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Clinic Evaluation of The Destrovir Spray Effectiveness in SARS-CoV-2 Disease

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ABSTRACT

In late 2019 on 11th March 2020 a novel coronavirus, SARS-CoV-2 causing Coronavirus disease 2019 (COVID-19) appeared in Wuhan, China and World Health Organisation declared it to have developed pandemic status. Although there are many detection techniques for the disease to control the pandemic such as RT-PCR, serological methods, or fast antigen tests, the actual problem is the prevention of the disease. The nasal cavity and rhino pharynx are key sites of the initial replication of SARS-CoV-2. In this article, the effectiveness of destrovir spray was investigated by creating a mechanical barrier in the nasal and oral mucosa, which are the entry points of the virus into the body, and to destroy the virus that comes into contact with this barrier. In the presented study, quantitation cycle (Cq) and irradiation values (RFU) of 12 different high-positive patients (Cq≤25) after application of both RT-PCR and destrovir spray were determined at different concentrations (10%, 20%, 30%, 40%, and 50%) aimed to evaluate its effectiveness. According to the results obtained by comparing the effectiveness of disinfectant with 70% ethyl alcohol on the 46 patient samples with Cq<20, 46 patient samples with Cq values between 20-25, and 46 patient samples with Cq≥25 including different variants are examined. A total of 138 COVID-19 patient samples were treated with 5% and 10% ratios of destrovir spray. Its effectiveness on Cq values and RFU was evaluated and it was aimed to compare the effectiveness of this evaluation with 96% ethyl alcohol used in the same samples.

Keywords: SARS-CoV-2, COVID-19, nasal spray, destrovir, ethyl alcohol, disinfectant

INTRODUCTION

In December 2019, an emerging of a mysterious infectious outbreak, which was reported as pneumonia of unknown cause, appeared in Wuhan city, Hubei Province, China which called SARS-CoV-2 (genus Betacoronavirus, subgenus Sarbecoronavirus). Although there are many airborne viruses including influenza-, rhino-, adreno-, entero-, and coronavirus, the newly emerged type of the SAR-CoV-2 receives much attention due to its devastating impact within the pandemic [1]. Globally, 10 November 2021, there have been 250,715,502 confirmed cases of COVID-19, including 5,062,106 deaths, reported to WHO. As of 7 November 2021, a total of 7,084,922,999 vaccine doses have been administered [2]. Additionally, disease is called COVID-19. The SARS-CoV-2 is a positively stranded enveloped RNA virus with a fragile outer membrane that is the type of beta coronavirus and only affected on mammalian. Its structural form composes of the 30 kb genome with 14 open reading frames encoded to the spike protein (S), nucleocapsid protein (N), a small membrane protein (SM), and membrane glycoprotein (M) with an additional membrane glycoprotein (HE) [3]. The most important part is the spike protein (S) which is integrating part of the virus to the host receptor (ACE2). S protein also has two subunits as first an amino-terminal subunit (S1) and a carboxyl-terminal subunit (S2) by host furin-like proteases. Moreover, the C-terminal of the S1 subunit (S1 CTD) consists of the receptor-binding domain (RBD) which takes the critical role in recognizing and binding the host receptor as shown in **Figue 1**. Moreover, S protein also takes an important role in recognizing host range and tissue tropism, alongside being responsible for inducing many of the host immune responses [4,5]. On the other hand, ACE2 receptor plays a crucial role in regulating oxygen/carbon dioxide transfer, commonly found within the respiratory epithelia. Especially SARS-CoV-2 has been targeted the ciliated and goblet cells where the spreading of the viral loads is happened, within the upper respiratory tract [6]. The symptoms of the disease are the lower respiratory tract such as fever, cough, dyspnea, and chest tight-ness. On the other hand, upper respiratory symptoms like sore throat, nasal congestion, rhinorrhea, and olfactory dysfunction can be observed [7]. The loss of smell is the other symptom of the COVID-19 and it can be the presenting symptom before others (coughing, fever, and dyspnea). Thus, sudden onset loss of smell should be considered to be COVID-19 positive [8].

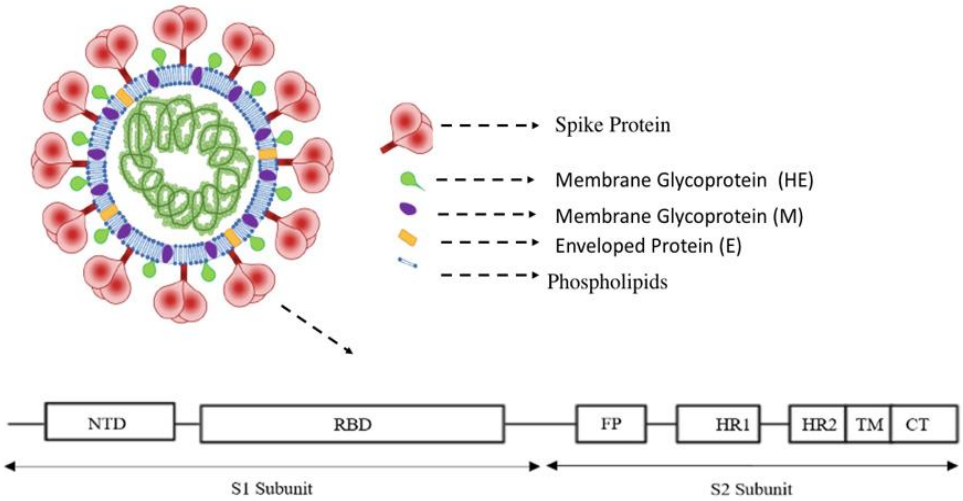

Figure 1. General structure of SARS-CoV-2

Generally, inhaled air is firstly routed through the nose. On average ≈10,000 L of air are inhaled by a healthy human per day although the nasal cavity presents the highest resistance to airflow. For this result of the resistance, the nasal cavity shows the two significant roles; first air conditioning, creating the correct levels of humidity and air temperature, second removal of foreign particles including dust, airborne droplets, and pathogens [9]. The major challenge of the COVID-19 is that the diagnosis of the disease. Saliva is the actual source for the RT-PCR tests that contains a high viral load in COVID-19 with up to 1.2×10^8 infective copies/ml. The recent studies showed that the nasopharynx appears to have a higher viral load than that found in the oropharynx in RT-PCR tests [6]. In literature, several advances within the nasal spray field have been given. Many of these attempts can be crudely categorized into two main areas: active targeting of the virus (e.g., products such as SaNOtize) and passively protecting the mucosa from viral uptake (e.g., Taffix and Vicks First Defence). Although the first one is expensive, it prefers in SARS-CoV-2 treatment studies [10]. Thus, we though that reduction of nasal viral titres is at least as much importance as in the oral cavity/oropharynx [11]. In this study aimed that destrovir nasal spray can be used for routine use during the care of COVID-19 patients, particularly before any procedure that involves the upper aerodigestive tract, including intubation, nasal and oral procedures, endoscopy and bronchoscopy. Addtionally we suggest that it should be used in daily life without having any COVID-19 disease to make barrier to the disease.

MATERIAL AND METHOD

Sample Collection, Transportation, and Storage

Nasopharyngeal swabs of SARS-CoV-2 patients were collected by trained personnel and transferred to Kanuni Sultan Suleyman Training and Research Hospital in a VTM solution tube. Patient's swap samples were transferred to our COVID-19 Diagnostic Center within 1h. Samples are detected by IAGNOVITAL DIAGNO5plex NS SARS-CoV-2 Real Time PCR kit on Biorad CFX96 platform and all Cq and RFU results values were evaluated. All swab samples were stored in VTM solution in test tubes.

Spray Treatment

Destrovir spray 100/20ul (10% spray), 100/40ul (20% spray), 100/60ul (30% spray), 100/80ul (40% spray), 100/100ul (%) on 12 COVID-19 positive patients 50 sprays were treated sequentially and vortexed for 10 seconds. Each concentration was placed in order A to G in a 96 well plate. In the G series, the patient samples were treated with 70% ethyl alcohol at a ratio of 1/1 and vortexed for 10 seconds. Sorting available in well plate; A:100:100 Sample+100:0 Spray, B:100:100 Sample+100:20 Spray, C:100:100 Sample+100:40 Spray, D:100:100 Sample+100:60 Spray, E:100:100 Sample+100:80 Spray, F:100:100 Sample+100:100 Spray, G:100:100 Sample+100:100 Alcohol (70% ethyl alcohol). The results obtained were recorded as the results of the 1st hour. The samples placed in the well plate prepared for the experiment on the same day were stored under 2 different conditions at +4°C and -20°C for 14 days. At the end of 14 days, the samples were reworked by providing the same conditions.

