ACIBADEM MEHMET ALI AYDINLAR UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

THE EFFECT OF CONCENTRATION OF BIOLOGICAL FLUIDS BY HYDROPHILIC ELASTIC POLYMER BEADS ON THE SENSITIVITY OF SARS-CoV-2 DIAGNOSIS BY REAL-TIME PCR

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M.Sc. THESIS

DEPARTMENT OF MEDICAL BIOTECHNOLOGY

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Department:	Medical Biotechnology
Program:	Medical Biotechnology
Thesis Title:	The effect of concentration of biological fluids by hydrophilic elastic polymer beads on the sensitivity of SARS-CoV-2 diagnosis of Real Time-PCR.
Student's name and Surname:	Dilara Çankaya.
Date of Defence:	23/06/2022

This is to certify that I have examined this copy of master thesis. I have found that she/he prepared after fulfilling the specified requirements in the associated legislations before the final examining committee whose signatures are below.

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DECLARATION

I declare that this thesis work is my own work, I had no unethical behavior at any stages from the planning to the writing of the thesis, I obtained all the information in this thesis in accordance with academic and ethical rules, I cited all the information and comments that were not obtained with this thesis work, and I provided resources in the list of references. I also declare that there was no violation of any patents and copyrights during the study and writing of this thesis.

23.06.2022

Dilara Çankaya

PREFACE AND ACKNOWLEDGEMENT

I would like to thank my dear advisor, Prof. Dr.Zühtü Tanıl Kocagöz for their guidance, contributions, encouragement, and support during this research.

Furthermore, I am grateful to my family. First and special thanks, to my mother Nilay ÇAKMAK. She is the one who encouraged me for my dreams, and to support any time for me to not give up. My father Mustafa Ertan ÇAKMAK for his endless support and help when I needed most.

I would like to thank my lovely son Atahan ÇANKAYA who is my one and only. Also my lovely husband Uğurcan ÇANKAYA, he always encouraged me with my dreams. Their presence inspired me to finish this thesis. Also, I feel lucky to have Hilal ŞENTÜRK and Gözde Ervin KÖLE as my best friends and a companions in this adventure. Again, I would like to thank Ümit FIRAT for his contribution to my work. I am grateful to all my family, dear lab mates whom we worked together until night, and my dear close friends for being with me in this process.

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LIST OF SYMBOLS AND ABBREVIATIONS

µl	Microliter
D	Deionized water
D mmc	Deionized water Concentrated with MyMagiCon–RW100®
DNA	Deoxyribonucleic acid
E Protein	Envelope Protein
ER	Endoplasmic reticulum
iPZT	İzlenebilir polimeraz zincirleme tepkimesi
M	Mineral water
M mmc	Mineral water Concentrated with MyMagiCon–RW100®
M Protein	Membrane protein
MERS	Middle East Respiratory Syndrome Coronavirus
ml	Mililiter
mmc	Concentrated with MyMagiCon–RW100®
RNA	Ribonucleic acid
RT-PCR	Real Time Polymerase Chain Reaction
S	0,9% NaCl (saline) solution
S mmc	0,9% NaCl solution Concentrated with MyMagiCon–RW100®
S Protein	Spike Protein
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
T	Tap water
T mmc	Tap water Concentrated with MyMagiCon–RW100®
W1	Bottle Drinking Water Hayat
W1 mmc	Bottle Drinking Water 1 Concentrated with MyMagiCon–RW100®
W2	Bottle Drinking Water Hamidiye
W2 mmc	Bottle Drinking Water 2 Concentrated with MyMagiCon–RW100®
WHO	World Health Organization

ÖZET

Hidrofilik Elastik Polimer Boncuklar Tarafından Biyolojik Sıvıların Konsantrasyonunun İzlenebilir Polimeraz Zincirleme Tepkimesi kullanılarak SARS-CoV-2 Tanısının Duyarlılığına Etkisi

Tanımlanmasından bu yana SARS-CoV-2, insanlık tarihindeki en ölümcül pandemilerden birine neden oldu. Şu anda, COVID-19 tanısı için en yaygın kullanılan yöntem nazofaringeal sürüntü örneklerinde SARS-CoV-2 RNA'sının izlenebilir polimeraz zincirleme tepkimesi (iPZT) ile saptanmasıdır. Nazofaringeal sürüntü örneği alınması, hastada olumsuz sonuçlar oluşturabilecek rahatsız edici ve invaziv bir yöntemdir. Bu araştırmada, COVID-19 tanısı için nazofaringeal sürüntü örneğine alternatif olarak gargara ve ağız çalkama suyunu konsantre eden yeni bir yöntemin kullanılma etkinliğini araştırdık. İlk olarak, oda sıcaklığında ve 4°C'de 10 gün sonra bile viral RNA miktarında önemli bir kayıp olmadığını belirledik. Ardından, gargara için kullanılan suyun bileşiminin PZT duyarlılığına etkisini belirlemek için altı farklı su çeşidi kullandık. iPZT'de en duyarlı saptamanın deiyonize su ile sağlandığını belirledik. Klinik çalışmamızda, 363 hastanın nazofaringeal, gargara ve ağız çalkama suyu örnekleri, konsantrasyon öncesi ve sonrasında SARS-CoV-2 RNA varlığı açısından iPZT ile incelendi. Nazofaringeal sürüntü örneklerinin 76'sı (%66.7), konsantre edilmemiş gargara/ağız çalkalama suyu örneklerinin 67'si (%58.8) ve konsantre edildikten sonraki gargara/ağız çalkalama suyu örneklerinin 101'i (%88.6) olmak üzere toplam 114 hastada SARS-CoV-2 virüsü saptandı. MyMagiCon–RW100®, nazofaringeal sürüntü yerine gargara/ağız çalkalama suyu kullanılarak hastanelerde veya evde hasta başı tanı için solunum yolu patojenlerinin hızlı saptanmasını sağlayabilir.

Anahtar Sözcükler: COVID-19, SARS-CoV-2, Polimeraz Zincirleme Tepkimesi, Ağız Çalkalama Suyu , Virüs konsantrasyonu, Mikroorganizma konstransyonu

ABSTRACT

The effect of concentration of biological fluids by hydrophilic elastic polymer beads on the sensitivity of SARS-CoV-2 diagnosis by Real-Time PCR

Severe Acute Respiratory Syndrome-2 (SARS-CoV-2) caused one of the deadliest pandemics in human history since its discovery in 2019. Currently, the gold standard approach for the diagnosis of COVID-19 is by identification of SARS-CoV-2 RNA in nasopharyngeal swab samples by Real Time Polymerase Chain Reaction (RT-PCR). Nasopharyngeal swab sampling is an uncomfortable and invasive sampling method that may have negative consequences. In this research, we studied the possibility of using gargle/mouthwash samples for the diagnosis of COVID-19 as an alternative to nasopharyngeal swab sampling, after concentrating them with a novel liquid concentrator. We have first determined that there was no loss of viral RNA and approximately a tenfold decline after 10 days at room temperature and 4 °C. Then, six different water sources were used to determine the effect of composition of water used for mouthwash, on the sensitivity of PCR. Deionized water produced the most sensitive result on RT-PCR. In our clinical study, 363 patients' nasopharyngeal, gargle/mouthwash samples were analyzed by RT-PCR for the presence of SARS-CoV-2 before and after concentration. The SARS CoV-2 virus was identified in a total of 114 patients, 76 (66.7%), 67 (58.8%), and 101 (88.6%) of nasopharyngeal swab, gargle/mouthwash samples before and after concentration, respectively. MyMagiCon–RW100® may enable rapid detection of respiratory pathogens from gargle/mouthwash in hospitals or at home for point of care testing.

Keywords: COVID-19, SARS-CoV-2, PCR, Mouthwash, Gargle, Virus concentration, Microorganism concentration

2 INTRODUCTION

Over the years, a lot of diseases occurred that caused numerous illnesses and millions of people death. These diseases are mostly caused by bacteria or viruses and spread among people via contagion. They are classified by their spread rate, area and also their effect on the human body. Most of these diseases were epidemics. On the other hand, there are pandemics that spread wider area and is defined as “an epidemic occurring worldwide, or over a very wide area, crossing international boundaries and usually affecting a large number of people” (1). The previously reported coronavirus based pandemics were caused by viral zoonotic pathogens as SARS-CoV (severe acute respiratory syndrome coronavirus) and MERS (Middle East respiratory syndrome coronavirus). These viruses caused severe respiratory diseases in humans (2) .

2.1 Severe Acute Respiratory Syndrome Coronavirus-2

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a novel coronavirus, the cause of the ongoing 2019 global pandemic. This pandemic had named the COVID-19. The first case was identified in Wuhan, a city in China, around December 12, 2019 (3). The first case started at an animal market in the city, via transmission of the SARS-CoV-2 virus from a bat to a human, which was identified by the whole genome sequencing of the first patient samples. Coronavirus and most of its subtypes can jump between species, and cause a variety of diseases (4).

According to WHO, until 1 June 2022, there have been 527,211,631 cases of coronavirus diseases 2019 (COVID-19), and 6,289,371deaths globally. In Turkey, there has been 15,071,772 confirmed cases and 98,961deaths until the 1 June 2022 (5).

2.1.1 Severe acute respiratory syndrome coronavirus-2 symptoms

The most common clinical symptoms of SARS-CoV-2 disease are dry cough, fever and shortness of breath in the majority of patients (6). Also, they might have headache, sore throat, chills and diarrhea. At these mild cases, patients have pulmonary signs as ground-glass lung opacity on chest X-ray (7). Patients may also experience, decreased white blood cell or lymphocyte number (8) and increased at some stages of infection (9, 10). At more critical cases, patients experienced severe pulmonary infection, respiratory failure, along with organ damage and dysfunction. With the

extra-pulmonary system dysfunctions, such as derangements in hematologic and digestive system, sepsis and septic shock risks increases dramatically, which results in increased risk of fatality rate.

