RT-PCR. The relatively lower sensitivity determined in the present study may be partly due to the patient characteristics, lack of antibody response or disease severity. Lower sensitivity might also be attributed to the high number of patients with mild disease, which probably yielded a moderate antibody response. Literature analysis revealed sensitivity results for Abbott assay between 77.8-100.00%; Beckman Coulter assay between 76.5-81.5, Euroimmun S IgG between 70.7-93.8, Euroimmun NCP IgG between 88.89-100%, Roche assay between 75.60-97.20% and Siemens assay between 85.9-98.1% (5-13).

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Although RT-PCR is the gold standard for the definite diagnosis of SARS-CoV-2 infection, we also analyzed the data of a small group of RT-PCR negative patients with typical symptoms and/or positive chest CT. It has been previously documented that RT-PCR may yield false-negative results and additional tools such as chest CT and/or X-ray may aid diagnosis in such cases (14-16). As a matter of fact antibody response was detected in seven of the 10 RT-PCR negative patients, indicating past infection.

IDSA and ECDC recommend that SARS-CoV-2 antibody tests may help the diagnosis of RT-PCR negative, COVID-19 symptomatic patients (2, 17). Antibody assays may support RT-PCR in the diagnosis of SARS CoV-2 infections, especially when patients present at a late stage of COVID-19 where it is usually hard to get positive nasopharyngeal swabs. In a cohort study performed in Shenzen, China RT-PCR negative contacts of SARS-CoV-2 RT-PCR confirmed cases were tested for specific antibodies and it was found that 4.5% of 880 RT-PCR-negative close contacts were positive on total antibody ELISA (18). Long QX et al had reported that serial antibody testing may be helpful to diagnose RT-PCR negative COVID-19 suspected patients (19). Although the sample size is small, the findings in the RT-PCR negative group in this study provided evidence on the importance of antibody testing as an additional tool for the definite diagnosis of RT-PCR negative individuals. There is still lack of data related to the rate of seropositivity in RT-PCR negative individuals, however, large scale seroepidemiological studies may help to figure out the exact number of people with contact to SARS-CoV-2.

The sensitivity of the immunoassays also varies according to the SARS-CoV-2 antigen targeted. Higher sensitivity was detected for the assays targeting spike antigens in this study. The sensitivity variation was not pronounced within the group of assays using the

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same antigen as the antibody target. While several other studies revealed higher sensitivity rates for the spike antigen-based assays (10, 20, 21), data from other studies indicate higher sensitivity for NP antigen-based assays (7, 9). Turbett et al reported that the two nucleocapsid antibody tests (Abbott and Roche) were more sensitive than the spike protein antibody test (Diasorin) overall with pronounced differences observed using samples collected 7 to 14 days after symptom onset (15). However, these three assays had comparable sensitivity using samples collected >14 days post-symptom onset (22). This is in accordance with our results since we only included samples after 14 days symptom onset. Andrey et al reported that S-assays tended to display slightly better sensitivity and NPV when they compared Roche S and Roche N (21). These discrepancies are not surprising since there is a large diversity between the serologic assay comparisons in terms of patient selection, age, severity of disease, type of antibodies detected, chemical structure of the antigen targeted and the sampling time. It is not surprising to observe higher sensitivity with immunoassays detecting total antibody in samples obtained <14 days, since IgM type of antibodies are also detected. This was also observed in our study with the Siemens assay which detected total antibodies targeting S antigen.

The concordance between the assays was highest for Siemens and Beckman Coulter which targeted S antigen. In the second rank were Abbott and Euroimmun NCP, targeting NP antigen. This result supported the approach that the performance of the immunoassays should be evaluated according to the type of antigen targeted.

Our results indicated that all of the assays achieved high specificity as declared by the manufacturers. High specificity results about SARS-CoV-2 antibody assays were also reported by several other study groups (5, 6). In the setting of COVID-19 high specificity is

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more important than high sensitivity since anti-SARS-CoV-2 antibody tests are not intended to be used for screening and a false positive result might lead to a false sense of security. High specificity is essential to achieve a high PPV. Although sensitivity is important for test performance, PPV and NPV are more useful to estimate the probability of the disease and interpret the test result. Sensitivity, specificity and the prevalence of COVID-19 in a specific population have important impact in determining the validity of a test result. PPV generated for an assumed 10%, 15% and 20% prevalence in the current study, respectively, revealed that PPV was highest for Siemens, followed by Beckman Coulter, Euroimmun S, Roche and Euroimmun NCP. In a multi-center comparison of seven immunoassays Oved et al reported that narrow differences in the specificity of the assays had profound effect on PPV in case of low prevalence scenarios (8). With the progression of the pandemic and increasing number of vaccinations, higher prevalence settings will become likely. The NPV determined for 20% prevalence in this study showed that any of the tests evaluated could be reliably considered in high prevalence setting.