According to the results obtained after first study, a total of 138 COVID-19 patient samples were taken by 46 patient samples Cq<20, 46 patient samples Cq between 20-25, and 46 patient samples Cq≥25 also each of the Cq group were treated with 100/5ul (5% spray), 100/10ul (10% spray), and 100/100ul ethyl alcohol (96%) samples, respectively. Then, a 96-well plate was prepared and all samples including negative-positive controls were carefully placed. To the rows on the 96-well plate; A:100:100 Sample+100:0 Spray, B:100:100 Sample+100:0 Spray, C:100:100 Sample+100:5 Spray, D:100:100 Sample+100:5 Spray, E:100:100 Sample+100:10 Spray, F:100:100 Sample+100:10 Spray, G:100:100 Sample+100:100 Alcohol (where 96% ethyl alcohol is used in 1/1 ratio, H:100:100 Sample+100:100 Alcohol (96% ethyl alcohol was used in 1/1 ratio) was placed. All the results obtained were re-examined and recorded in the following time zones under the same conditions: in the range of 0-1 hours, 1-72 hours and 72-120 hours. The samples placed in the wellplate prepared for the experiment on the first day were stored at +4 °C throughout the study period.

RT-PCR Tests

The extra RNA extraction step is not required because of the VTM solution usage with nucleic acid extraction property. Proper vigorous vortexing is enough for RNA extraction step.

The IAGNOVITAL DIAGNO5plex NS SARS-CoV-2 Real Time PCR kit is utilized. The primers of the kit were designed based on the conserved regions of ORF1ab and RNaseP genes of SARS-CoV-2. 6-carboxy- fluorescein (FAM) and phosphoramidite (HEX) channels were used for ORFlab and RNaseP gene, respectively. Additionally, with this kit, mutations can be detected in the tetramethylindo(di)-carbocyanines (Cy5 and Cy5.5) and carboxyrhodamine (ROX) channel information; however, in this study only positivity was examined. According to the kit protocol, 2.5 µl patient samples with VTM were added to a 7.5 µl ready kit mixture to achieve 20 µl PCR mixture in total. Thermal cycle parameters of RT-PCR amplification were as follows: 52°C for 5 min for reverse transcription, 95°C for 20 s for holding, then 40 cycles of 95°C for 1 s and 60°C for 1 s for denaturation, annealing, and extension, respectively.

Test Interpretation

In the Biorad CFX96 platform, threshold was arranged as 200 according to the kit protocol. The positive results of SARS-CoV-2 made sense as sigmoids with Cq values below 36 for FAM channel irrespective of HEX values. Nonsigmoidal signals and sigmoidal signals with Cq values above 36 in the FAM channel and sigmoidal signals with Cq values below 36 in the HEX channel were interpreted as negative based on the kit protocol. Nonsigmoidal signals and sigmoids below 36 Cq on both FAM and HEX channels were interpreted as an invalid result. The test also targets a conserved region of SARS- CoV-2 Rnase P as an internal control in HEX channel.

Statistical Analyses

NCSS (Number Cruncher Statistical System) program was used for statistical analysis. Descriptive statistical methods (mean, standard deviation, median, frequency, percentage, minimum, and maximum) were used while evaluating the data. The conformity of the quantitative data to the normal distribution was tested with the Shapiro-Wilk test and graphical examinations. The Mann-Whitney U test was used for comparisons between two groups of quantitative variables that did not show normal distribution. One-way analysis of variance and binary evaluations with Bonferroni correction were used for comparisons between groups of more than two normally distributed quantitative variables. Kruskal-Wallis test and Dunn-Bonferroni test were used for comparisons between groups of more than two quantitative variables that did not show normal distribution. Dependent groups t-test was used for within-group comparisons of normally distributed quantitative variables. Wilcoxon signed-ranks test was used for in-group comparisons of quantitative variables that did not show normal distribution. Statistical significance was accepted as $p<0.05$.

Statement of Ethics

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. This study protocol was reviewed and approved by Ethics Committee of Kanuni Sultan Suleyman Training and Research Hospital No: 2021.10.264, Subject No: KAEK/2021.10.264 Date: 19.10.2021 – 14:20 – E-80929729-000-18087 and Republic of Turkey, Ministry of Health, COVID-19 Scientific Research Studies Approval No: YakupArtik-2021-08-26T01_46_44.

RESULTS

Findings Obtained in the Preliminary Study

The sample Cq measurement values of the cases participating in the study on the first day were found to be statistically significantly lower than the sample measurements stored at +4°C ($p=0.001$; $p<0.01$) and -20°C ($p=0.001$; $p<0.01$) on the 14th day. At the same time, when these two different degrees were compared, no statistically significant difference was obtained ($p>0.05$). In addition, 10% destovir sprayed Cq values of the samples were found to be statistically significantly lower than the measurements stored at +4°C on the 14th day ($p=0.001$; $p<0.01$). No statistically significant difference was described between the Cq measurements sprayed with 10% destovir spray on the first day and the measurements stored at -20°C and +4°C ($p>0.05$). On the other hand, the Cq measurement samples, which were sprayed with 20% destovir spray on the first day, were found to be statistically significantly lower than the measurements stored at +4°C and -20°C on the 14th day ($p=0.030$; $p<0.05$). When these two temperatures were compared among themselves, no statistically significant difference was found between Cq measurements ($p>0.05$). Same situation is obtained as 30% ($p=0.009$; $p<0.01$), 40% ($p=0.013$; $p<0.05$), and 50% ($p=0.005$; $p<0.01$) destovir sprayed Cq measurement values ($p>0.05$). From another point of view, the Cq values of samples mixed with 70% ethyl alcohol on the first were found to be statistically significantly lower than the Cq measurement stored at +4°C ($p=0.006$; $p<0.01$) and -20°C ($p=0,010$; $p<0,05$) on the 14th day. It was found to be statistically significantly lower than the stored CT measurement ($p=0.010$; $p<0.05$). There was no statistically significant difference between the Cq measurements of the cases stored at +4°C and the Cq measurements stored at -20°C on the 14th day ($p>0.05$) which are mixed with 70% ethyl alcohol. All information is summarized in **Table 1**.

The Cq measurement of the sample on the first day of the subjects participating in the study was avarage 3.34±1.59 according to the measurements sprayed with 10% destovir, 3.25±2.02 sprayed with 20% destovir, 3.36±1.39 sprayed with 30% destovir, 3.28±1.18 sprayed with 40% destovir, 2.39±0.82 sprayed with 50% destovir, and 3.99±1.12 mixed with 70% ethyl alcohol that were found statistically significant, respectively; $p=0.003$; $p=0.002$; $p=0.002$; $p=0.003$; $p=0.005$; $p=0.002$; $p<0.01$. In addition, it is found statistically significant that on the first day, 70% ethanol mixed Cq measurement of the subjects participating in the study was 0.86±1.34 higher than 40% destovir sprayed samples, and 1.48±0.88 higher than 50% destovir sprayed samples (respectively; $p=0.049$; $p=0.005$; $p<0.05$). The sample Cq measurement of the subjects included in the study, stored at +4°C on the 14th day was 4.55±1.99 according to the 10% destovir sprayed measurement, 2.73±1.01 compared to the 50% destovir spray sprayed group, and 2.99±1.87 compared to the 70% ethyl alcohol mixed respectively; $p=0.008$; $p=0.018$; $p=0.008$; $p<0.05$. At the same time, it was found statistically significant that the Cq value of the samples stored at -20°C on the 14th day was 3.36±1.09 on average compared to the 20% destovir sprayed samples, and 3.72±2.75 on average compared to the 30% destovir sprayed samples, respectively; $p=0.043$; $p=0.043$; $p<0.05$.