2.2 Severe Acute Respiratory Syndrome Coronavirus-2 Structure

Coronaviruses are single-stranded, RNA enveloped viruses (11) and have the largest known RNA genome (GenBank no. MN908947) ranging from 26 to 32 kilobases in length, encoding 9860 amino acids (12, 13). They are spherical virions. The main structure consist of a core shell and a surface that resembles a solar corona based on its surface protein projections which gives the Latin name corona. (14). There are four main subfamilies: alpha, beta, gamma and delta-coronaviruses. SARS-CoV-2 belongs to the beta-coronavirus group, which also includes MERS-CoV and SARS-CoV (10).

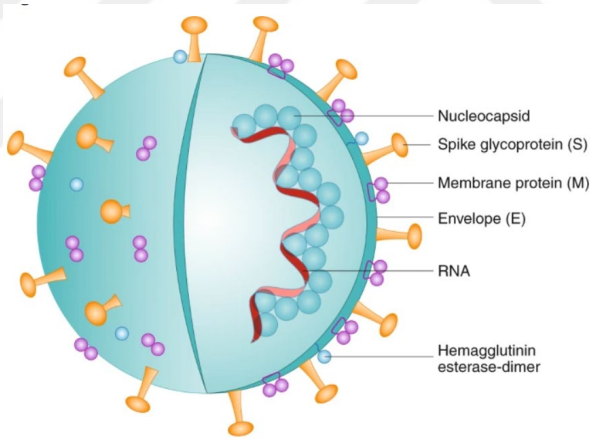


Figure 1. The structure of SARS-COV-2 (21).

Viruses consist of main proteins which build their structure and important ones among them are envelope proteins. Beta-coronaviruses have three important envelope proteins (Figure 1). Those are spike protein, membrane protein, and envelope protein (15). There are also nonstructural proteins which are inside the virus core. Proteases and RNA-dependent RNA polymerase are nonstructural units of SARS-CoV-2 and they are encoded by the ORF region site of the RNA viruses (16). The spike proteins have high importance, since they remain in the structure of the virus

and allow the virus to recognize the host cell that they fuse into. The recognition and fusion is done by the help of the S protein (17). After the viruses attach to the host cells they fuse the membrane, penetrate inside and release their genomic content. The RNA of the virus transcribes into mRNA and it translates the information to generate the required content of the new virus. These elements are carried through the endoplasmic reticulum (ER) to the Golgi apparatus and lead the assembly and release of the new virus itself. Spike protein is 180–200 kDa, and it consists of different domains such as extracellular N-terminus, a transmembrane (TM) domain anchored in the viral membrane, and a short intracellular C-terminal segment (18). When the virus attaches to the host cell, spike protein undergoes a structural rearrangement, this allows the virus to fuse with the host cell (19) (Figure 2). The coronavirus disease 2019 can easily escape from the immune defense system because the spike proteins are coated with polysaccharide molecules. This structure supports the virus to camouflage themselves, thus the virus can gateway from the immune system of the host organism (20, 21).

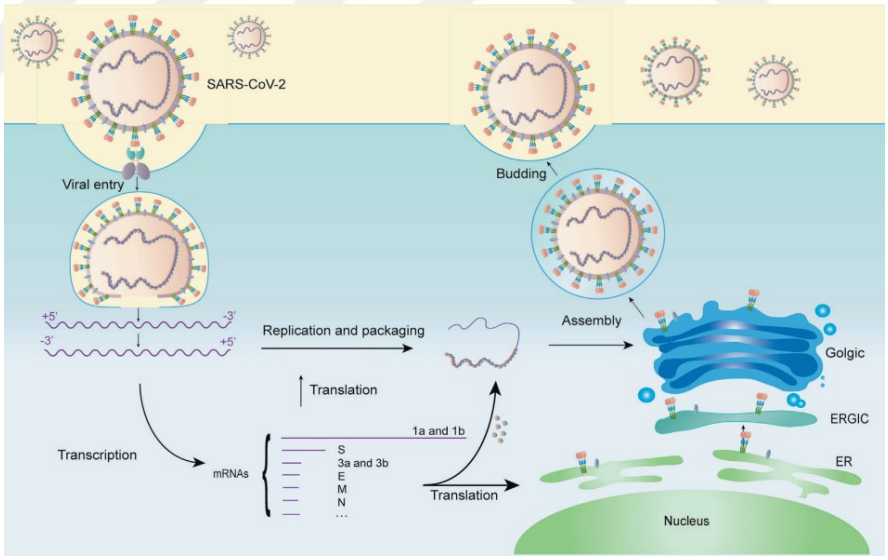
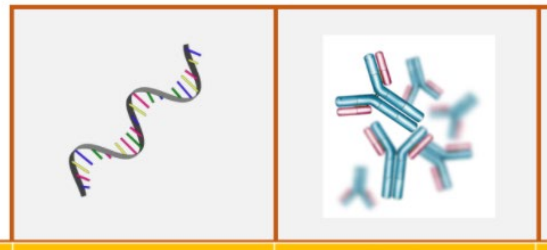


Figure 2. The fusion and replication of the virus in the host cell (Modified- (22)).

2.3 Diagnosis of Severe Acute Respiratory Syndrome Coronavirus-2

It is very important to identify the virus at the beginning of the illness to prevent the increase of the symptoms and the fatality, as explained earlier. Moreover, early diagnosis is important aspect so that the spread of the virus is prevented at early stages by keeping the infected people in lockdown and lowering person to person connections. The SARS-CoV-2 virus can be identified with different techniques. These can be CT scans to image the lungs to identify the damage that is created via the virus. Additionally, the diagnosis can be done by laboratory tests on samples acquired from the patients, by using different diagnostic tools. Next-generation sequencing and microarray analyses are two of the main tools but they are not currently used for diagnosis because of the expenses of the tests. On the other hand, these techniques are research tools to identify the virus and its evolution (23).



	PCR Test	Antibody Test	Antigen Test
Detects	Virus RNA	Antibodies	Virus antigens
Sample	Nose/ Throat swab	Blood	Nose/ throat swab
Indicates	Current Infection	Past Infection	Current Infection

Figure 3. The workflow of the different COVID-19 detection (Modified - (24)).

There are tools to identify the SARS-CoV-2 virus load (Figure 3).The easiest diagnostic tools to identify the disease from the patients are the antigen tests. These tests are easier to apply than RT-PCR test and also faster to get the result. But their sensitivity and specificity are low with respect to the RT- PCR assays (33). Antigen based tests are not sensitive enough to obtain accurate result especially when the virus load is lower than 10^4 virus RNA copies per/ml (34). This occurs especially in patients who are at the early stages of the disease. Thus, it is very limiting tool for diagnosis. It may result to lose the early stage coronavirus disease 2019 patients leading to spread

of the virus to other health people. Because of this, antigen tests are very limiting method for COVID-19 diagnosis.

The most popular tool to identify the SARS-CoV-2 virus from the patients is real-time polymerase chain reaction (RT-PCR). This laboratory-based approach is approved by the World Health Organization (WHO) and followed by all countries and test centers. Because it is respectively fast and easy to obtain results from a big set of patients' samples. Samples are collected by the educated personnel via a cotton swab sampling through the nasopharyngeal swab. The samples then later prepared to run a RT-PCR test by the responsible personnel.

2.3.1 The risk of the nasopharyngeal swab sampling and alternative methods

The samples from the patients are being collected by the nasopharyngeal swab sampling. The main aim of the sampling is to collect the secretions which are enriched by the viruses that can be identified by the nucleic acid-based detections. This procedure is painful and uncomfortable for the patient. To obtain the samples, it requires trained personnel. Moreover, sampling poses a risk of infection for the person who does the sampling, because there is a risk of the sample contamination to the personnel (25, 26). Also, the health of the trained personnel and medical staff is very important especially during such pandemics, since it is not possible to train healthcare staff, in short period of time. Also collecting the samples, is a time-consuming application. This situation results in, creating waiting lines in front of the testing centers (27). Hence, the sampling is standing as the possible cause the spread of the virus, during time from infected people to the healthy people at the sampling time and in the waiting lines (28). SARS-CoV-2 cause respiratory malfunctioning because the major virus load is present in respiratory epithelial cells. These are the areas that need to be kept humid for healthier respiration, thus they produce the body fluids like mucus and saliva. The main transmission route of COVID-19 is from the body fluids spread during talking, coughing and sneezing. These transmissions are all based to respiratory secretion contamination to the other people (29).

As mentioned, the nasopharyngeal sampling is dangerous procedure as it spreads the virus to healthcare personnel (30, 31). On the other hand, detection from the mouthwash sample is the most effective sampling method (32). Since during the pandemic, correct and timely identification of acute and past infections with SARS-CoV-2 are an urgent need (33, 34). With respect to disadvantages of nasopharyngeal sampling, other sampling methods should be developed to prevent the spread of the disease and discomfort to the patient. The mouthwash sample can also be collected by patient himself. These self-collected samples are easy to handle for any age of patient including the children. They are non-invasive and safe for healthcare workers (30).

Before this study, the sensitivity of identification of COVID-19 from mouthwash samples by RT-PCR was lower than nasopharyngeal swab samples. In this study, we have investigated the possibility of using gargle/mouthwash samples, after concentrating them by a new product named MyMagiCon® (GigaBioMol, Bio-T, Istanbul, Turkey) that we have recently developed, for the diagnosis of COVID-19, as an alternative to nasopharyngeal swab sampling. MyMagiCon® is a powder containing a unique polymer that rapidly removes small molecules from solutions. The elastic polymer beads expand rapidly by absorbing water and other small molecules and concentrating microorganisms and macromolecules.

MyMagiCon–RW100® concentrates gargle/mouthwash samples 10 to 20 times for the diagnosis of infectious agents such as SARS-CoV 2, influenza virus, and other agents causing infections in the respiratory system. Microorganisms are concentrated when they are intact. Even if the organism is lysed and its nucleic acids and antigens are released into the solution, these large molecules will be concentrated as well (27).

