Simple Concentration Method Enables Using Gargle and Mouthwash Instead of Nasopharyngeal Swab Sampling for the Diagnosis of Covid19 by PCR

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Abstract

Since its emergence in December 2019, SARS-CoV-2 is causing one of the most devastating pandemics in human history. Until majority of populations will be immunized with an effective vaccine, one of the most important measures to break the chain of transmission will continue to be early diagnosis and isolation of people infected with SARS-CoV-2. Currently, the most important method for definitive diagnosis of COVID-19 is identification of SARS-CoV-2 RNA in nasopharyngeal swab samples by Real-Time Polymerase Chain Reaction. Nasopharyngeal swab sampling is a discomforting procedure sometimes with adverse effects, which also poses a risk for infection for the personnel performing the sampling. We have developed a new method and tool for concentrating liquid biological samples, which enabled us to use gargle and mouth-wash samples to be used in RT-PCR, for the diagnosis of COVID-19, as an alternative to nasopharyngeal swab samples. We have analyzed the nasopharyngeal and gargle and mouthwash samples, before and after concentration, of 174 patients by RT-PCR for the presence of SARS-CoV-2. Among 72 patients in which SARS-CoV-2 was identified in at least one of their samples, the virus was identified in 57 (79.2%), 45 (62.5%) and 63 (87.5%) of nasopharyngeal swab, gargle and mouth-wash samples before and after concentration, respectively. When concentrated by our new method, gargle and mouthwash samples can be used instead of nasopharyngeal samples in identification of SARS-CoV-2 by RT-PCR, with the same or even better sensitivity. Eliminating the need for nasopharyngeal sampling, will save the patients from an invasive and painful procedure and will lower the risk of infection for the healthcare personnel taking the sample. This easy sampling procedure may decrease the workload of hospitals, shorten the turn-around time of obtaining test results and thus enable rapid isolation of infected patients.

Introduction

Severe acute respiratory syndrome (SARS-CoV-2) is a positive-sense, and single-stranded RNA (ssRNA) virus (Siddell SG). Since its emergence in December 2019, SARS-CoV-2 is continuing to cause one of the most devastating pandemics in human history. COVID-19 led entire world to face an economic crisis, which additionally makes it harder to control the pandemic. Although several promising vaccine candidates are under development it is estimated that it will take at least until the end of 2021 to control the pandemic. Until majority of world population develops immunity by effective vaccination or by being infected, protective masks, social distancing, and quarantine rules will continue to be most important measures to slow down the speed of transmission in order to stop to overwhelm the health systems (Nicola M et al.).

Rapid diagnosis and isolation of people carrying SARS-CoV-2 before they transmit the virus to uninfected people is the key to break the chain of transmission. Currently, the most important diagnostic method for definitive diagnosis of COVID-19 is identification of SARS-CoV-2 RNA in nasopharyngeal swab samples by RT-PCR (Tang YW et al; Afzal A). Nasopharyngeal swab sampling is a painful process for patients, sometimes leading to serious complications. It requires trained personnel and poses a risk of infection for the person who does the sampling (Gopaul R et al.; Gupta K et al; Mughal Z et al.). It is a time consuming application which created waiting lines in front of the testing centers in many parts of the world, by the people who wait for hours to give a nasopharyngeal swab sample. In this study, we have investigated the possibility of using gargle and 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 mixture that contains a special polymer that removes small molecules quickly from solutions. The elastic polymer beads swell quickly by absorbing water and other small molecules, concentrating microorganisms and macromolecules. *MyMagiCon-RW100*® is intended for concentrating gargle and mouthwash, for the diagnosis of infectious agents like SARS-CoV-2, Influenza virus and other agents causing infection in the respiratory system. Microorganisms are concentrated if they are in intact form. However, even if the organisms are lysed and their nucleic acids and antigens are released in the solution, these will be also concentrated, since molecules larger than approximately 0.5kD will stay outside the polymer beads while small molecules, that can penetrate the pores of polymer meshes, will be

removed. Thus, *MyMagiCon-RW100*® concentrates the microorganisms and their macromolecules 10 to 20 times.