Table 1. Comparisons of Cq values by time and spray levels

Cq		First day[1]	14th day +4°C stored[2]	14th day-20°C stored[3]	[1-2]p	[1-3]p	[2-3]p
Sample	n	12	11	12	[a]**0.001****	[a]**0.001****	[a]**0.460**
	N/A	**0(0.0)**	**1(8.3)**	**0(0.0)**			
	Avarage±Ss	20.81±2.97	27.02±4.11	28.20±3.95			
	Median(Min-Max)	20.9(16.9-26.1)	27.8(19.2-33.2)	29.2(20.3-35.8)			
Spray 10%	N	11	9	4	[a]**0.001****	[a]**0.090**	[a]**0.217**
	N/A	**1(8.3)**	**3(25.0)**	**8(66.7)**			
	Avarage±Ss	23.67±2.53	31.20±4.77	28.06±4.54			
	Median(Min-Max)	24.5(19.1-26.4)	31.8(22.6-38.9)	29.0(22.4-31.9)			
Spray 20%	N	12	3	5	[a]**0.030***	[a]**0.027***	[a]**0.655**
	N/A	**0(0.0)**	**9(75.0)**	**7(58.3)**			
	Avarage±Ss	24.06±4.16	29.45±3.00	29.21±3.80			
	Median(Min-Max)	23.7(17.0-34.4)	29.7(26.3-32.3)	28.9(24.4-35)			
Spray 30%	n	12	3	5	[a]**0.009****	[a]**0.002****	[a]**0.456**
	N/A	**0(0.0)**	**9(75.0)**	**7(58.3)**			
	Avarage±Ss	24.17±2.36	32.99±3.29	31.23±1.97			
	Median(Min-Max)	24(20.9-29.4)	33.2(29.6-36.2)	31.4(28.1-33.5)			
Spray 40%	n	11	4	4	[a]**0.013***	[a]**0.013***	[a]**0.773**
	N/A	**1(8.3)**	**8(66.7)**	**8(66.7)**			
	Avarage±Ss	23.79±2.45	29.19±3.89	29.27±2.46			
	Median(Min-Max)	23.8 (20.1-29.3)	28.1 (25.8-34.7)	28.4 (27.4-32.8)			
Spray 50%	n	10	7	3	[a]**0.005****	[a]**0.011***	[a]**0.732**
	N/A	**2(16.7)**	**5(41.7)**	**9(75.0)**			
	Avarage±Ss	23.06±1.94	29.04±4.02	30.94±0.39			
	Median(Min-Max)	23.3(19.8-25.7)	29.6(23.3-33.6)	30.9(30.6-31.4)			
Ethly alcohol 70%	n	12	9	10	[a]**0.006****	[a]**0.010***	[a]**0.744**
	N/A	**0(0.0)**	**3(25.0)**	**2(16.7)**			
	Avarage±Ss	24.80±2.75	29.46±3.14	29.97±4.18			
	Median(Min-Max)	24.9(21.4-30.9)	29.5(25.9-35.8)	29.5(24.4-36.2)			
Sample-spray 10%	**Difference**	-3.34±1.59	-4.55±1.99	-2.17±1.98			
	p	[b]**0.003****	[b]**0.008****	[b]**0.068**			
Sample-spray 20%	**Difference**	-3.25±2.02	-7.08±1.11	-3.36±1.09			
	p	[b]**0.002****	[b]**0.109**	[b]**0.043***			
Sample-spray 30%	**Difference**	-3.36±1.39	-10.62±5.72	-3.72±2.75			
	p	[b]**0.002****	[b]**0.109**	[b]**0.043***			
Sample-spray 40%	**Difference**	-3.28±1.18	-4.45±2.76	-4.59±3.93			
	p	[b]**0.003****	[b]**0.068**	[b]**0.068**			
Sample-spray 50%	**Difference**	-2.39±0.82	-2.73±1.01	-4.80±0.88			
	p	[b]**0.005****	[b]**0.018***	[b]**0.109**			
Sample-70% ethly alcohol	**Difference**	-3.99±1.12	-2.99±1.87	-1.19±3.03			
	p	[b]**0.002****	[b]**0.008****	[b]**0.221**			
70% ethly alcohol-spray 10%	**Difference**	0.58±1.19	-1.30±3.77	-4.73±2.55			
	p	[b]**0.091**	[b]**0.310**	[b]**0.109**			
70% ethly alcohol-spray 20%	**Difference**	0.74±1.85	-2.94±3.18	-4.40±3.69			
	p	[b]**0.060**	[b]**0.109**	[b]**0.144**			
70% ethly alcohol-spray 30%	**Difference**	0.63±1.16	-6.48±3.96	-3.22±4.45			
	p	[b]**0.071**	[b]**0.109**	[b]**0.144**			
70% ethly alcohol -spray 40%	**Difference**	0.86±1.34	-0.16±0.92	-3.43±0.85			
	p	[b]**0.049***	[b]**1.000**	[b]**0.109**			
70% ethly alcohol-spray 50%	**Difference**	1.48±0.88	0.71±1.58	-5.01±1.79			
	p	[b]**0.005****	[b]**0.686**	[b]**0.109**			

[a]Mann-Whitney U test; [b]Wilcoxon Signed Ranks test; *p<0.05; **p<0.01

According to the RFU values of samples on the first day, 10% destovir sprayed RFU value was 16.91±12.50, in 20% as 18.58±11.36, 30% as 16.00±9.29, 40% as 14.91±11.47, 50% as 15.40±9.25 and in the mixed group with 70% ethyl alcohol as 14.08±10.01 that was found statistically significant respectively, p= 0.003; p=0.002; p=0.002; p=0.003; p=0.005; p=0.002; p<0.01. It was found statistically significant that the RFU measurement with 70% ethanol mixed on the first day was 4.50±3.68% higher than the 20% destovir spray sprayed RFU sample (p=0.007; p<0.01). According to the RFU values of samples study stored at +4 degrees on the 14th day, the mean of 10% destovir sprayed RFU sample measurement was 15.50±7.39, the mean of 50% destovir sprayed group was 15.57±5.59 and 70% ethyl alcohol was 10.67±5, respectively; p=0.012; p=0.018; p=0.008; p<0.05. According to the measurement of RFU samples stored at -20°C on the 14th day, it was obtained statistically significant that the mean of RFU values of samples with 20% destovir spray was 10.60±5.86, and the mean was 13.00±3.46 in the 40% destovir sprayed group, respectively p=0.043; p=0.042; p<0.05. In addition, it was found statistically significant that the RFU values of samples mixed with 70% ethyl alcohol, stored at -20°C, 10.17±9.89 higher on average compared to the RFU values of samples sprayed with 30% destovir (p=0.027; p<0.05). The distribution of Cq and RFU values of the samples is shown in **Figure 2**.

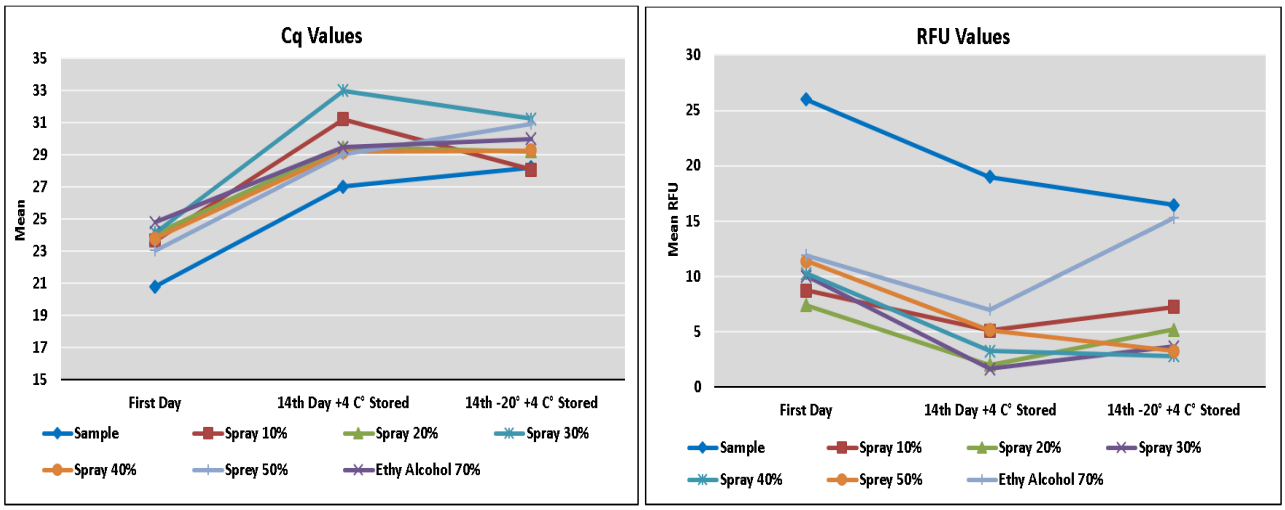

Figure 2. Distribution of Cq (left) and RFU (right) values of the samples

No statistically significant difference was obtained between the RFU measurement values of the samples stored at +4°C on the 14th day of the cases participating in the study (p>0.05). At the same time, no statistically significant difference was found between 10% and 20% destovir sprayed RFU measurements stored at +4 °C on the same day (p>0.05). RFU measurement values sprayed with 30% destovir spray on the first day were found to be statistically significantly higher than RFU measurements stored at +4 °C on the 14th day (p=0.019; p<0.05). RFU sample measurement values sprayed with 30% destovir spray on the first day were described to be statistically significantly higher than the RFU sample measurement stored at -20°C on the 14th day (p=0.042; p<0.05). Also no statistically significant difference was obtained between the measurements of 30% destovir spray sprayed RFU samples stored at +4°C and -20°C (p>0.05). There is statistically significant compared to the RFU values of samples with 40% destovir on the first day and RFU values of samples stored at +4°C (p=0.015; p<0.05) and -20°C (p=0.005; p<0.01) on the 14th day. No statistically significant difference was found between the measurements of 40% destovir sprayed RFU values of the samples stored at +4°C and -20°C on the 14th day (p>0.05). RFU values of samples sprayed with 50% destovir spray on the first day were statistically significant compared to the RFU sample measurements stored at +4°C (p=0.012; p<0.05) and -20°C (p=0.019; p<0.05) on the 14th day. No statistically significant difference was found between the measurements of 50% destovir spray sprayed RFU values of samples stored at +4°C and -20°C on the same day (p>0.05). RFU sample measurements mixed with 70% ethanol on the first day were found to be statistically significantly higher than RFU measurements stored at +4°C on the 14th day (p=0.022; p<0.05). No statistically significant difference was found between the measurements of RFU mixed with 70% ethyl alcohol stored at -20°C on the first day and the 14th day (p>0.05). Moreover, no statistically significant difference was described between the RFU measurements mixed with 70% ethyl alcohol stored at +4°C and -20°C on the 14th day (p>0.05) as summarized in **Table 2**.