The aim of this study was to investigate if concentrated gargle/mouthwash can be used as efficiently instead of nasopharyngeal swab samples for the diagnosis of SARS-CoV-2 by RT-PCR.

3 MATERIALS AND METHODS

3.1 Buffers and Chemicals

All SARS-CoV-2 viruses taken from patient, inactived and cell culture in Acibadem Labcell, İstanbul,Turkey). Final concentration of SARS-CoV-2 virus was 10^{15}. For RT-PCR step, DIAGNOVITAL® HS SARS-CoV-2 Real-Time PCR Kit was used (A1 life sciences, Istanbul, Turkey).

3.2 Stability of SARS CoV-2 virus in Mouthwash Samples

To determine if gargle/mouthwash may be used instead of nasopharyngeal swab samples for COVID-19 diagnosis, we first looked at the stability of SARS-CoV-2 in mouthwash samples. For this purpose, 10 negative SARS-CoV-2 volunteers were selected for taking mouthwash samples. Volunteers gargled and rinsed mouth with soft drinking water named Hamidiye (Hamidiye Spring Water Company, pH = 6,92, hardness = 36 ppm) vigourously at least 10 seconds and put it back into empty disposable cups. Samples were aliquoted into 1 ml and was spiked with SARS-CoV-2. The concentration adjusted to the final of 10^{15} copies/ml for five volunteer's samples and the other five with 10^{14} copies/ml. Each aliquot was split into two equally, in the micro-centrifuge tubes and they were kept either at room temperature or at 4 °C for several different days and checked at days 0, 3, 5, 7 and 10. Later, the concentration of virus titer in each sample was quantified by using RT-PCR. DIAGNOVITAL® HS SARS-CoV-2 Real-Time PCR Kit. The average copy number in samples stored at room temperature and 4°C was calculated for each of these days, and the change in copy number of viruses was determined.

3.3 Effect of Collecting Gargle/Mouthwash Samples with Different Types of Liquid to the Sensitivity of RT-PCR in the Diagnosis of SARS- CoV-2

Mouthwash samples were taken from 4 negative SARS- CoV-2 volunteers. Those samples were spiked with same amount of inactivated coronavirus cultured in the laboratory conditions and with the dilution factor of 1/1000. Final virus concentration was 10^{12} copy/mL. Different liquids used to collect mouthwash samples.

- Drinking Water: Two different bottled drinking water were used for purpose.
- Deionized water: Elkay- electric water fountain connected to deionized water.
- Tap water: Tap water was obtained from Acibadem Mehmet Ali Aydinlar University.
- Mineral water: Akmina brand mineral water was used for purpose.
- Saline solution: 0,9% NaCl (Saline) solution had prepared by using deionized water.

3.3.1 Bottled drinking water (W1)

A commercial drinking water was obtained from a market (HAYAT, Danone Hayat Beverage and Food Industry and Trade Inc.) and named as W1. The hardness and pH of liquid were measured by pH 50 Violab Benchtop pH meter - 201T Electrode. Other content information was taken from the label on the bottle. pH was specified as 8,0 on the label.

Table 1: The content and pH of the W1

Content	Amount
Sulfate	6 mg/L
Sodium	4,4 mg/L
Calcium	19,3
Chloride	1,2 mg/L
Magnesium	2,4
Hardness	30 ppm
pH	7,9

3.3.2 Bottled drinking water (W2)

A commercial drinking water was obtained from a market (HAMİDİYE, Hamidiye Spring Water Company). Only the hardness and pH of liquid were measured. Other content information was taken from the label on the bottle. pH was specified as 7,0 on the label. pH was measured by using same product (see 3.3.1).

Table 2: The content and pH of the W2

Content	Amount
Sulfate	7,18 mg/L
Sodium	7,8 mg/L
Calcium	-
Chloride	7,9 mg/L
Magnesium	-
Hardness	36 ppm
pH	6,92

3.3.3 Electric water fountain connected to deionized water (D)

The distilled water filtered to obtain distilled deionized water by using machine brand the Elkay. Only hardness and pH of liquid were measured for this sample.

Table 3: The pH of the deionized water (D)

Content	Amount
Hardness	19ppm
pH	7,2

3.3.4 Tap water (T)

The tap water was obtained at Acibadem Mehmet Ali Aydinlar University. Only hardness and pH of liquid were measured (see 3.3.1).

Table 4: The hardness of the tap water (T)

Content	Amount
Hardness	167 ppm
pH	7,7

3.3.5 Mineral water (M)

A commercial mineral water was obtained from a market (Danone Beverage and Food Industry and Trade Inc). The content information was taken from label on the bottle.

Table 5: The content and the pH of the mineral water (M)

Content	Amount
Sulfate	9,52 mg/L
Sodium	22,2 mg/L
Calcium	393,2
Chloride	5,05 mg/L
Magnesium	28,8
Hardness	40 ppm
pH	5,9

3.3.6 Saline (0,9% NaCl) solution (S)

NaCl (Sigma-Aldrich, #31434-1KG-R) solution was prepared with deionized water. Hardness and pH of the solution were measured (see 3.3.1).

Table 6: The hardness and pH of the Saline Solution (S)

Content	Amount
Hardness	2700 ppm
pH	6,12

3.4 Sample Collection for Clinical Study

For this research, 3 types of sample types were used. Those samples are nasopharyngeal swab, mouthwash sample and concentrated mouthwash samples. Total of 363 volunteers above the age of 18, who were admitted to the Acibadem Altunizade Hospital (Istanbul) by symptoms of respiratory infection, were included in

the study. SARS-CoV-2 was studied in three samples of each patient, nasopharyngeal swab sample, gargle/mouthwash before and after concentration using direct RT-PCR without RNA extraction and commercial PCR kit A1 Lifesciences, Istanbul, Turkey). To eliminate false-positive and false-negative results, each batch of samples studied by PCR included negative and positive controls.

3.4.1 Nasopharyngeal swab collection

Nasopharyngeal swab samples were obtained by a nucleic acid collection and transport medium, Nucliswab (Inovatif Biotechnology Organisation Tic. Ltd. Şti. Istanbul, Turkey) which contains 3 mL of viral nucleic acid extracting and preserving solution liquid. Clinical specimens suspected of respiratory tract infection are transferred in this tube, the liquid inside the tube can be used directly in real-time PCR reactions. After collecting nasopharyngeal swab samples, these samples were stored at +4°C.

3.4.2 Mouthwash and concentrated mouthwash sample collection

Since mouthwash is easily collected and clinically informative for disease detection, the consideration that maximizes the benefit of using mouthwash a diagnostic fluid deserves more attention. After collecting nasopharyngeal swab samples, patients were instructed to take a few sips of water (different types of water had been used for the purpose as tap water, distilled water, deionized water, mineral water, and saline solution) and then to gargle and rigorously rinse their mouth forcefully with this water for at least 10 seconds and put it back to a disposable cup. After collecting nasopharyngeal swab samples, these samples were stored at +4°C until studied by RT-PCR.

3.5 Sample Preparation

3.5.1 Nasopharyngeal swab sample preparation

Nasopharyngeal swab samples were processed in biosafety level 2 cabinet. Samples known to be positive for SARS-CoV2 and coming from Acibadem Altunizade Hospital were listed. After vortex, 500 µl of sample was taken and transferred to a micro-centrifuge tube placed on ice.

3.5.2 Gargle/mouthwash sample preparation

Gargle/mouthwash samples are processed in biosafety level 2 cabinet. Firstly, all gargle/mouthwash samples are listed and vortexed.

3.5.3 Concentrated mouthwash sample preparation

Gargle/mouthwash samples which are taken from patients were concentrated using MyMagiCon–RW100® as instructed in the user guide. Briefly, 20 mL of samples were put from cup into the tube. MyMagiCon–RW100® powder was poured into the tube. The cap of the tube was closed and the sample was mixed with absorbing powder by swirling the tube gently. The bottom of the tube was tapped a few times on the bench to ensure that all liquid was collected at the bottom. It was waited for 5 min for the concentration of the sample. Then, a sterile pipette was used for pulling out 500 µl concentrated sample from the bottom of the tube. Then the concentrated samples were transferred to micro-centrifuge tubes.

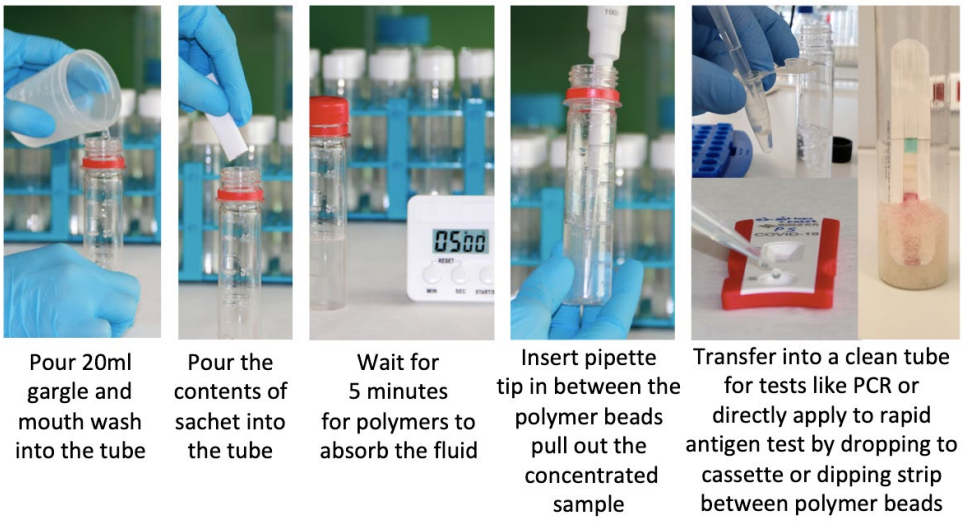


Figure 4. The protocol for the MyMagiCon–RW100® .