Methods

Stability of SARS-CoV-2 in mouthwash samples

To evaluate gargle and mouthwash as an alternative to nasopharyngeal swab samples for the diagnosis of COVID-19, we first evaluated the stability of SARS-CoV-2 in mouthwash samples. For this purpose, mouthwash samples were collected from 10 healthy volunteers. From five of these samples, 1 mL aliquots were spiked with SARS-CoV-2, grown in cell culture and inactivated, to a final concentration of 10^{15} and the other five with 10^{14} copies/mL. Each aliquot was split into two equal parts in micro-centrifuge tubes and one set of these were kept at room temperature while the other set at 4°C. The concentration of virus in each sample was quantified by RT-PCR using a commercial kit (Bioeksen, Istanbul, Turkey) at days 0, 3, 5, 7 and 10. 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 (Figure 1).

Determination of the sensitivity of RT-PCR in identification of SARS-CoV-2 in nasopharyngeal and concentrated mouthwash samples

A total of 174 volunteers above the age of 18, who admitted to the Acibadem Altunizade Hospital (Istanbul) by symptoms of respiratory infection, were included in the study. After collecting nasopharyngeal swab samples, patients were instructed to take a few sips of regular drinking water, and then to gargle and rigorously rinse their mouth with this water for at least 10 seconds and put it back to an empty cup.

Gargle and mouthwash samples were concentrated using *MyMagiCon-RW100*® as instructed in the user guide. Briefly, 20 mL of sample was put into the tube and waited for 5 minutes for the absorbent beads to swell and absorb most of the fluid and the mixture turned in a gel-like form. The concentrated sample was collected with an automatic pipette by inserting the pipette tip in between the beads and aspirating the fluid.

Nasopharyngeal swab sample, gargle and mouthwash before and after concentration, were analyzed for the presence of SARS-CoV-2 using commercial PCR kits (Bioeksen and A1 Lifesciences, Istanbul, Turkey).

Results

The stability of SARS-CoV-2 RNA in mouthwash samples, stored at room temperature and 4°C, is shown in Figure 1. There was no significant change in the copy number of SARS-CoV-2 RNA after three days of storage at both room temperature and 4°C. There was about one log decrease after storing 10 days at both of these temperatures. Storing mouthwash samples either at room temperature or 4°C, showed no significant difference in the stability of viral RNA.

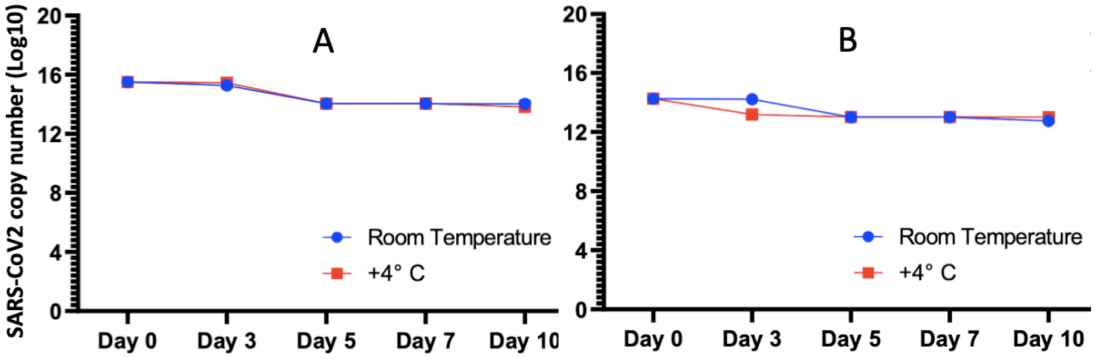

Figure 1. The stability of SARS-CoV-2 RNA in mouthwash samples at room temperature and 4°C. The starting number of RNA copies in samples shown in graph A was 10^{15} and in samples shown in graph B was 10^{14}.