Second Group Study Findings

A statistically significant difference was obtained between the 0-1-hour sample Cq values of the samples according to the

Table 2. Comparisons of RFU values by time and spray levels

Cq		First day[1]	14th day +4°C stored[2]	14th day-20°C stored[3]	[1-2]p	[1-3]p	[2-3]p
Sample	n	12	11	11	[a]0.440	[a]0.165	[a]0.576
	N/A	**0(0.0)**	**1(8.3)**	**1(8.3)**			
	Avarage±Ss	26.00±13.57	19.00±7.89	16.45±4.52			
	Median(Min-Max)	26.5(10-47)	17(11-36)	16(11-25)			
Spray 10%	N	11	8	4	[a]0.239	[a]0.793	[a]0.165
	N/A	**1(8.3)**	**4(33.3)**	**8(66.7)**			
	Avarage±Ss	8.73±6.74	5.13±2.95	7.25±2.06			
	Median(Min-Max)	7(2-23)	3.5(3-11)	7.5(5-9)			
Spray 20%	N	12	3	5	[a]0.067	[a]0.489	[a]0.362
	N/A	**0(0.0)**	**9(75.0)**	**7(58.3)**			
	Avarage±Ss	7.42±6.36	2.00±1.00	5.20±4.92			
	Median(Min-Max)	6(1-20)	2(1-3)	3(1-13)			
Spray 30%	n	12	3	6	[a]0.019*	[a]0.042*	[a]0.431
	N/A	**0(0.0)**	**9(75.0)**	**6(50.0)**			
	Avarage±Ss	10.00±5.86	1.67±1.15	3.67±3.14			
	Median(Min-Max)	10 (1-20)	1 (1-3)	3.5 (0-8)			
Spray 40%	n	11	4	5	[a]0.015*	[a]0.005**	[a]0.530
	N/A	**1(8.3)**	**8(66.7)**	**7(58.3)**			
	Avarage±Ss	10.27±5.97	3.25±1.26	2.80±1.64			
	Median(Min-Max)	8(2-22)	3(2-5)	2(1-5)			

[a]Mann-Whitney U test; [b]Wilcoxon Signed Ranks test; *p<0.05; **p<0.01

Table 2 (continued). Comparisons of RFU values by time and spray levels

Cq		First day[1]	14th day +4°C stored[2]	14th day-20°C stored[3]	[1-2]p	[1-3]p	[2-3]p
Spray 50%	n	10	7	4	[a]**0.012***	[a]**0.019***	[a]**0.502**
	N/A	**2(16.7)**	**5(41.7)**	**8(66.7)**			
	Avarage±Ss	11.40±5.56	5.14±3.80	3.25±2.75			
	Median (Min-Max)	12(5-20)	3(2-11)	3.5(0-6)			
Ethly alcohol 70%	n	12	9	11	[a]**0.022***	[a]**0.829**	[a]**0.106**
	N/A	**0(0.0)**	**3(25.0)**	**1(8.3)**			
	Avarage±Ss	11.92±5.45	7.00±2.87	15.27±11.32			
	Median(Min-Max)	11(2-20)	8(2-11)	12(2-40)			
Sample-spray 10%	**Difference**	16.91±12.50	15.50±7.39	7.50±1.91			
	p	[b]**0.003****	[b]**0.012***	[b]**0.066**			
Sample-spray 20%	**Difference**	18.58±11.36	15.67±1.53	10.60±5.86			
	p	[b]**0.002****	[b]**0.109**	[b]**0.043***			
Sample-spray 30%	**Difference**	16.00±9.29	16.00±1.73	13.40±3.46			
	p	[b]**0.002****	[b]**0.102**	[b]**0.043**			
Sample-spray 40%	**Difference**	14.91±11.47	11.25±2.99	13.00±3.46			
	p	[b]**0.003****	[b]**0.068**	[b]**0.042***			
Sample-spray 50%	**Difference**	15.40±9.25	15.57±5.59	12.25±3.95			
	p	[b]**0.005****	[b]**0.018***	[b]**0.066**			
Sample-70% ethly alcohol	**Difference**	14.08±10.01	10.67±5.17	0.80±15.30			
	p	[b]**0.002****	[b]**0.008****	[b]**0.767**			
70% ethly alcohol-spray 10%	**Difference**	3.00±5.37	1.33±3.14	14.00±9.17			
	p	[b]**0.099**	[b]**0.279**	[b]**0.109**			
70% ethly alcohol-spray 20%	**Difference**	4.50±3.68	4.33±3.79	11.25±11.81			
	p	[b]**0.007****	[b]**0.180**	[b]**0.068**			
70% ethly alcohol-spray 30%	**Difference**	1.92±2.84	4.67±3.21	10.17±9.89			
	p	[b]**0.057**	[b]**0.109**	[b]**0.027***			
70% ethly alcohol - spray 40%	**Difference**	1.55±4.01	1.67±2.31	14.25±9.88			
	p	[b]**0.259**	[b]**0.276**	[b]**0.068**			
70% ethly alcohol-spray 50%	**Difference**	1.30±3.06	2.60±2.51	14.00±10.52			
	p	[b]**0.231**	[b]**0.102**	[b]**0.068**			

[a]Mann-Whitney U test; [b]Wilcoxon Signed Ranks test; *p<0.05; **p<0.01

Cq groups (p=0.001; p<0.01). Those with Cq <20 were found to be significantly lower than those with Cq value between 20-25 and Cq≥25 (p=0.001; p=0.001; p<0.01). Likewise, the sample value of those in the Cq between 20-25 group was found to be significantly lower than those with a Cq ≥25 (p=0.001; p<0.01). A statistically significant difference was found between the measurements of the subjects with destrovir spray mixed at a rate of 5% (p=0.001; p<0.01) and 10% (p=0,008; p<0,01) for 0-1 hour according to the Cq groups. Based on the results of the pairwise comparison made to determine the difference; sample values with 5% destrovir in those Cq<20 were found to be significantly lower than those with Cq value between 20-25 and Cq≥25 (p=0.001; p=0.001; p<0.01). Likewise, the sample value with 5% destrovir in those with Cq 20-25 was found to be significantly lower than those with Cq≥25 (p=0.005; p<0.01). Moreover, a statistically significant difference was found between the measurements of the samples mixed with ethyl alcohol at a rate of 96% for 0-1 hour according to the Cq groups (p=0.008; p<0.01). Sample values of 96% ethyl alcohol of those Cq<20 were found to be significantly lower than those with Cq≥25 (p=0.001; p<0.01) as summarized in **Table 3**.

Samples with a Cq<20 in the trial performed between 0-1 hours mean 5.28±2.44 compared to samples mixed with 5% destrovir, mean 5.25±2.50 compared to samples mixed with 10% destrovir, mean 8.28±3.61 compared to samples mixed with 96% ethanol was lower on average compared to the mixed samples and was named as statistically significant. For 1-72 hours, these values mean 4.93±2.70 for samples mixed with 5% destrovir, 5.75±3.29 for samples mixed with 10% destrovir, and 6.70±2.69 for samples mixed with 96% ethanol (respectively; p=0.001; p=0.001; p=0.001; p<0.01). In addition, the samples mixed with 96% ethyl alcohol were 1.61±2.29 higher on average than the samples mixed with 5% destovir that was found to be statistically significant (p=0.001; p<0.01). When the same calculations were calculated for 72-120 hours, the mean was 4.06±2.28 for the samples mixed with 5% destrovir, 4.56±3.01 for the samples mixed with 10% destrovir, and 6.34±3.62 for the samples mixed with 96% ethanol that was considered statistically significant. The samples mixed with 96% ethanol were 3.14±3.83 higher than the samples mixed with 5% destovir and 2.67±4.17 higher than the samples mixed with 10% destovir (p=0.001; p=0.004), p<0.01).

Among the samples studied between 0-1 hours, the average of the samples with a Cq value between 20-25 is lower than 5.19±3.41 compared to the samples with 5% destrovir mixed, 5.93±3.57 for 10% destrovir, and 5.35±4.82 for 96% ethyl alcohol mixed samples that were found statistically significant (respectively; p=0.001; p=0.001; p=0.001; p<0.01). For 1-72 hours, mean 4.93±3.22 for samples mixed with 5% destrovir, 6.32±3.69 for samples mixed with 10% destrovir, and 4.20±1.98 for samples mixed with 96% ethanol. The samples mixed with 96% ethyl alcohol were on average 2.79±3.69 lower than the samples mixed with 10% destovir (p=0.011; p<0.05). For 72-120 hours, the mean is 4.06±2.87 for samples mixed with 5% destrovir, 4.48±2.98 for samples mixed with 10% destrovir, and 6.27±4.58 for samples mixed with 96% ethyl alcohol. In addition, the samples mixed with 96% ethanol were 1.61±2.29 higher on average than the samples mixed with 5% destovir (p=0.001; p<0.01).