3.6 RT-PCR Set up and Analysis

DIAGNOVITAL® HS SARS-CoV-2 Real-Time PCR Kit (Table 1) detects the presence of N1 and N2 regions of the Nucleocapsid gene which are different and highly specific gene sequences of SARSCoV-2. The PCR master mix also contains primers and probe for an endogenous human target (RNaseP), which is extracted from the swab. The positive control is used to confirm the functionality of the assays and overall PCR performance, the negative human extraction control is included to evaluate the quality of the RNA isolation independently from the SARS-CoV-2 assays.

To begin the reaction, all equipment and workplaces decontaminated. Then, all components of DIAGNOVITAL® HS SARS-CoV-2 Real-Time PCR Kit which are PCR master mix, NTC (negative control), positive control were thawed on ice and vortexed. The reaction was set for the each patient and all their samples since each patient have 3 types of samples (swab, mouthwash and concentrated mouthwash). Then, 15 µl PCR Master mix was put into the Biorad 96-Well PCR Plates' wells as much as the number of samples. Then, 5µl of patients' sample added into the wells. Biorad Microseal 'B' PCR Plate Sealing Film used to cover well plate. Reaction was run according to RT-PCR (BİO-RAD C100 Real-Time Thermal Cycler CFX96 Optics, Serial No:785BR181139) conditions.

Table 7. DIAGNOVITAL® HS SARS-CoV-2 Real-Time PCR Kit conditions.

Step	Cycles	Temperature	Duration
Reverse Transcription	1	45°C	10 minutes
Initial Denaturation	1	95°C	2 minutes
Amplification	40	95°C	3 seconds
		60°C*	10 seconds

*Enable Data Collection for

For analysis of RT-PCR results, instructions of the kit manual was used. Negative template control (dH_2O) controls must not give a positive Ct for any assay.

If the NTC negative control returns a positive result, the reaction has been contaminated by sample RNA. To be sure positive for SARS-CoV-2, its amplification in the FAM channel N1 / N2 must give positive Ct values. If the HEX channel which is internal control fails to amplify, the sample must still be considered positive.

For negative for SARS-CoV-2 result, the FAM channel (N1 / N2) must not give positive Ct values. The internal control in the HEX channel (RNAseP) must give a positive Ct value (< 40 cycles) for these samples to be sure that sample material of suitable quality was present.

For the internal control assay, all reactions involving RNA isolate must have positive Ct values.The Ct values should be< 40 cycles. For the positive control, a positive Ct at the FAM channel must be observed. The Ct value for the positive control should be 28<Ct <32. If no amplification signal is observed for any assay, that meant that PCR was inhibited.

Table 8. The explanation from the RT-PCR results by using the DIAGNOVITAL® HS SARS-CoV-2 Real-Time PCR Kit

N1-N2 (FAM)	RNAseP (HEX)	Interpretation
+	-	Sample is positive for SARS-CoV-2
+	+	Sample is positive for SARS-CoV-2
-	+	Only internal control was amplified which means sample is negative for SARS-CoV-2
-	-	PCR was inhibited, results are invalid.
+	-	Positive control result
-	-	Negative control result

4 RESULTS

4.1 Stability of SARS-CoV-2 in mouthwash samples stored at different temperatures for different days by using RT-PCR

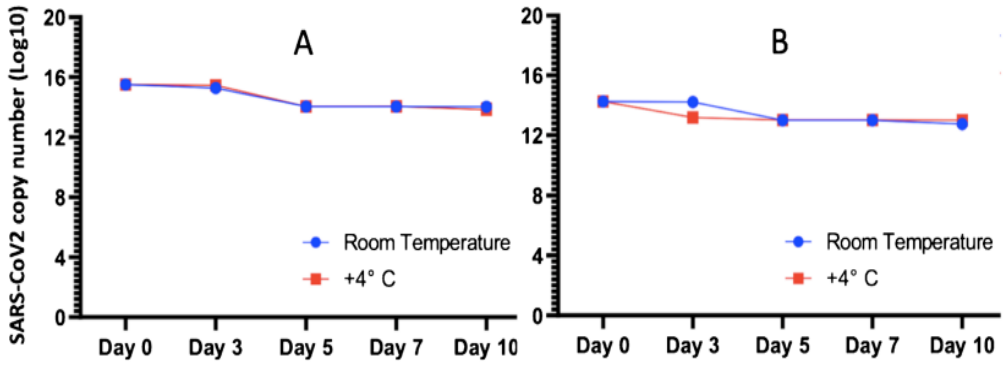


Figure 5. The stability graphics of SARS-CoV-2 RNA obtained from mouthwash samples at room temperature and 4°C. Graph A indicate the starting number of RNA copies was 10^{15}/mL, and graph B 10^{14}/mL in samples.

4.2 Comparison of the mouthwash samples collected by using different types of liquids to analyze the sensitivity of PCR

Purpose of this study was the determination of the effect of different fluids which are two different brands of waters (W1 and W2), deionized water (D), tap water (T), mineral water (W) and 0,9% NaCl (saline) solution (S) on the sensitivity of PCR. Firstly, four negative SARS-CoV-2 volunteers gave six mouthwash samples with different water types which are bottle drinking water (W1), bottle drinking water (W2), deionized water (D), tap water (T), mineral water (W), and 0,9% NaCl solution (S). After the first step, RT-PCR was done directly on the mouthwash samples. Then, these mouthwash samples were concentrated by MyMagiCon–RW100® (3.5.3)

4.2.1 Volunteer 1's mouthwash results with six different water types

Both mouthwash and concentrated mouthwash samples which belong to the volunteer 1 with different types of water Ct results are in Table 9.

Table 9. Volunteer 1's mouthwash and concentrated mouthwash samples with six different water types Ct result comparison

Volunteer 1 Sample	**W1**	**W2**	**D**	**T**	**M**	**S**
Mouthwash	**-**	**38,32**	**32,44**	**36,57**	**-**	**-**
Concentrated Mouthwash	**33,13**	**30,81**	**26,52**	**30,66**	**33,01**	**37,76**

Both mouthwash and concentrated mouthwash samples belong to Volunteer 1 RT-PCR results with different types of water comparison in Figure 6.

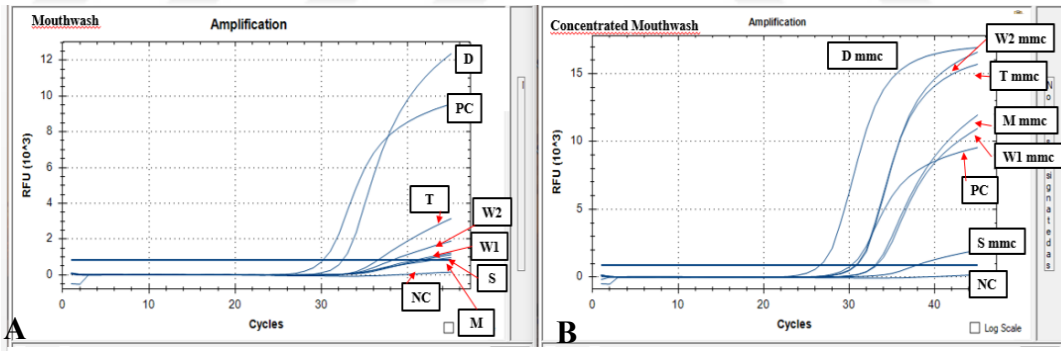


Figure 6. Comparison of the Volunteer 1's mouthwash (A) and concentrated mouthwash (B) samples with six different water types on RT-PCR. (Bottled drinking water (W1) and Bottled drinking water (W2), deionized water (D), tap water (T), mineral water (W) and 0,9% NaCl (saline) solution (S), concentrated with MyMagiCon–RW100® (mmc), positive control (PC), negative control (NC)).

4.2.2 Volunteer 2's mouthwash results with six different water types

Both mouthwashes and concentrated mouthwash samples belong to Volunteer 2 but different types of water Ct result in Table 10.

Table 10. Volunteer 2's mouthwash and concentrated mouthwash Ct results with six different water types

Volunteer 2 Sample	**W1**	**W2**	**D**	**T**	**M**	**S**
Mouthwash	**-**	**36,69**	**29,77**	**36,84**	**-**	**38,23**
Concentrated Mouthwash	**34,38**	**32,03**	**23,85**	**34,51**	**39,33**	**-**

Both mouthwash and concentrated mouthwash samples belong to Volunteer2 RT-PCR results with different types of water comparison in Figure 7.

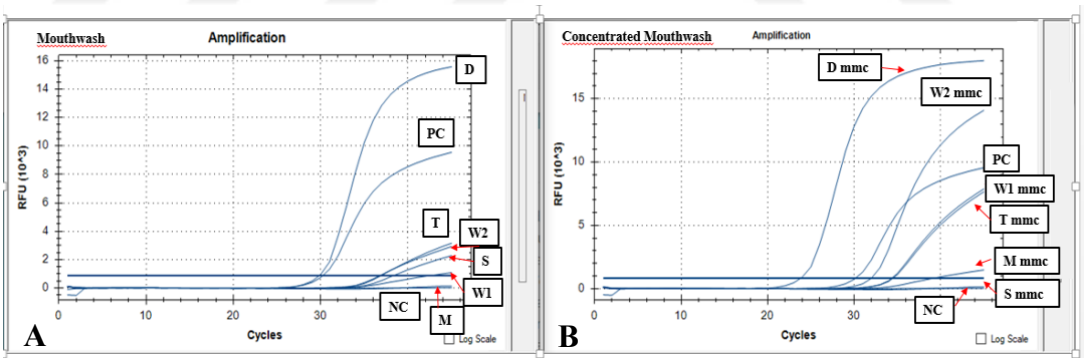


Figure 7. Comparison of the Volunteer 2's mouthwash (A) and concentrated mouthwash (B) samples with six different water types on RT-PCR. (Bottled drinking water (W1) and Bottled drinking water (W2), deionized water (D), tap water (T), mineral water (W) and 0,9% NaCl (saline) solution (S), concentrated with MyMagiCon–RW100® (mmc), positive control (PC), negative control (NC)).