In 72 samples, SARS-CoV2 RNA was identified in at least one of the three sample types. The viral RNA was detected in 57 (79.2%) nasopharyngeal, in 45 (62.5%) gargle and mouthwash before concentration and in 63 (87.5%) after concentration among the total RT-PCR positive patients (Figure 2). Concentration of samples increased the number of samples in which SARS-CoV-2 RNA was detected by 25%. The effect of concentration on the detection of SARS-CoV-2 RNA is shown in Figure 3. In samples having high concentration of virus, compared to the original sample, the amplification curve passed the cut off threshold (CT) value several cycles earlier after concentration by *MyMagiCon*® (Figure 3-A). In samples which contained the virus at a concentration of below the limit of detection, it became possible to detect the virus after concentration (Figure 3-B).

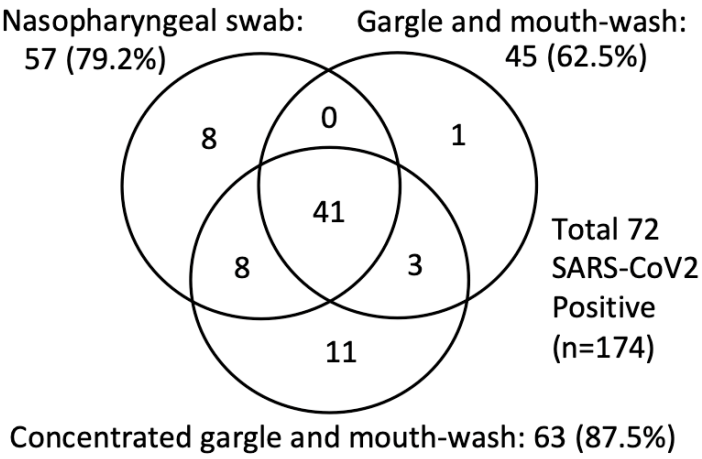

Figure 2. Detection of SARS-CoV-2 RNA by RT-PCR in nasopharyngeal swab samples, in gargle and mouthwash samples before and after concentration by *MyMagiCon-RW100*®.

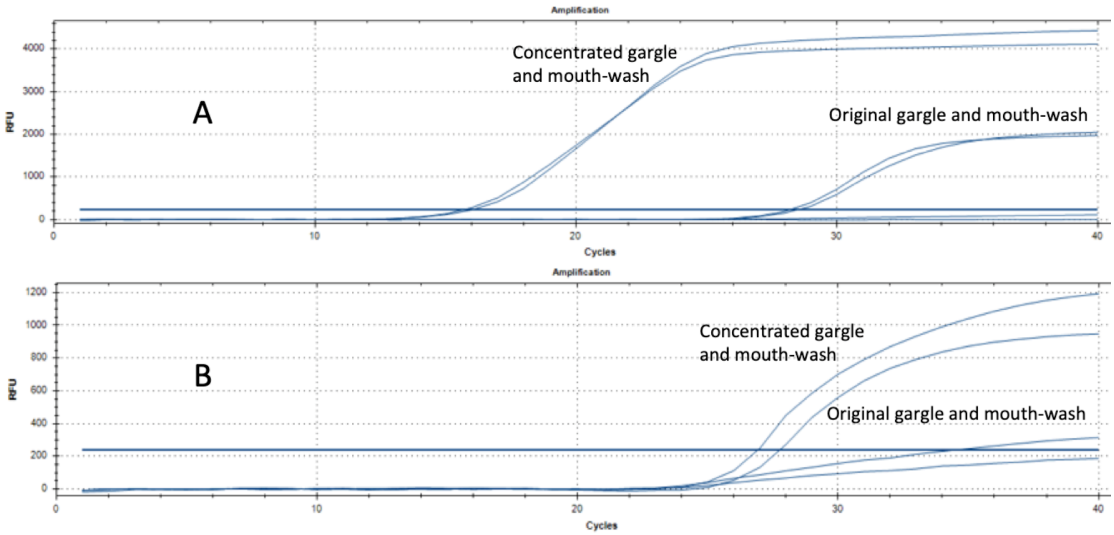

Figure 3. The effect of concentration of gargle and mouthwash samples on the detection limit of SARS-CoV-2 by RT-PCR. Each sample was evaluated in duplicate. In graph A, it was possible to detect SARS-CoV-2 without concentration at later cycles, however, the detection of the virus was not possible without concentration in the example shown in graph B.