The samples studied between 0-1 hours, the average of the samples with Cq≥25 is lower than the samples mixed with 5% destrovir as 5.46±3.41, an average of 4.08±2.32 compared to the

Table 3. Comparisons of Cq values of samples at 0-1 hours

0-1 hour		Cq group			
Cq		**<20**	**20-25**	**≥25**	**p**
Sample	n	46	45	43	[a]**0.001****
	N/A(+)	**0(0.0)**	**1(2.2)**	**3(6.5)**	
	Avaragt±Ss	19.61±2.49	23.43±2.20	27.65±3.27	
	Median (Min-Max)	19.5(15.4-29.4)	23.1(19.1-27.2)	27.1(22.1-33.2)	
Destrovir %5	n	45	41	25	[a]**0.001****
	Avarage±Ss	**1(2.2)**	**5(10.9)**	**21(45.7)**	
	Median(Min-Max)	24.78±2.99	28.48±4.25	31.36±3.20	
	Avaraget±Ss	24.6(18.2-31.1)	27.2(22.6-39.6)	31(26.7-38.1)	
Destrovir 10%	n	43	38	25	[a]**0.001****
	N/A(+)	**3(6.5)**	**8(17.4)**	**21(45.7)**	
	Avaragt±Ss	24.83±3.07	29.05±3.99	30.62±2.77	
	Median(Min-Max)	25.1(18.4-34.7)	27.5(24.1-38.3)	30.1(26.1-36.0)	
Ethly alcohol 96%	n	21	28	18	[a]**0.008****
	N/A(+)	**25(54.3)**	**18(39.1)**	**28(60.9)**	
	Avarage±Ss	27.17±4.33	28.58±4.00	31.53±4.54	
	Median(Min-Max)	26.3(21.7-35.3)	28.5 (15.6-36.9)	30.8(20.2-39.6)	
Sample-destrovir 5%	**Difference**	-5.28±2.44	-5.19±3.41	-5.46±3.41	
	p	[b]**0.001****	[b]**0.001****	[b]**0.001****	
Sample-destrovir 10%	**Difference**	-5.25±2.50	-5.93±3.57	-4.08±2.32	
	p	[b]**0.001****	[b]**0.001****	[b]**0.001****	
Sample-ethly alcohol 96%	**Difference**	-8.28±3.61	-5.35±4.82	-4.85±5.46	
	p	[b]**0.001****	[b]**0.001****	[b]**0.002****	
Destovir 5%-ethly alcohol 96%	**Difference**	-2.64±4.26	-0.79±5.11	-0.78±3.37	
	p	[b]**0.010***	[b]**0.438**	[b]**0.369**	
Destovir 10%-ethly alcohol 96%	**Difference**	-2.94±4.92	0.30±5.98	-1.88±2.36	
	p	[b]**0.018***	[b]**0.806**	[b]**0.019***	

[a]Oneway ANOVA; [b]Paired Samples test; **p<0.01

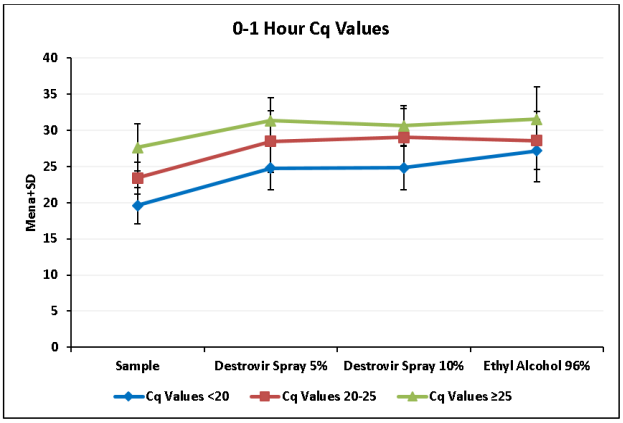

Figure 3. Distribution of 0-1-hour Cq values

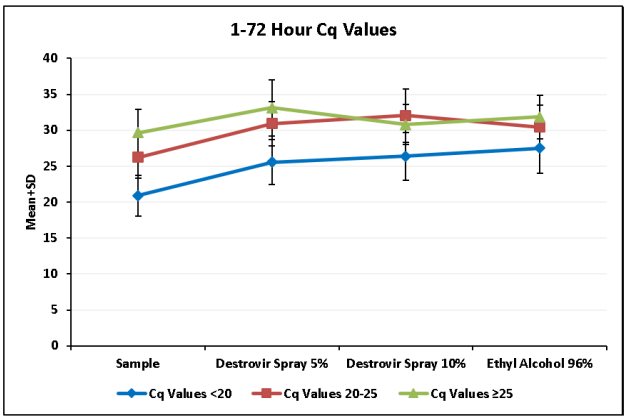

Figure 4. Distribution of 1-72-hour Cq values

samples mixed with 10% destrovir, 4.85±5.46 compared to the samples mixed with 96% ethanol that were statistically significant. The values were calculated for 1-72 hours which 5.46±3.14 average compared to samples mixed with 5% destrovir, 4.18±2.51 on average compared to samples mixed with 10% destrovir, and 16±3.83 compared to samples mixed with 96% ethanol. In addition, for the samples studied within 72-120 hours, the mean was 4.52±2.32 lower than the 5% destrovir mixed samples and 5.62±3.39 lower than the 10% destrovir mixed samples (respectively; p=0.001; p=0.001; p=0.002; p<0.01). Distribution of 0-1, 1-72, and 72-120-hour Cq values are shown in **Figure 3**, **Figure 4**, and **Figure 5**, respectively.

A statistically significant difference was described between the measurements of the samples at 1-72 hours, 5% and 10% mixed with destrovir spray of the cases according to the Cq groups (p=0.001; p<0.01). Sample values of those with a Cq<20

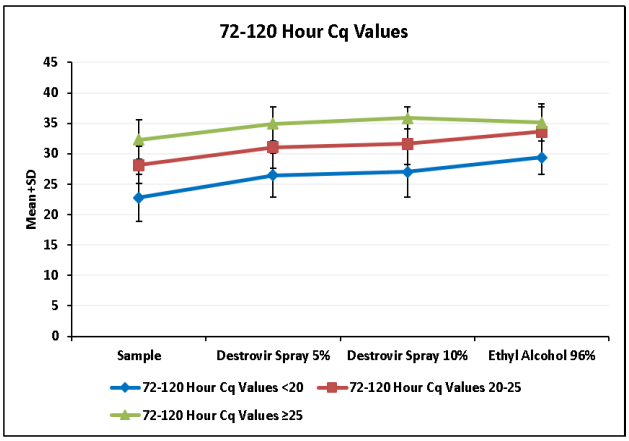

Figure 5. Distribution of 72-120-hour Cq values

Table 4. Comparisons of Cq values of samples at 1-72 hours

1-72 hours		**CT**			
Cq		**<20**	**20-25**	**≥25**	**p**
Sample	n	46	45	38	[a]**0.001****
	N/A(+)	**0(0.0)**	**1(2.2)**	**8(17.4)**	
	Avarage±Ss	20.92±2.82	26.24±2.91	29.65±3.24	
	Median(Min-Max)	20.6(15.9-27.8)	26.4(21.2-32.6)	29.2(22.9-36.7)	
Destrovir 5%	n	43	35	17	[a]**0.001****
	N/A(+)	**3(6.5)**	**11(23.9)**	**29(63.0)**	
	Avarage±Ss	25.55±3.14	30.92±3.06	33.13±3.91	
	Median(Min-Max)	25.5(18.2-32.8)	31.2(26.1-37.2)	31.7(27.9-39.5)	
Destrovir 10%	n	42	31	10	[c]**0.001****
	N/A(+)	**4(8.7)**	**15(32.6)**	**36(78.3)**	
	Avarage±Ss	26.37±3.3	32.04±3.71	30.8±2.76	
	Median(Min-Max)	26.2(19.2-36.7)	31.3(26.8-38.4)	30.7(27.5-37.4)	
Ethyl alcohol 96%	n	43	23	11	[c]**0.001****
	N/A(+)	**3(6.5)**	**23(50.0)**	**35(76.1)**	
	Avarage±Ss	27.49±3.47	30.45±3.07	31.85±3.01	
	Median(Min-Max)	26.8(21.6-36.9)	30.8(25.1-36.9)	32.7(25.1-35.3)	
Sample-destrovir 5%	**Difference**	-4.93±2.70	-4.93±3.22	-5.46±3.14	
	p	[d]**0.001****	[d]**0.001****	[d]**0.001****	
Sample-destrovir 10%	**Difference**	-5.75±3.29	-6.32±3.69	-4.18±2.51	
	p	[d]**0.001****	[d]**0.001****	[d]**0.008****	
Sample–ethly alcohol 96%	**Difference**	-6.70±2.69	-4.20±1.98	-4.16±3.83	
	p	[d]**0.001****	[d]**0.001****	[d]**0.026***	
Destovir 5%-ethly alcohol 96%	**Difference**	-1.61±2.29	0.76±3.67	0.04±2.79	
	p	[d]**0.001****	[d]**0.494**	[d]**0.917**	
Destovir 10%-ethly alcohol 96%	**Difference**	-0.61±2.64	2.79±3.69	1.13±2.43	
	p	[d]**0.174**	[d]**0.011***	[d]**0.500**	

[a]Oneway ANOVA; [c]Kruskal Wallis test; [d]Wilcoxon Signed Ranks test; **p<0.01

were found to be significantly lower than those with a Cq values between 20-25 and a Cq≥25 (p=0.001; p=0.001; p<0.01). Likewise, the sample value of those with Cq between 20-25 was found to be significantly lower than those with a Cq≥25 (p=0.001; p<0.01). A statistically significant difference was found between the measurements of the samples mixed with 96% ethanol for 1-72 hours (p=0.008; p<0.01). Sample values of 96% ethyl alcohol of those with a Cq<20 were found to be significantly lower than those with a Cq of 20-25 and a Cq≥25 (p=0.004; p=0.001; p<0.01) as summarized in **Table 4**.