4.2.3 Volunteer 3's mouthwash results with six different water types

Both mouthwash and concentrated mouthwash samples which are belong to Volunteer 3 Ct results are in Table 11.

Table 11. Volunteer 3's mouthwash and concentrated mouthwash Ct results with six different water types

Volunteer 3 Sample	**W1**	**W2**	**D**	**T**	**M**	**S**
Mouthwash	-	**35,53**	**29,11**	**38,08**	-	-
Concentrated Mouthwash	**31,61**	**27,79**	**23,80**	**32,43**	-	-

Both mouthwash and concentrated mouthwash samples belong to Volunteer 3 RT-PCR results with different types of water comparison in Figure 8.

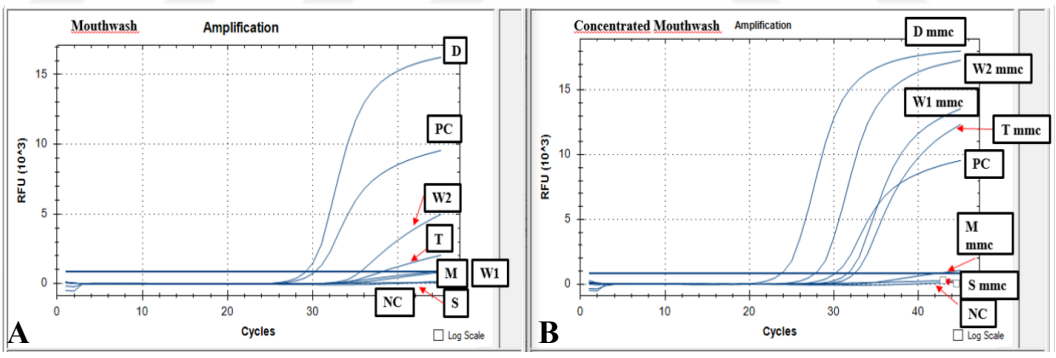


Figure 8. Comparison of the Volunteer 3's mouthwash (A) and concentrated mouthwash (B) samples with six different water types on RT-PCR. (Bottled drinking water (W1) and Bottled drinking water (W2), deionized water (D), tap water (T), mineral water (W) and 0,9% NaCl (saline) solution (S), concentrated with MyMagiCon–RW100® (mmc), positive control (PC), negative control (NC)).

4.2.4 Volunteer 4's mouthwash results with six different water types

Both mouthwash and concentrated mouthwash samples belong to Volunteer 4 but different types of water Ct value results are in Table 12.

Table 12. Volunteer 4 mouthwash and concentrated mouthwash Ct value results with six different water types

Volunteer 4 Sample	**W1**	**W2**	**D**	**T**	**M**	**S**
Mouthwash	-	-	**34,42**	**37,42**	**38,80**	**39,86**
Concentrated Mouthwash	**33,32**	**32,50**	**28,48**	**33,07**	-	-

Both mouthwash and concentrated mouthwash samples belong to Volunteer 4 RT-PCR results with different types of water comparison in Figure 9.

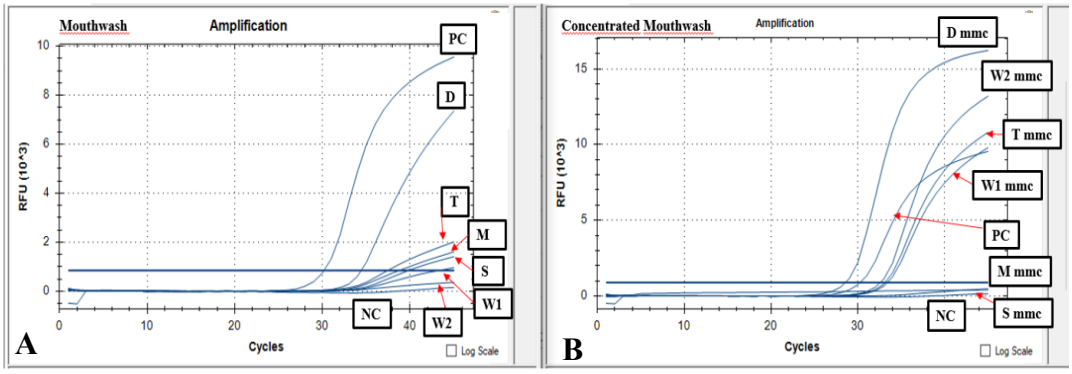


Figure 9. Comparison of the Volunteer 4's mouthwash (A) and concentrated mouthwash (B) samples with six different water types on RT-PCR. (Bottled drinking water (W1) and Bottled drinking water (W2), deionized water (D), tap water (T), mineral water (W) and 0,9% NaCl (saline) solution (S), concentrated with MyMagiCon–RW100® (mmc), positive control (PC), negative control (NC)).

4.3 Comparison of volunteers for same liquid type.

The purpose of this study is that compare the effect of the same fluids for 4 volunteers' mouthwash and concentrated mouthwash samples on the sensitivity of PCR used for the diagnosis of Covid19. The same protocol were used in 4.2. For RT-PCR step, DIAGNOVITAL® HS SARS-CoV-2 Real-Time PCR Kit was used.

4.3.1 RT-PCR results of 4 volunteers mouthwash and concentrated mouthwash samples obtained with bottled drinking water W1

Both mouthwashes and concentrated mouthwash samples obtain from bottle drinking water 1 belong to all four volunteers Ct result in Table 13

Table 13. Bottled drinking water's (W1) mouthwash and concentrated mouthwash Ct results comparison obtained from 4 different volunteers.

SAMPLE	Volunteer 1	Volunteer 2	Volunteer 3	Volunteer 4
Mouthwash	-	-	-	-
Concentrated Mouthwash	**33,13**	**34,38**	**31,61**	**33,32**

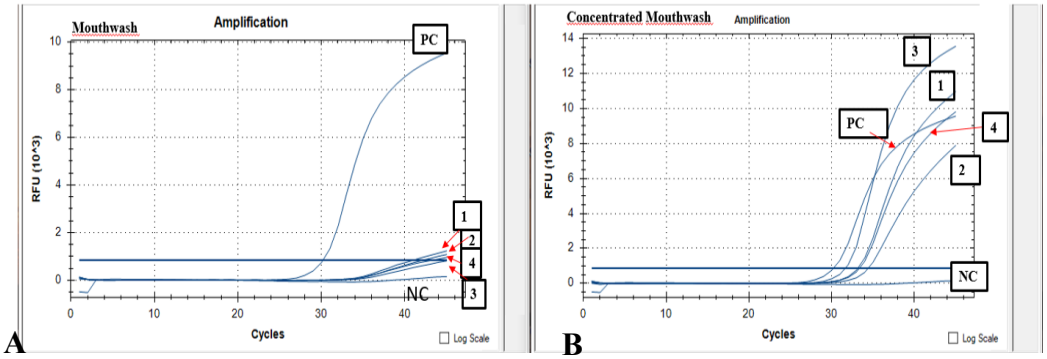


Figure 10. RT-PCR results of 4 volunteers' mouthwash and concentrated mouthwash samples comparison obtained with bottled drinking water W1 (Volunteer 1 (1), volunteer 2 (2), volunteer 3 (3), volunteer 4 (4), positive control (PC), negative control (NC)).

4.3.2 RT-PCR results of 4 volunteers mouthwash and concentrated mouthwash samples obtained with electric water fountain (D)

Elkay- electric water fountain connected to deionized water. Both mouthwashes and concentrated mouthwash samples obtain from deionized water belong to all four volunteers Ct result in Table 14.

Table 14. Electric Water Fountain deionized water's (D) mouthwash and concentrated mouthwash Ct results comparison obtained from 4 volunteers

SAMPLE	Volunteer 1	Volunteer 2	Volunteer 3	Volunteer 4
Mouthwash	**32,44**	**29,77**	**29,11**	**34,42**
Concentrated Mouthwash	**26,52**	**23,85**	**23,80**	**28,48**

Both mouthwash and concentrated mouthwash samples belong to all four volunteers RT-PCR results with deionized water comparison in Figure 11.

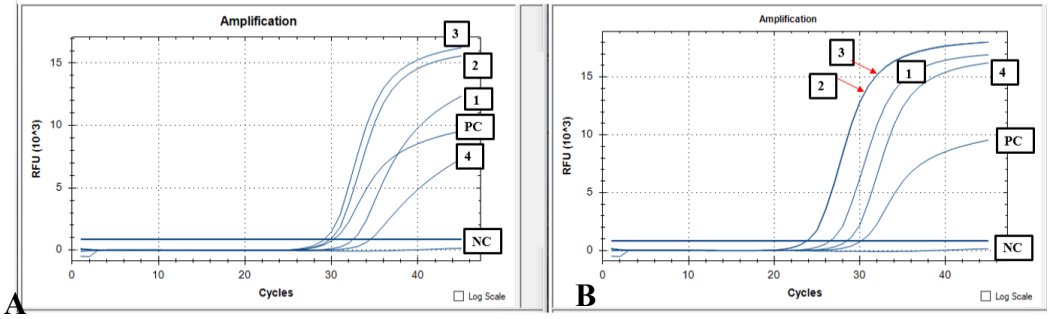


Figure 11. RT-PCR results of 4 volunteer's mouthwash (A) and concentrated mouthwash (B) samples comparison obtained with electric water fountain deionized water (D) on RT-PCR. ((Volunteer 1 (1), volunteer 2 (2), volunteer 3 (3), volunteer 4(4), positive control (PC), negative control (NC)).

4.3.3 RT-PCR results of 4 volunteers mouthwash and concentrated mouthwash samples obtained with Tap water (T)

Both mouthwashes and concentrated mouthwash samples obtain from tap water in Acibadem Mehmet Ali Aydinlar University belong to all four volunteers Ct result in Table 15

Table 15. Tap water's (T) mouthwash and concentrated mouthwash Ct results comparison obtained from 4 volunteers

SAMPLE	Volunteer 1	Volunteer 2	Volunteer 3	Volunteer 4
Mouthwash	**36,57**	**36,84**	**38,08**	**37,47**
Concentrated Mouthwash	**30,66**	**34,51**	**32,43**	**33,07**

Both mouthwash and concentrated mouthwash samples belong to all four volunteers RT-PCR results with tap water comparison in Figure 12.