Timing of sample collection in relation to the onset of specific symptoms affects the positivity rate of SARS-CoV-2 specific antibodies (6, 9). The rate of antibody positivity was relatively low (87.30%) for <20 days post-symptom onset samples than for ≥20 days (94.80%) samples. In agreement with the literature the current study revealed that sensitivity increases in line with the time-dependent production of specific antibodies (5,10). To reach maximum sensitivity it would be ideal to test IgG after 20 days of infection.

There is still limited data related to the effect of disease severity on the rate of antibody production. It was observed in the current study that the lowest antibody response was in the asymptomatic group. Although the sample size was small, this result was in accordance

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to the previous results (5). It is important to notice that Sbased assays detected significantly less antibody than NP based assays in the severe group ($p<0.005$). This finding might explain the development of severe disease due to the lack of neutralizing anti-S antibodies, while N antibodies are still produced. This should be further analyzed to reach a conclusion about the relationship between disease severity and the type of antibody developed. Therefore, severity of COVID-19 should also be taken into account for the interpretation of seroprevalence data and disease outcome data emerging nowadays.

The higher rate of antibody positivity in a relatively younger age group detected in the current study might be attributed to the failure of humoral immune response to new antigens in an aging immune system. In their comprehensive review Bajaj et al relates the relatively decreased antibody response to SARS-CoV-2 to the limited capability of binding to a new antigen in the elderly, leading to the production of lower affinity antibodies in a longer time (23).

Antibody tests were detected negative by all the assays in nine RT-PCR positive patients. This may be attributed to the antibody test performances, early stage infection or mild disease in this group. However, early-stage infection was not the case in our study since all the samples were obtained at least 15 days post-symptom or RT-PCR positivity. Although the rate of antibody negative samples was higher in N antigen based assays than S antigen based ones, the difference was not significant. This is in accordance with some of the previous studies (24-26) indicating that differences in sensitivity among the immunoassays during acute SARS-CoV-2 infection are only partly associated to the type of target antigen. The observation of negative antibody result in RT-PCR proven cases once again proved that negative antibody result does not rule out previous COVID-19 infection.

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Strengths of this study is the head-to-head comparison of identical well-characterized samples to allow unbiased comparison of the performance of commonly used SARS-CoV-2 immunoassays.

Our data revealed that mild course of disease usually leads to the development of a moderate IgG response while asymptomatic disease is related to a weak immune response. Clinical performance of different SARS-CoV-2 immunoassays is influenced by disease severity and the target antigen of the immunoassays. There is still a need for evidence on the performance of antibody tests since there are risks of heterogeneity regarding the population included, the severity of infection, serum sampling time and the target antigen of the assay. These findings emphasize the importance of validation in the real life setting and draws attention to the understanding of antibody test limitations for the appropriate use and interpretation of serological tests.

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Conflict of Interest

The authors declare no conflict of interest.

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Table 1. Characteristics of the immunoassays evaluated.

	Architect SARS-CoV-2	Access Anti-SARS-CoV-2	Atellica-IM SARS-CoV-2	Elecsys Anti-SARS-CoV-2	Euroimmun SARS-CoV-2	Euroimmun SARS-CoV-2 NCP
Antibody type	IgG	IgG	Total	Total	IgG	IgG
Target antigen	NP	S1-RBD	S1-RBD	NP	S1	NP
Assay principle	CMIA	CMIA	CMIA	ECLIA	ELISA	ELISA
Result calculation	Index (S/Co)	Index (S/Co)		Index (S/Co)	Index (S/Co)	Index (S/Co)
Manufacturer	Abbott Diagnostics	Beckman Coulter	Siemens Healthcare	Roche Diagnostics	Euroimmun	Euroimmun

NP: Nucleoprotein; S: Spike protein; RBD: Receptor Binding Domain; CMIA: Chemiluminescent microparticle immunoassay; ECLIA: Electro chemiluminescent immunoassay; S/Co: Signal to cut-off ratio

Table 2. Performance characteristics of six SARS-CoV-2 immunoassays with the data of 133 RT-PCR positive patients.