A statistically significant difference was descibed between 1-72-hour sample measurements of the cases according to the Cq groups (p=0.001; p<0.01). Sample Cq<20 values were found to be significantly lower than those with Cq value between 20-25 and Cq≥25 (p=0.001; p=0.001; p<0.01). Likewise, the sample value of those with Cq 20-25 was found to be significantly lower than those with Cq≥25 (p=0.001; p<0.01). Statistically significant difference was obtained between the measurements of samples mixed with Destrovir spray at 5% and 10% hours (p=0.001; p<0.01). Sample values with 5% destrovir in those below CT<20 were found to be significantly lower than those with CT 20-25 and CT≥25 (p=0.001; p=0.001; p<0.01). A statistically significant difference was found between the measurements of samples mixed with 0-1 hour and 96% ethyl alcohol (p=0.008; p<0.01). Sample values of 96% ethyl alcohol of those with a Cq<20 and Cq values between 20 was found to be significantly lower than those with Cq≥25(p=0.007; p=0.014; p<0.05) as summarized in **Table 5**.

Table 5. Comparisons by Cq values at 72-120 hours

72-120 hours		**Cq**			
Cq		**<20**	**20-25**	**≥25**	**p**
Sample	n	46	43	39	[a]**0.001****
	N/A(+)	**0(0.0)**	**3(6.5)**	**7(15.2)**	
	Avarage±Ss	22.77±3.88	28.16±3.06	32.33±3.2	
	Median(Min-Max)	21.7(15.8-33.2)	27.5(21.1-36.3)	32.4(26.7-39.7)	
Destrovir 5%	n	42	26	18	[a]**0.001****
	N/A(+)	**4(8.7)**	**20(43.5)**	**28(60.9)**	
	Avarage±Ss	26.48±3.65	31.05±3.5	34.9±2.85	
	Median(Min-Max)	25.7(19.3-37.9)	30.4(25.7-36.8)	35.3(30.5-39.3)	
Destrovir 10%	n	44	24	10	[c]**0.001****
	N/A(+)	**2(4.3)**	**22(47.8)**	**36(78.3)**	
	Avarage±Ss	27.03±4.21	31.65±3.37	35.86±1.83	
	Median(Min-Max)	25.8(20.4-37.9)	31.6(27-38.1)	36.4(32.4-38.6)	
Ethly alcohol 96%	n	24	13	4	[c]**0.001****
	N/A(+)	**22(47.8)**	**33(71.7)**	**42(91.3)**	
	Avarage±Ss	29.37±2.74	33.64±4.11	35.12±3.06	
	Median(Min-Max)	28.8(26.1-36.9)	32.1(28.3-38.8)	34.6(32.5-38.8)	

[a]Oneway ANOVA; [c]Kruskal Wallis test; [d]Wilcoxon Signed Ranks test; **p<0.01

Table 5 (continued). Comparisons by Cq values at 72-120 hours

72-120 hours		Cq			
Cq		**<20**	**20-25**	**≥25**	**p**
Sample-destrovir 5%	**Difference**	-4.06±2.28	-4.06±2.87	-4.52±2.32	
	p	**[d]0.001****	**[d]0.001****	**[d]0.001****	
Sample-destrovir 10%	**Difference**	-4.56±3.01	-4.48±2.98	-5.62±3.39	
	p	**[d]0.001****	**[d]0.001****	**[d]0.008****	
Sample-ethly alcohol 96%	**Difference**	-6.34±3.62	-6.27±4.58	-5.42±2.80	
	p	**[d]0.001****	**[d]0.003****	**[d]0.068**	
Destovir 5%-ethly alcohol 96%	**Difference**	-3.14±3.83	-0.44±2.71	-1.47±2.79	
	p	**[d]0.001****	**[d]0.386**	**[d]0.273**	
Destovir 10%-ethly alcohol 96%	**Difference**	-2.67±4.17	1.61±3.85	2.39±2.19	
	p	**[d]0.004****	**[d]0.398**	**[d]0.285**	

[a]Oneway ANOVA; [c]Kruskal Wallis test; [d]Wilcoxon Signed Ranks test; **p<0.01

Table 6. Comparisons by RFU values at 0-1 hours

0-1 hour		Cq			
RFU		**<20**	**20-25**	**≥25**	**p**
Sample	n	46	45	41	**[a]0.001****
	N/A(+)	**0(0.0)**	**1(2.2)**	**5(10.9)**	
	Avarage±Ss	27.80±9.29	22.36±8.34	15.41±10.65	
	Median(Min-Max)	27.5(8-50)	24(4-46)	16(1-36)	
Destrovir 5%	n	45	40	20	**[c]0.001****
	N/A(+)	**1(2.2)**	**6(13.0)**	**26(56.5)**	
	Avarage±Ss	6.62±3.63	5.65±4.36	3.65±3.73	
	Median(Min-Max)	6 (2-20)	4 (1-20)	2.5 (1-17)	
Destrovir 10%	n	42	30	20	**[c]0.809**
	N/A(+)	**4(8.7)**	**16(34.8)**	**26(56.5)**	
	Avarage±Ss	5.21±2.96	5.10±2.89	4.80±3.33	
	Median(Min-Max)	5(1-13)	4(1-13)	5(1-13)	
Ethly alcohol 70%	n	18	22	12	**[c]0.043***
	N/A (+)	**28(60.9)**	**24(52.2)**	**34(73.9)**	
	Avarage±Ss	8.28±4.91	5.50±3.08	5.67±6.91	
	Median(Min-Max)	8(2-19)	4.5(2-11)	3(2-26)	
Sample-destrovir 5%	**Difference**	21.44±8.03	17.15±7.86	15.10±8.74	
	p	**[d]0.001****	**[d]0.001****	**[d]0.001****	
Sample-destrovir 10%	**Difference**	22.69±8.93	18.93±7.26	13.20±7.30	
	p	**[d]0.001****	**[d]0.001****	**[d]0.001****	
Sample-ethly alcohol 96%	**Difference**	21.89±7.70	18.86±6.61	13.08±10.55	
	p	**[d]0.001****	**[d]0.001****	**[d]0.008****	
Destovir 5%-ethly alcohol 96%	**Difference**	-1.56±5.96	0.29±4.16	-1.82±3.84	
	p	**[d]0.254**	**[d]0.777**	**[d]0.081**	
Destovir 10%-ethly alcohol 96%	**Difference**	-2.94±6.08	-0.14±2.82	1.44±3.68	
	p	**[d]0.064**	**[d]0.615**	**[d]0.197**	

[a]Oneway ANOVA; [c]Kruskal Wallis test; [d]Wilcoxon Signed Ranks test; *p<0.05; **p<0.01

A statistically significant difference was described between the RFU values of the samples examined between 0-1 hours with different Cq values (p=0.001; p<0.01). According to the results of the pairwise comparisons made to determine the difference, the RFU sample values of those with a Cq<20 were found to be significantly higher than those with a Cq between 20-25 and a Cq≥25 (p=0.020; p=0.001; p<0.05). Likewise, the RFU sample value of those with a Cq between 20-25 was found to be significantly higher than those with a Cq≥25 (p=0.003; p<0.01). In addition, a statistically significant difference was found between the RFU values of the subjects in which Destrovir spray was mixed at a rate of 5% from the samples studied within 0-1 hour according to the Cq groups (p=0.001; p<0.01). The RFU values of 5% Destrovir with Cq≥25 were found to be significantly lower than those with Cq<20 and Cq value between 20-25 (p=0.001; p=0.045; p<0.05) as shown in **Table 6**.