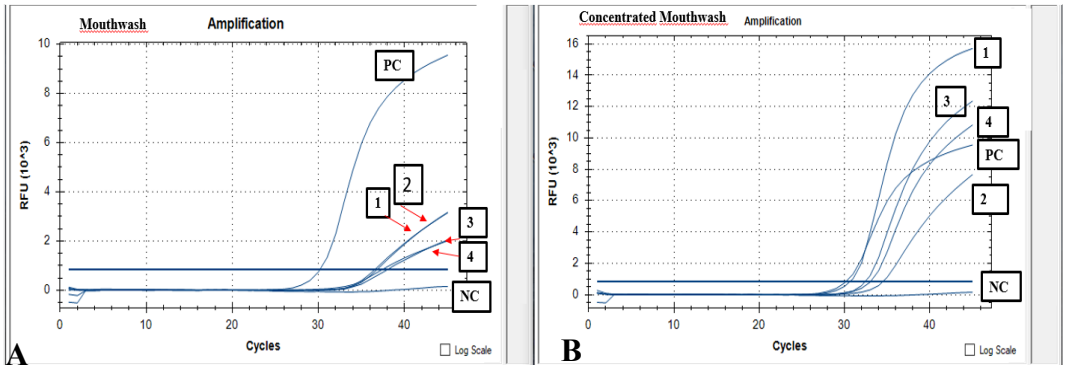


Figure 12. RT-PCR results of 4 volunteer's mouthwash (A) and concentrated mouthwash (B) samples comparison obtained with tap water (T) on RT-PCR. (Volunteer 1 (1), volunteer 2 (2), volunteer 3 (3), volunteer 4(4), positive control (PC), negative control (NC)).

4.3.4 RT-PCR results of 4 volunteers mouthwash and concentrated mouthwash samples obtained with Mineral Water (M)

Both mouthwashes and concentrated mouthwash samples obtain from mineral water belong to all four volunteers Ct result in Table 16

Table 16. Mineral water's (M) mouthwash and concentrated mouthwash sample Ct results comparison obtained from 4 volunteers

SAMPLE	Volunteer 1	Volunteer 2	Volunteer 3	Volunteer 4
Mouthwash	-	-	-	**38,80**
Concentrated Mouthwash	**33,01**	**39,33**	-	-

Both mouthwash and concentrated mouthwash samples belong to all four volunteers RT-PCR results with tap water comparison in Figure 13.

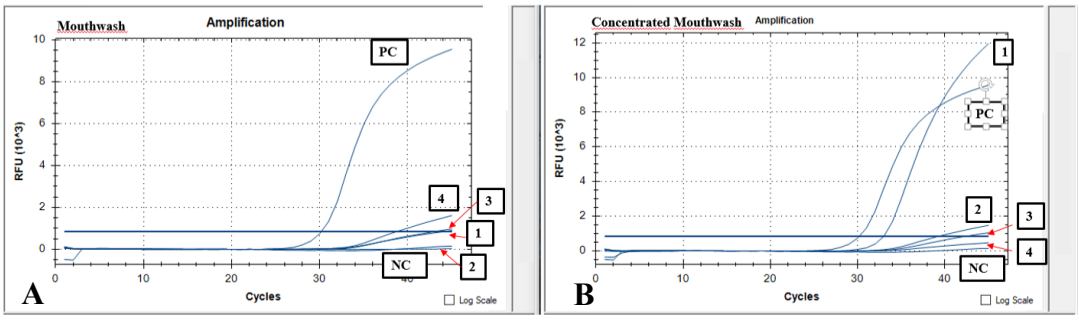


Figure 13. RT-PCR results of 4 volunteer's mouthwash (A) and concentrated mouthwash (B) samples comparison obtained with mineral water (M) on RT-PCR. (Volunteer 1 (1), volunteer 2 (2), volunteer 3 (3), volunteer 4(4), positive control (PC), negative control (NC)).

4.3.5 RT-PCR results of 4 volunteers mouthwash and concentrated mouthwash samples obtained with 0,9% NaCl Solution (S)

NaCl solution was prepared with distilled water.

Table 17. 0,9% NaCl Solution (S) mouthwash and concentrated mouthwash sample comparison obtained from 4 different volunteers

SAMPLE	Volunteer 1	Volunteer 2	Volunteer 3	Volunteer 4
Mouthwash	-	**38,23**	-	**39,86**
Concentrated Mouthwash	**37,76**	-	-	-

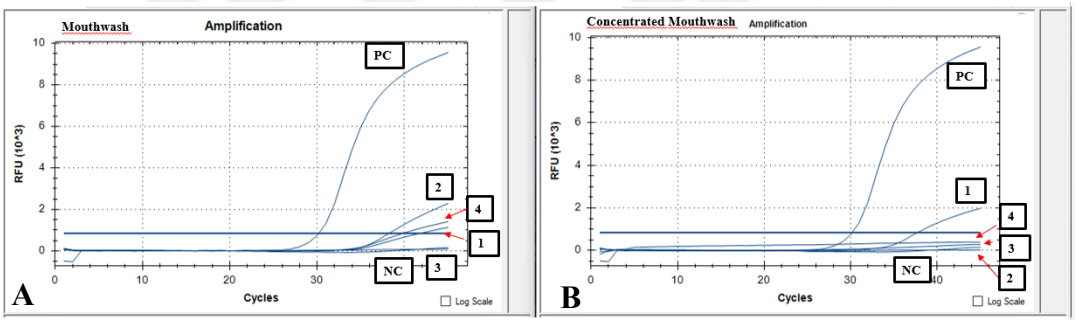


Figure 14. RT-PCR results of 4 volunteer's mouthwash and concentrated mouthwash samples comparison obtained with 0,9% NaCl Solution (S) on RT-PCR. (Volunteer 1 (1), volunteer 2 (2), volunteer 3 (3), volunteer 4 (4), positive control (PC), negative control (NC)).

4.4 Comparison of liquids merged on the same graph

4.4.1 All mouthwash and concentrated mouthwash results of volunteer 1

Table 18. Volunteer 1 mouthwash and concentrated mouthwash samples Ct values with different water sources comparison

SAMPLE	W1	W2	D	T	M	S
Mouthwash	-	38,32	32,44	36,57	-	-
Concentrated mouthwash	33,13	30,81	26,52	30,66	33,01	37,76

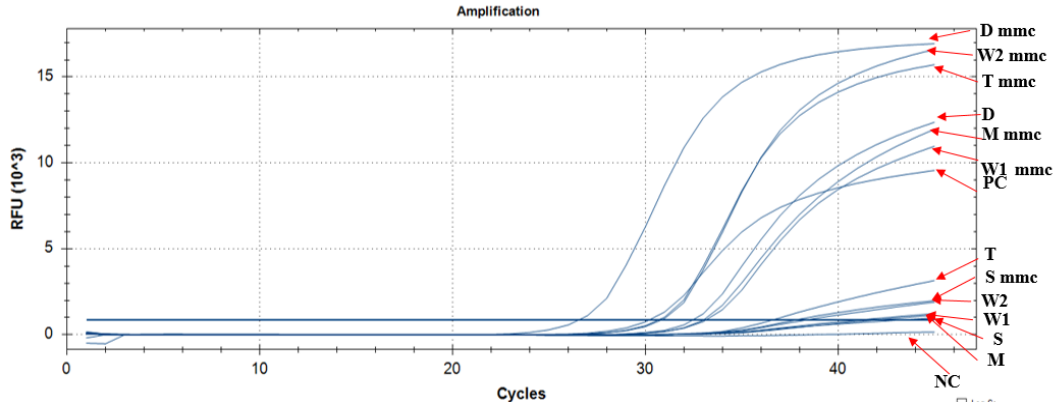


Figure 15. Comparison of RT-PCR results of volunteer 1 all mouthwash and concentrated mouthwash samples with different water types. (Bottled drinking water (W1) and bottled drinking water (W2), deionized water (D), tap water (T), mineral water (W) and 0,9% NaCl (saline) solution (S), concentrated with MyMagiCon–RW100® (mmc), positive control (PC), negative control (NC)).

4.4.2 All mouthwash and concentrated mouthwash results of volunteer 2

Table 19. Volunteer 2 mouthwash and concentrated mouthwash comparison Ct value with different water sources

SAMPLE	W1	W2	D	T	M	S
Mouthwash	-	36,69	29,77	36,84	-	38,23
Concentrated mouthwash	34,38	32,03	23,85	34,51	39,33	-

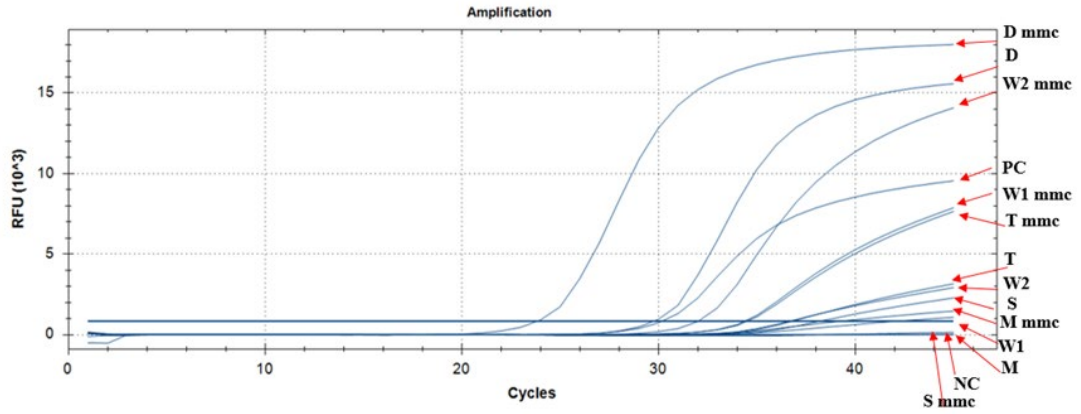


Figure 16. Comparison of RT-PCR results of volunteer 2 all mouthwash and concentrated mouthwash sample with different water types. (Bottled drinking water Hayat (W1) and Bottled drinking water Hamidiye (W2), deionized water (D), tap water (T), mineral water (W) and 0,9% NaCl (saline) solution (S), concentrated with MyMagiCon–RW100® (mmc), positive control (PC), negative control (NC)).