Discussion

COVID-19 became one of the most devastating pandemic in human history. As in all pandemic diseases rapid diagnosis of people carrying the infectious agent and their quarantine before they transmit the disease to healthy people

is the key measure to control the disease in large populations (Nicola M et al.). Currently, PCR is the main diagnostic tool for rapid and sensitive diagnosis of COVID-19 (Tang YW et al; Afzal A).

The gold standard test for detecting SARS-CoV-2 is considered to be the analysis by RT-PCR of a sample obtained by nasopharyngeal swab. Therefore, the most widely used application for obtaining samples for PCR is by a nasopharyngeal swab. However, besides being very discomforting, many adverse effects associated with nasopharyngeal swab sampling, including epistaxis in 8.3% of cases, have been reported. Additionally, nasopharyngeal swab sampling poses an important risk of transmission of the virus to healthcare personnel who have to do tens hundreds of sampling every day, in centers with high admission rate (Gopaul R et al.; Gupta K et al; Mughal Z et al.).

It is expected to find SARS-CoV-2 in the oral secretions of COVID-19 patients. Epithelial cells in the oral cavity have been shown to express large amount of ACE2 receptors, which plays a key role in the entry and replication of SARS-CoV-2 (Xu K et al.). Nasopharynx and oropharynx are not separated from each other anatomically and it is logical to think that the secretions in the nasopharynx will be mixed into the oral secretions. Additionally virus particles in the blood may pass into exudates produced in the oral cavity.

Several studies revealed the presence of SARS-CoV-2 in saliva of Covid19 patients. The sensitivity of RT-PCR analysis of saliva specimens was 66% to 92% for COVID-19 as compared with the standard diagnosis with nasopharyngeal swabs (Azzi L et al; Fernandes LL et al.). In this study, we have shown that SARS-CoV-2 RNA is pretty stable in oral secretions if it is stored at room temperature. There was not any loss of viral RNA after storing the mouthwash samples for 3 days at room temperature and only ten-fold decrease at the end of 10 days. Storing at 4°C preserved the viral RNA as well as room temperature but it did not improve the quantity of viral RNA that can be detected by RT-PCR. Inspired by these data we investigated the possibility of using concentrated gargle and mouthwash samples instead of nasopharyngeal swab samples with the same or better efficiency in the diagnosis of COVID-19.

Recently, we have developed a method for concentrating biological fluids by the help of elastic polymer meshes that absorb water and other molecules and thus concentrate microorganisms and macromolecules. In this study we have evaluated the efficiency of *MyMagiCon–RW100*®, which is intended for concentrating gargle and mouthwash.

The results of this study showed that gargle and mouthwash samples can be used as efficiently as nasopharyngeal swab samples, after concentrating by *MyMagiCon–RW100*®. Among all 72 patients in whom SARS-CoV-2 RNA was identified by RT-PCR in at least one of the nasopharyngeal or gargle and mouthwash samples, 57 (%79,2) was identified in nasopharyngeal swab and 45 (%62,5) in gargle and mouthwash samples. However, when gargle and mouthwash samples were concentrated by *MyMagiCon–RW100*®, it was possible to identify SARS-CoV-2 RNA in 63 (%87,5) of samples making them better samples than nasopharyngeal samples for the diagnosis of COVID-19. An interesting finding was the presence of SARS-CoV-2 RNA in only nasopharyngeal samples of 8 patients and in only concentrated gargle and mouthwash samples of 11 patients. If the virus was really present in only nasopharyngeal or oropharyngeal cavity at the time of sampling, or there was a problem with collecting the samples properly, needs further investigation.

Using gargle and mouthwash samples instead of nasopharyngeal swab samples will increase the patient compliance, eliminate the adverse effects of nasopharyngeal swab sampling, significantly decrease the infection risk of health personnel obtaining the samples and prominently lower the workload of healthcare centers. When the rapid antigen tests with sensitivities close to RT-PCR become available, *MyMagiCon–RW100*® may enable rapid diagnosis from mouthwash samples which may be applied in hospitals or even at homes for self-testing.

Acknowledgments

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Conflict of Interest

Authors, Tanıl Kocagöz and Özge Can are the founders and shareholders of GigaBioMol and Bio-T companies.

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