The RFU values of the samples with a Cq<20 studied within 0-1 hours were 21.44±8.03 on average compared to the samples mixed with 5% destrovir, 22.69±8.93 on average compared to 10% destrovir samples, 21.89±7.70 on average compared to 96% ethyl alcohol. Samples studied within 1-72 hours were mean 21.42±6.30 compared to 5% destrovir mixed samples and 22.46±7 average compared to 10% destrovir mixed samples and 17.60±7.01 compared to 96% ethyl alcohol. In addition, the samples mixed with 96% ethyl alcohol were calculated as 3.44±4.21 on average compared to the samples mixed with 5% destovir, and 4.76±4.74 on average compared to the samples mixed with 10% destovir (p=0.001; p= 0.001; p<0.01). Samples in 72-120 hours were an average of 24.05±7.99, 25.34±8.85, and 17.83±9.96 according to the samples mixed with 5% destrovir, 10% destrovir, and an 96% ethyl alcohol, respectively; p=0.001; p=0.001; p=0.001; p<0.01. It was found statistically significant that the samples mixed with 96% ethanol were on average 2.19±4.26 higher than the samples mixed with 5% destovir, and 4.30±3.85 higher than the samples mixed with 10% destovir (p=0.016; p =0.001; p<0.05).

Among the samples studied within 0-1 hours, in cases with Cq between 20-25, the mean of RFU sample measurements is

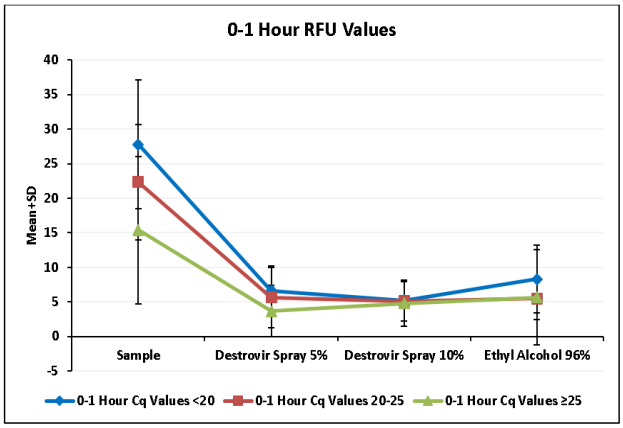

Figure 6. Distribution of 0-1-hour RFU values

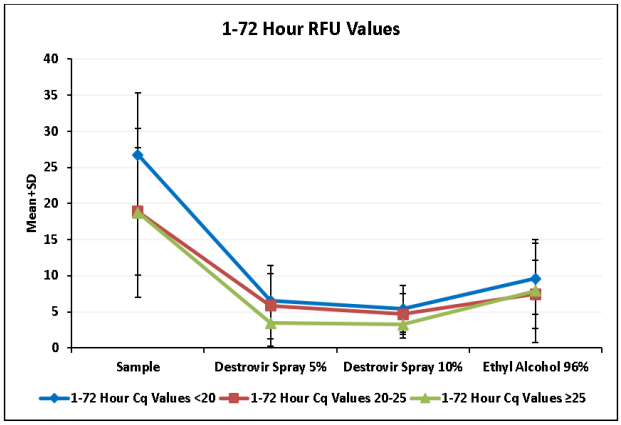

Figure 7. Distribution of 1-72-hour RFU values

17.15±7.86 units according to the 5% destrovir mixed sample measurement, and 18.93±7.26 according to the 10% destrovir mixed sample measurement. unit and 96% ethyl alcohol mixed sample measurement was 18.86±6.61 units higher on average (respectively; p=0.001; p=0.001; p=0.001; p<0.01). In samples studied within 1-72 hours, on the other hand, in cases with CT between 20-25, the mean of RFU sample measurements is 14.93±7.69 units according to 5% destrovir mixed sample measurement, 17.00±7 according to 10% destrovir mixed sample measurement and 11.91±8.20 units based on 96% ethyl alcohol mixed (respectively; p=0.001; p=0.001; p=0.001; p<0.01). It was found statistically significant that the samples mixed with 96% ethanol were 4.55±3.83 units higher on average compared to the measurement of the samples mixed with 10% destovir (p=0.005; p<0.01). In the samples studied within 72-120 hours, the mean of RFU sample measurements is 16.42±8.95 units according to the 5% destrovir mixed sample measurement, and 15.16±9.88 according to the 10% destrovir mixed sample measurement in cases with CT between 20-25. unit and 96% ethyl alcohol mixed sample measurement was 18027±8.17 units higher on average (respectively; p=0.001; p=0.001; p=0.005; p<0.01). Finally, in cases with CT≥25 of the samples studied within 0-1 hours, an average of 15.10±8.74 units according to the RFU sample measurements, according to the 5% destrovir mixed sample measurement, and 13.20±7 according to the 10% destrovir mixed sample measurement and 13.08±10.55 units for 96% ethyl alcohol mixed sample measurement (respectively; p=0.001; p=0.001; p=0.008; p<0.01). In cases with CT≥25 of the samples studied within 72-120 hours, the mean of RFU sample measurements is 16.54±9.04 units according to the 5% destrovir mixed sample measurement, 19.75±10.95 units average according to the 10% destrovir mixed sample measurement, and 11.33±4.69 based on 96% ethyl alcohol mixed sample measurement (respectively; p=0.001; p=0.012; p=0.008; p<0.05). It was found statistically significant that the RFU sample measurements of the samples studied within 72-120 hours were 15.58±4.98 units higher than the 5% destrovir mixed sample measurement in cases with CT 25 and above (respectively; p=0.002; p<0.01). Distribution of 0-1, 1-72, and 72-120-hour RFU values are summarized in **Figure 6**, **Figure 7**, and **Figure 8**, respectively.

A statistically significant difference was obtained between the RFU values of the samples studied between 1-72 hours of the cases according to the Cq groups (p=0.001; p<0.01). Based on to the results of the pairwise comparison made to determine the difference; RFU values of the samples of those with Cq<20 were found to be significantly higher than those

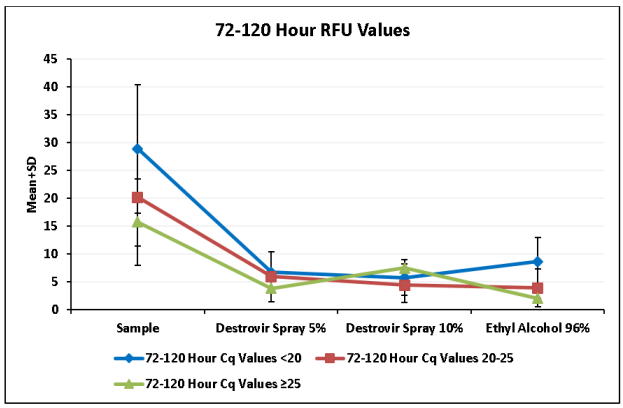

Figure 8. Distribution of 72-120-hour RFU values

with Cq between 20-25 and Cq≥25 (p=0.001; p=0.001; p<0.01). A statistically significant difference was found between the samples mixed with Destrovir spray at 5% and 10% (p=0.018; p<0.05). The RFU values of samples with 5% Destrovir were found to be significantly higher in those with a Cq<20 than those with a Cq≥25 and above (p=0.019; p<0.05) as summarized in **Table 7**.

A statistically significant difference was obtained between 1-72 hour RFU sample values of the cases according to the Cq groups (p=0.001; p<0.01). According to comparisons, the RFU values of the samples with a Cq<were found to be significantly higher than those with a Cq between 20-25 and a Cq≥25 (p=0.001; p=0.001; p<0.01). A statistically significant difference was found between the Cq groups and the samples mixed with 5% Destrovir spray for 1-72 hours (p=0.037; p<0.05). The RFU values of the samples with 5% Destrovir in the samples with a Cq<20 were found to be significantly higher than those with a Cq ≥25 (p=0.049; p<0.05). RFU sample measurements mixed with 96% ethyl alcohol for 1-72 hours in cases with Cq<20 were found to be statistically significantly higher than those with Cq between 20-25 (p=0.002; p<0.01) as summarized in **Table 8**.

DISCUSSION

The nasal passage plays the frontline defense, filtearing harmful bacteria, and viruses. Additionally, it increases the sinonasal pathways to high risk, in terms of infection. Loss of smell after COVID-19 disease, is the crucial for the life standards [12]. The respiratory droplet and aerosols exhaled from infected individuals are the main reason for the SARS-CoV-2