4.4.3 All mouthwash and concentrated mouthwash results of volunteer 3

Table 20. Volunteer 3 mouthwash and concentrated mouthwash comparison Ct value with different water sources

SAMPLE	W1	W2	D	T	M	S
Mouthwash	-	35,53	29,11	38,08	-	-
Concentrated mouthwash	31,61	27,79	23,80	32,43	-	-

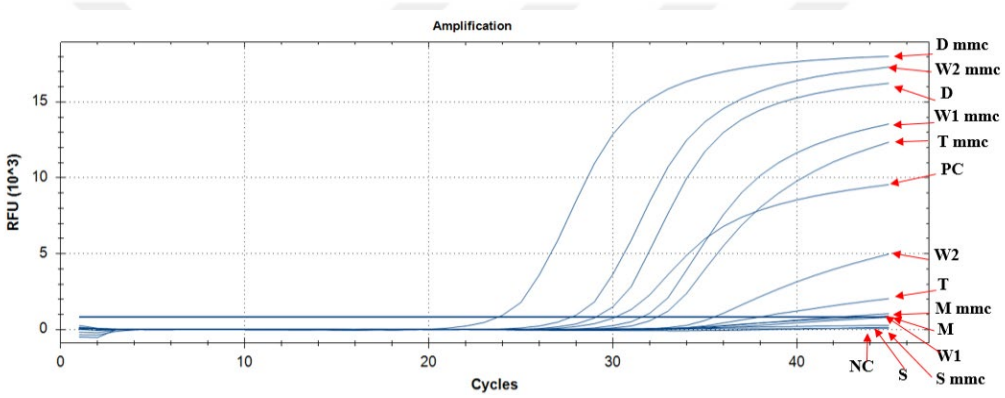


Figure 17. Comparison of RT-PCR results of volunteer 3 all mouthwash and concentrated mouthwash sample with different water types.

4.4.4 All mouthwash and concentrated mouthwash results of volunteer 4

Table 21. Volunteer 4 mouthwash and concentrated mouthwash comparison Ct value with different water sources

SAMPLE	W1	W2	D	T	M	S
Mouthwash	-	-	34,42	37,42	38,80	39,86
Concentrated mouthwash	33,32	32,50	28,48	33,07	-	-

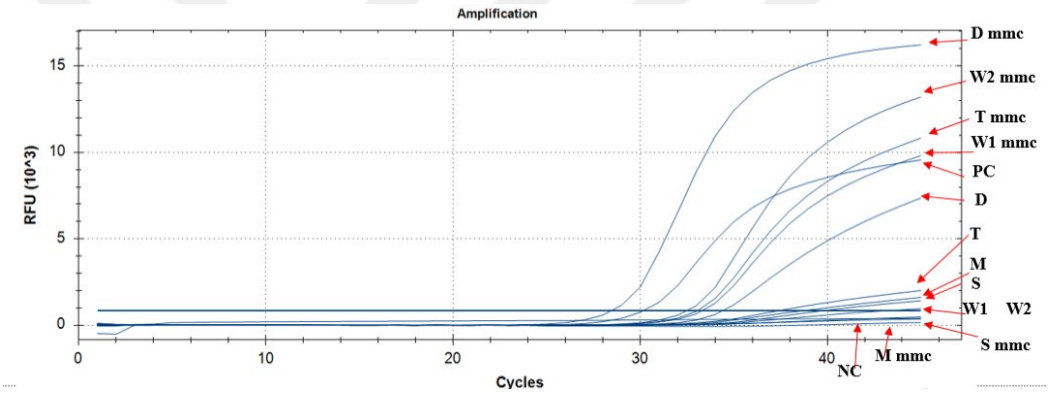


Figure 18. Comparison of RT-PCR results of volunteer 4 all mouthwash and concentrated mouthwash sample with different water types.(Bottled drinking water Hayat (W1) and Bottled drinking water Hamidiye (W2), deionized water (D), tap water (T), mineral water (W) and 0,9% NaCl (saline) solution (S), concentrated with MyMagiCon–RW100® (mmc), positive control (PC), negative control (NC)).

5 THE CONCENTRATION EFFECT OF MyMagiCon®

In 114 samples, SARS-CoV-2 RNA was identified in at least one of the three sample types which are nasopharyngeal, gargle, and mouthwash before concentration, after concentration with MyMagiCon®. Among the entire RT-PCR-positive patients, viral RNA was found in 76 (66.7%) nasopharyngeal, 67 (58.8%) gargle/mouthwash before concentration, and 101 (88.6%) after concentration (Figure 23). Ten patients had positive nasopharyngeal swab samples for SARS-CoV-2, but negative concentrated gargle/mouthwash samples.

On the contrary, SARS-CoV-2 was detected in 35 concentrated gargle/mouthwash samples, but not in nasopharyngeal samples from the same individuals.

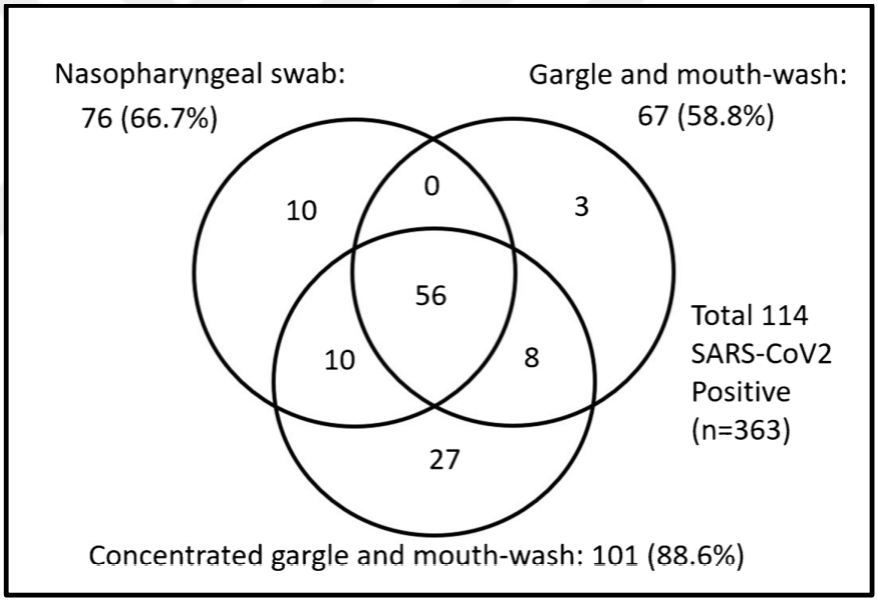


Figure 19. SARS-CoV-2 RNA detection using RT-PCR in nasopharyngeal swab samples, gargle, and mouthwash samples, before and after concentration by MyMagiCon–RW100® .

6 DISCUSSION

SARS-CoV-2 has been causing one of the deadliest pandemics in human history since its discovery in December 2019. COVID-19 triggered a global economic crisis, making it much more difficult to control the epidemic. Although various vaccinations are currently in use, it is expected that controlling the pandemic would take at least until the end of 2022. Protective masks, social distancing, and quarantine restrictions will continue to be the most significant methods to slow the pace of transmission in order to prevent overwhelming the health systems (35).The key to breaking the chain of transmission is rapid detection and isolation of infected patients before they spread the virus to uninfected people. The most crucial diagnostic approach for identifying the presence of COVID-19 is the detection of SARS-CoV-2 RNA in nasopharyngeal swab samples using RT-PCR (36, 37).

Nasopharyngeal swab sampling is a time-consuming application that has caused long lineups in front of testing facilities throughout the world, with people waiting for hours to deliver a nasopharyngeal swab sample. At the same time, it should be done carefully by well-trained healthcare workers. Nasopharyngeal specimen collection is often due to the waiting time for healthcare workers to collect nasopharyngeal swabs (NPS) which causes significant discomfort to patients, and it is associated with infection control risk to healthcare workers (38, 39). However, health care workers (HCW) must be worn with personal protective equipment (personal protective equipment: respirator, eye protection, gloves, and gowns) to get a swab sample, because it may trigger the gag reflex of the sampled person and cause coughing (40). SARS-CoV-2 virus is likely to be found in the oral secretions of COVID-19 patients. The oral cavity epithelial cells have been demonstrated to have a high level of ACE2 receptors, which play an important role in SARS-CoV-2 entrance and replication (41). SARS-CoV-2 virus was found in the saliva of COVID-19 patients in several research. When compared to the usual diagnosis of nasopharyngeal swabs, RT-PCR analysis of saliva specimens had a sensitivity of 66 to 92 percent for COVID-19 (42, 43). For all these reasons, there was a need for another diagnostic sampling method with the same sensitivity as an alternative to nasopharyngeal sampling. The most promising sampling method for the diagnosis of COVID-19 is mouthwash sampling which is much more

comfortable. The patient can obtain his own mouthwash sample. These self-collected samples are simple to handle for patients of all ages. They are non-invasive and risk-free for medical personnel. The use of a gargle/mouthwash sample instead of nasopharyngeal sampling would avoid patient discomfort and reduce the risk of transmission to the healthcare workers.