Immunoassay		% Sensitivity (95% CI)	% Specificity (95% CI)	PPV (95% CI)			NPV (95% CI)		
				10%*	15%*	20%*	10%*	15%*	20%*
S-based assays	Beckman Coulter (IgG)	89.60 (82.87-94.35%)	100.00 (92.89-100.00%)	100.00	100.00	100.00	98.86 (98.10-99.31%)	98.20 (97.02-98.91%)	97.47 (95.83-98.47%)
	Euroimmun S (IgG)	84.38 (76.91-90.19%)	98.00 (89.35-99.95%)	82.42 (40.21-97.03%)	88.16 (51.64-98.11%)	91.34 (60.21-98.66%)	98.26 (97.41-98.33%)	97.26 (95.95-98.16%)	96.17 (94.36-97.41%)
	Siemens (Total)	90.08 (83.63-94.61%)	100 (92.89-100.00%)	100.00	100.00	100.00	98.91 (98.19-99.35%)	98.28 (97.15-98.97%)	97.58 (96.01-98.54%)
NP-based assays	Abbott (IgG)	73.64 (65.16-81.01%)	100.00 (92.89-100.00%)	100.00	100.00	100.00	97.15 (96.24-97.85%)	95.56 (94.16-96.63%)	93.82 (91.92-95.29%)
	Euroimmun NCP (IgG)	76.56 (68.26-83.59%)	98.00 (89.35-99.95%)	80.96 (37.87-96.74%)	87.11 (49.19-97.92%)	90.54 (57.83-98.52%)	97.41 (96.48-98.10%)	95.95 (94.53-97.01%)	94.36 (92.42-95.82%)
	Roche (Total)	79.55 (71.65-86.07%)	100.00 (92.89-100.00%)	100.00	100.00	100.00	97.78 (96.92-98.40%)	96.52 (95.19-97.49%)	95.14 (93.32-96.48%)

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Table 3. Percentage agreement between the six immunoassays evaluated (for the 143 samples tested for sensitivity)

% Agreement (95% CI)	Beckman Coulter	Euroimmun S	Euroimmun NCP	Roche	Siemens
Abbott	56.6%	62.8%	85%	74%	50.5%
Beckman Coulter		80.4%	55.9%	57.7%	88.1%
Euroimmun S			57.9%	70.8%	83.3.%
Euroimmun NCP				66.4%	55.7%
Roche					62.5%

Table 4. The reactivity results of the RT-PCR negative patients.

Patient	Abbott	Beckman	Euroimmun		Roche	Siemens
			S	NCP		
S1	7.84	19.24	9.5	5.3	26.9	>10.00
S2	5.69	37.38	10.9	3.4	1.06	>10.00
S3	8.03	62.20	10.5	5	54.27	>10.00
S4	4.08	26.02	6.6	4.4	18.92	>10.00
S5	8.17	42.45	10.5	6.2	37.01	>10.00
S6	7.29	45.65	9	5.7	31.51	>10.00
S7	5.90	2.75	2.2	3.3	15.84	3.93
S8*	0.02	0.02	0.1	0.02	0.096	0.10
S9*	0.04	0.05	0.5	0.03	0.081	0.59
S10*	0.05	0.03	0.1	0.08	0.086	0.55

*: Negative result

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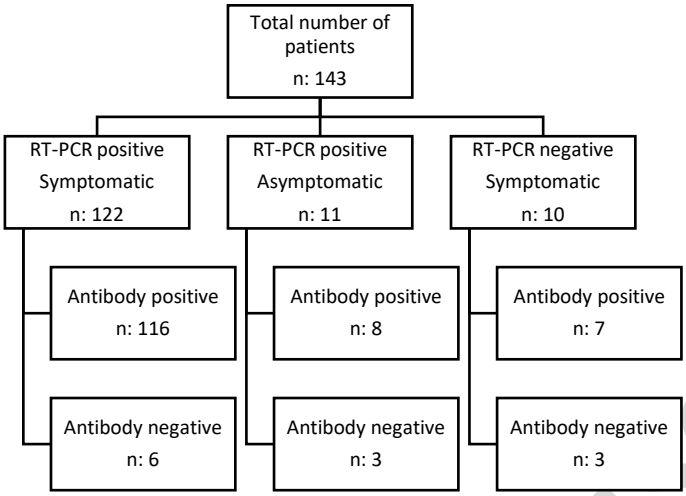
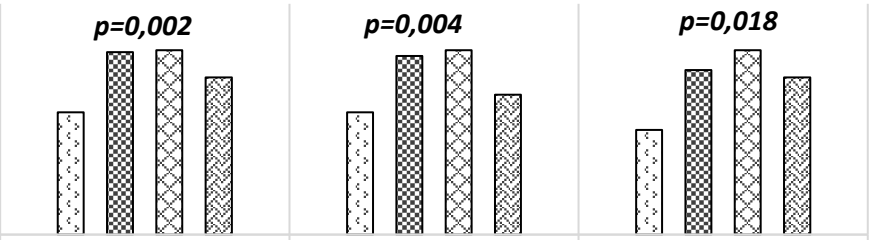


Fig.1. Characteristics of the patients and distribution of the antibody results according to the patient groups.

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	Any test	S-based assay	NP-based assay
Asymptomatic	63.6	63.6	54.5
Mild	94.8	92.8	85.6
Moderate	95.8	95.8	95.8
Severe	81.8	72.8	81.8

Fig. 2. Antibody positivity rates of S and NP antigen based assays according to the disease severity.

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