Table 7. Comparisons by RFU values at 1-72 hours

1-72 hours		Cq			
RFU		**<20**	**20-25**	**≥25**	**p**
Sample	n	46	45	37	[a]**0.001****
	N/A(+)	**0(0.0)**	**1(2.2)**	**9(19.6)**	
	Avarage±Ss	26.74±8.53	18.87±8.84	18.7±11.65	
	Median(Min-Max)	26(4-42)	20(2-34)	17(1-43)	
Destrovir 5%	n	43	30	13	[c]**0.018***
	N/A(+)	**3 (6.5)**	**16(34.8)**	**33(71.7)**	
	Avarage±Ss	6.51±3.79	5.83±5.6	3.46±2.18	
	Median(Min-Max)	6(1-19)	5(1-28)	3(1-7)	
Destrovir 10%	n	41	22	9	[c]**0.141**
	N/A(+)	**5(10.9)**	**24(52.2)**	**37(80.4)**	
	Avarage±Ss	5.41±3.21	4.68±2.83	3.22±1.86	
	Median(Min-Max)	5(1-15)	4(1-11)	3(1-6)	
Ethyl alcohol 96%	n	42	23	9	[c]**0.167**
	N/A(+)	**4(8.7)**	**23(50.0)**	**37(80.4)**	
	Avarage±Ss	9.57±4.94	7.43±4.69	7.89±7.11	
	Median(Min-Max)	9.5(2-20)	6(2-16)	6(1-24)	
Sample-destrovir 5%	**Difference**	21.42±6.30	14.93±7.69	16.54±9.04	
	p	[d]**0.001****	[d]**0.001****	[d]**0.001****	
Sample-destrovir 10%	**Difference**	22.46±7.79	17.00±7.19	19.75±10.95	
	p	[d]**0.001****	[d]**0.001****	[d]**0.012***	
Sample-ethyl alcohol 96%	**Difference**	17.60±7.01	11.91±8.20	11.33±4.69	
	p	[d]**0.001****	[d]**0.001****	[d]**0.008****	
Destovir 5%-ethyl alcohol 96%	**Difference**	-3.44±4.21	-2.63±6.21	-1.00±3.16	
	p	[d]**0.001****	[d]**0.070**	[d]**0.496**	
Destovir 10%-ethyl alcohol 96%	**Difference**	-4.76±4.74	-4.55±3.83	-3.25±2.22	
	p	[d]**0.001****	[d]**0.005****	[d]**0.102**	

[a]Oneway ANOVA; [c]Kruskal Wallis test; [d]Wilcoxon Signed Ranks test; **p<0.01

Table 8. Comparisons by RFU values at 72-120 hours

1-72 hours		Cq			
RFU		**<20**	**20-25**	**≥25**	**p**
Sample	n	46	42	38	[a]**0.001****
	N/A(+)	**0(0.0)**	**4(8.7)**	**8(17.4)**	
	Avarage±Ss	28.89±11.52	20.17±8.69	15.76±7.79	
	Median(Min-Max)	32.5(2-52)	20.5(3-38)	16(1-36)	
Destrovir 5%	n	38	24	13	[c]**0.037***
	N/A(+)	**8(17.4)**	**22(47.8)**	**33(71.7)**	
	Avarage±Ss	6.74±3.67	5.92±4.47	3.85±2.41	
	Median(Min-Max)	6(2-15)	5(1-17)	4(1-8)	
Destrovir 10%	n	41	19	2•	[e]**0.073**
	N/A(+)	**5(10.9)**	**27(58.7)**	**44(95.7)**	
	Avarage±Ss	5.78±3.21	4.42±3.08	7.5±0.71	
	Median(Min-Max)	5(1-15)	3(1-13)	7.5(7-8)	
Ethyl alcohol 96%	n	23	11	2•	[e]**0.002****
	N/A(+)	**23(50.0)**	**35(76.1)**	**44(95.7)**	
	Avarage±Ss	8.61±4.3	3.91±3.45	2±0	
	Median(Min-Max)	8(2-16)	3(1-13)	2(2-2)	
Sample-destrovir 5%	**Difference**	24.05±7.99	16.42±8.95	15.58±4.98	
	p	[d]**0.001****	[d]**0.001****	[d]**0.002****	
Sample-destrovir 10%	**Difference**	25.34±8.85	15.16±9.88	-	
	p	[d]**0.001****	[d]**0.001****		
Sample-ethyl alcohol 96%	**Difference**	17.83±9.96	18.27±8.17	-	
	p	[d]**0.001****	[d]**0.005****		
Destovir 5%-ethyl alcohol 96%	**Difference**	-2.19±4.26	0.33±4.72	-	
	p	[d]**0.016***	[d]**0.891**		
Destovir 10%-ethyl alcohol 96%	**Difference**	-4.30±3.85	-1.83±3.60	-	
	p	[d]**0.001****	[d]**0.273**		

[a]Oneway ANOVA; [c]Kruskal Wallis test; [d]Wilcoxon Signed Ranks test; [e]Mann Whitney U test; *p<0.05; **p<0.01

transmission. Thus, agents which reduce the viral loads in the throat and nasal cavity or protect mucosal tissue from initial infection can be affected on preventing infection and reducing virus spread between individuals [13].

Especially sprays are actual staff for preventing virus. Some contain decongestant compounds like xylometazoline, tramazoline, or oxymetazoline can be affected on reduce symptoms of nasal congestion [14]. This study aimed to address the challenges faced by nasal sprays by engineering

high-viscosity materials with apparent yielding behaviors (ensuring maximal retention in the nasal cavity).

It has been clearly determined that Destrovir spray has a significant increase in Cq values compared to positive COVID-19 samples and a serious suppression in RFU values in all samples where it is used, both in the studies on the first day and in the studies carried out on the 14th day with samples stored at +4°C and -20°C. Thus, we suppose that destrovir spray stops the reproduction of SARS-CoV-2, and this effect occurs as a result of the destruction of the virus's lipoprotein layer and RNA construction. 70% ethyl alcohol is prevented the reproduction of the virus by breaking down the lipoproteins in the outer surface structure of SARS-CoV-2 in the COVID-19 positive samples [15]. When the results of the samples of destrovir spray were compared with the results of the samples treated with 70% ethyl alcohol, the results were more positive than ethyl alcohol so as to destroy the SARS-CoV-2 structure [16]. The reason for this is that while 70% alcohol is effective on the samples stored at +4°C, however, there is no significant increase in the Cq values of the samples stored at -20°C. In samples stored at -20°C degrees, ethyl alcohol fixes the virus RNA and causes these fragments to replicate which cause to remain the Cq and RFU values as the first day values in q-RT-PCR. While normal samples kept the same Cq and RFU values on the first day, Cq values increased significantly and RFU values were significantly decreased in those kept both at +4°C and -20°C on the 14th day. This suggests that the reproductive process and replication power of SARS-CoV-2 decrease after a certain period of time. In literature, the authors in [17] examined the effectiveness of VTM solution on both temperature +4°C and -20°C to explain the preservation of positive ratio in SARS-CoV-2. The authors also presented that at the end of the 10 days, the positivity of the COVID-19 positive samples is decreased [18]. Moreover, in the results obtained in the second group, it was clearly determined that destrovir spray significantly increased Cq values compared to normal samples and significantly suppressed RFU values in all samples in which both 5% and 10% destrovir spray were used. According to these observed results, if the destrovir spray gives laboratory results in accordance with the stated mechanism of action, it inhibits the ability of the virus to multiply in the first use and destroy the outer lipoprotein structures and the RNA structure of the virus. If the spray is used by the nasal and oral route as specified, it can be inactivated by destroying the SARS-CoV-2 virus and its variants containing similar structures.

On the other side, it was thought that it would be beneficial to use Destrovir spray both as a prophylactic so that people do not infect each other the COVID-19 disease. It has caused to reduce virulence while the virus is still in the nose and mouth. There was no significant difference in effect between the 5% and 10% destrovir spray doses used in the study. It was determined that the effects in the samples where 5% and 10% levels of destrovir spray and 96% alcohol were used at 1/1 ratio were very close to each other. It has been observed that the table formed in the PCR results of the destructive and lethal effect of 96% ethyl alcohol on SARS-CoV-2 virus contains extremely similar values to the table formed by destrovir spray. This was considered as another proof that destrovir spray acts on the virus with an action mechanism similar to 96% ethyl alcohol and stops the reproduction by destroying the virus [18]. In all studies, it was checked that destrovir spray and alcohol did not adversely affect the working order of the PCR device with values such as HEX, FAM, and RFU controlled in all studies.

The proper operation of the PCR device in all of the samples treated with destrovir spray and ethyl alcohol and the numerical diversity of the obtained data were determined as other proof that the materials used did not cause any negative interactions on the device. It is known that in a negative interaction that may occur in the PCR device, it is not possible to obtain these values and especially in this arithmetic variety. In normal samples, especially those with Cq<25 preserved their Cq values, and RFU values until the end of the study, and in those with Cq> 25, a slight increase in Cq values and a slight decrease in RFU values. Especially after 72 hours, Cq and RFU values in the clinical course of COVID-19 disease gave an idea that values should also be kept in the foreground. Considering that Destrovir spray has a similar mechanism of action to ethyl alcohol, it is not only for SARS-CoV-2, but also for various viral, bacterial, etc. It has been thought that it can also be used in cases of infection, and that it can be an alternative in various situations where ethyl alcohol cannot be used for any reason.

Author contributions: **YA**: developed the protocol, determined the method, conducted the experiments, analyzed the data, created the administrative process, vouched for the entire experimental process, and wrote the article; **NPC**: wrote and revised the article. **MSK**: developed the protocol, determined the method, conducted the experiments, analyzed the data, vouched for the entire experimental process and the products used, and revised the article; **SZMK**: created the necessary experimental conditions, managed the administrative process, and analyzed the q-RT-PCR results and vouched for it; **CK**: created the necessary experimental conditions and directed the administrative process; **AK**: created the necessary experimental conditions and managed the administrative process. All authors have agreed with the results and conclusions.

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