In a recent study, Bennet et al. showed that gargle samples are more sensitive in detecting viral respiratory infections, and this is the first indication that gargle samples can be used to diagnose SARS-CoV-2 (44). In another study, Goldfarb et al. recently compared self-collected saline gargle samples to health professional nasopharyngeal swabs for SARS-CoV-2 diagnosis. Saliva and nasopharyngeal swab samples were found to be much more likely to be positive than mouthwash and gargle samples. They also demonstrated that SARS-CoV-2 RNA is much more stable in gargle/mouthwash samples than in nasopharyngeal swab samples, especially in long-term storage (45).

In this study, we have observed the possibility of using gargle/mouthwash samples, after concentrating them on a new product named MyMagiCon® for the diagnosis of COVID-19, as an alternative to nasopharyngeal swab sampling. MyMagiCon® is a powder combination with unique polymer beads that quickly remove small molecules from solutions. The elastic polymer beads expand rapidly by absorbing water and other small molecules and concentrating microorganisms and macromolecules. MyMagiCon–RW100® concentrates gargle/mouthwash samples 10 to 20 times for the diagnosis of infectious agents such as SARS-CoV 2, influenza virus, and other agents causing illness in the respiratory system. Microorganisms are concentrated when they are intact. Even if the organism is lysed and its nucleic acids and antigens are released into the solution, they will be concentrated as well (27).

Firstly, we studied the stability of SARS-CoV-2 in mouthwash samples. For determining the effect of storage conditions at different temperatures for different days, ten SARS-CoV-2 negative volunteers were chosen to sample mouthwash. After storing the mouthwash samples at room temperature for three days, there was no loss of viral RNA and only a tenfold drop after ten days. Storing viral RNA at 4°C conserved it as well as room temperature, but it did not increase the amount of viral RNA detectable by RT-PCR. Based on these findings, the possibility of using

concentrated mouthwash samples instead of nasopharyngeal swab samples would have the same or better efficiency, has arised (Figure 5).

Secondly, experiments were performed on 6 different water sources with 4 different volunteers to determine the effect of different liquid types which are used for mouthwash, on sensitivity of PCR results. Six different liquid types which are two different brands of water (W1 and W2), deionized water (D), tap water (T), mineral water (W), and 0,9% NaCl (saline) solution (S) were used. When the results of volunteer 1 are examined, bottle drinking water 1 (W1), W2, mineral water (M) and, 0,9% NaCl (saline) solution S mouthwash samples gave Ct values close to the negative control. In contrast, when these samples were concentrated with MyMagiCon–RW100®, all the concentrated mouthwash samples indicated the amplification of viral nucleic acids. Although the same amount of inactive virus was added in all mouthwash samples, the most sensitive Ct value (26,52) was obtained with mouthwash obtained with deionized water and concentrated (D mmc) with MyMagiCon–RW100® (Table 9). All concentrated mouthwash samples gave a much stronger amplification signal than mouthwash samples that are not concentrated. The difference in the RT-PCR results of the samples concentrated MyMagiCon–RW100® is seen clearly (Figure 6).

When mouthwash and concentrated mouthwash Ct results of volunteer 2 compare each other, deionized mouthwash concentrated (D mmc) with MyMagiCon–RW100® has the best sensitive Ct values (Table 10). As in volunteer 1, the concentrated mouthwash samples with MyMagiCon–RW100® in volunteer 2 results showed better amplification by RT-PCR (Figure 7). When we compared the data of volunteer 3, deionized mouthwash concentrated with MyMagiCon–RW100® (D mmc) once again produced the best outcome (Tablo 11). Mineral water (M) and salt water (S) had the non- detectable results. Because the ion load in mineral water and saline water was too high, the results in RT-PCR were the closest to negative control (Figure 8). As with the other volunteers, results the concentrated mouthwash samples with MyMagiCon–RW100 produced better amplification with RT-PCR in volunteer 4 (Table 12),(Figure 9). The highest sensitivity in detecting SARS-CoV-2 for all volunteers was with deionized water used for gargling, which was subsequently concentrated with MyMagiCon–RW100®. Overall, deionized water was shown to be

the best option for preserving the RNA collected from the patient's mouthwash. Despite the fact, that all concentrated samples produced a strong signal, deionized water performed well in both mouthwash and concentrated mouthwash. The reason why deionized water gives the best results is that effect of the hardness of the water used as mouthwash. Hardness of the deionized water is 19 ppm (Table 3). If the hardness of the water is low which means that the ion load is low in the water, the amplification with RT- PCR will be better since the ions in may be inhibiting the PCR reaction. Soft water with the lowest ionic charge should be used for the more sensitive RT- PCR results. As a conclusion of this experiment, it was determined that lower the ion concentration in water used for mouthwash, better was PCR amplification.

Thirdly, we examined each volunteer's mouthwash and concentrated mouthwash samples, we compared the effect of the same fluids for 4 volunteers' mouthwash and concentrated mouthwash samples on the sensitivity of PCR used for the diagnosis of COVID-19. The aim of this experiment was to determine whether different people mouthwash samples may differently affect PCR amplification or not. Four SARS-CoV-2 negative volunteers gave mouthwash sample. Then, inactive coronavirus was added to the collected samples. Half of the samples were concentrated by using MyMagiCon®, whereas other half kept as mouthwash without any process. RT-qPCR was applied to each of the samples in order to understand how concentrated samples' results differed from non-concentrated ones. As a result of the experiment, no amplification was obtained from mouthwash samples.

In contrast, all the concentrated mouthwash samples showed amplification is very close to the positive control (Figure 10). The CT values of each sample is shown in table 13. Similarly, Ct value of signals obtained from untreated mouthwash samples were non-detectable, however all concentrated samples produced quite powerful signals. This indicates that concentration process paved the way for detecting SARS-CoV-2 in patients' samples with low concentration of virus. With deionized water, the Ct values of samples concentrated with MyMagiCon–RW100® showed that amplification was started approximately 6 cycles ahead for all 4 volunteers (Table14). Despite using the same amount of inactive virus, Volunteer 2 gave the best result in mouthwashes using deionized water than other 3 volunteers Figure (11). For tap water, concentrated mouthwash sample of Volunteer 1 gave the most sensitive result than

others (Figure 12). These showed that there may be variability of the composition of mouth secretions between different people. For mineral water results, when the direct mouthwashes of 4 volunteers are performed by RT-PCR, the values obtained are very close to each other and also to negative control, but the difference with the volunteer 1 was very prominent when they are concentrated (Figure 13).

For saline solution, the results of four participants were nearly identical in the negative control group. The PCR is affected by the excessive ion load in the saline solution (Figure 14). At the conclusion of this study, it was observed that if participants drank water before giving mouthwash, this had an impact on the outcomes since it probably changed both the mouthwash's ion and viral load.

When we compare all the results of volunteer 1 within itself, it was observed that the Ct value of the deionized water used for gargling and then concentrated with MyMagiCon–RW100®) sample is the lowest, which means the PCR amplification is the best. Concentrated mouthwash samples with MyMagiCon–RW100® worked much better compared to untreated mouthwash samples. All in all, solution D was found to be best options to keep the RNA obtained from patient's mouthwash. Despite the fact that all concentrated samples gave significant amplification signal, (except solution S), deionized water worked quite well both untreated mouthwash and concentrated samples (Figure 15).

The same results were obtained in volunteers 2, 3, and 4 (Figure 16-18). A concentrated sample of each of them produced more sensitive results. As mentioned before, the best choice for retaining the RNA obtained from the patient's mouthwash was concentrated deionized water. Since deionized water does not contain any molecule other than water it can be assumed that mouth secretions are themselves very good preservatives for SARS-CoV-2 virus. In conclusion of this experiment, the water solution that will be used by patient to wash their mouth is playing a crucial role in protecting RNA of interest inside the solution. Since RNA is quite fragile, the solution must provide a buffer effect between the time interval of taking the samples and introducing the samples into the qPCR machine.

In this research, it is determined that the efficiency of MyMagiCon–RW100® as an alternative to a nasopharyngeal swab in the diagnosis of Covid19. After being

concentrated by MyMagiCon–RW100®, the findings of this research showed that gargle/mouthwash samples can be used as effectively as nasopharyngeal swab samples, in the diagnosis of COVID-19.

Among the 114 patients who had SARS-CoV-2 RNA identified by RT-PCR in at least one of the nasopharyngeal or gargle/mouthwash samples, 76 (66.7 percent) had SARS-CoV-2 RNA identified in nasopharyngeal swab and 67 (58.8 percent) had SARS-CoV-2 RNA identified in gargle/mouthwash samples (Figure 25). When gargle/mouthwash samples were concentrated using MyMagiCon–RW100®, SARS-CoV-2 RNA could be detected in 101 (88.6%) of them, making them better samples for COVID-19 diagnosis than nasopharyngeal samples. SARS-CoV-2 positive nasopharyngeal swab samples from ten individuals were negative in concentrated gargle/mouthwash samples. At the same time, SARS-Cov-2 was positive in 35 concentrated gargle/ mouthwash samples, which were negative in nasopharyngeal samples of the same patients. (Figure 19).

Using mouthwash samples instead of nasopharyngeal swab samples will improve patient comfort, eliminate the negative effects of nasopharyngeal swab sampling, significantly reduce the infection risk of health personnel obtaining the samples, and significantly reduce the workload of healthcare facilities. Moreover, it has been determined that using MyMagiCon–RW100® for concentration of samples is a very good alternative sampling method for diagnosis. It can also be used in tuberculosis diagnosis to improve the sensitivity of antigen assays for tuberculosis antigens in tuberculosis patient urine samples

7 CONCLUSION

In conclusion, using gargle/mouthwash samples instead of nasopharyngeal swab samples will improve patient compliance, eliminate the negative effects of nasopharyngeal swab sampling, significantly reduce the risk of infection among health personnel obtaining the samples, and significantly reduce the workload of healthcare facilities. MyMagiCon–RW100® may enable quick diagnosis from mouthwash samples when rapid antigen tests with sensitivities near to RT-PCR become available, which might be used in hospitals or even at home for self-testing.

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9 APPENDIX

APPENDIX 1

10 CURRICULUM VITAE