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Evaluation and Validation of a New Sample Collection Kit Based on RT-PCR for the Detection of Coronavirus (SARS-COV-2)

R. N. Jawad (PhD)[1], H. K. Abdul-Sada (PhD)[*2]

1.Department of Nursing Technical, Technical Institution of Basra, Southern Technical University, Basra, Iraq.
2.Al-Zahraa College of Medicine, University of Basrah, Basrah, Iraq.

•Corresponding Author: H. K. Abdul-Sada (PhD)
Address: Al-Zahraa College of Medicine, University of Basrah, Basrah, Iraq.
Tel: +964 (773) 3975916. **E-mail:** Hussein.abdul-sada@uobasrah.edu.iq

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ABSTRACT

Background and Objective: During the COVID-19 crisis, all countries faced a major challenge in providing resources to isolate the new coronavirus, which was due to lack of required materials. A new cough kit has recently been developed that is easier to use than a nasopharyngeal or pharyngeal swab. The aim of this study is to evaluate and validate a new sample collection kit based on RT-PCR for detection of coronavirus (SARS-COV-2).

Methods: In this cross-sectional study, the sputum of 40 patients and 20 healthy individuals were collected through a new sample collection kit for coronavirus (SARS-COV-2) and nasopharyngeal swab. The kit consists of a conical sterile paper mask that covers the patient's mouth and nose. The conical filter contains a polyester polymer swab to collect coughed sputum droplets for sputum extraction. The swab is separated from the cone and placed in the virus transmission medium. The results based on RT-PCR for detection of coronavirus (SARS-COV-2) in two methods of sample collection were analyzed and compared.

Findings: The new sample collection kit was able to isolate SARS-COV-2 by RT-PCR from sputum and saliva through cough. The results were positive in 32 of 40 (80%) patients using the cough kit and 21 of 40 (52.5%) samples were positive with nasopharyngeal swab examination. The number of samples that were positive using both methods was 16 (40%). The number of samples that were negative by examining nasopharyngeal swabs and positive using cough swabs was 16 samples (40%). This indicates that cough swabs were 40% more accurate than nasopharyngeal swabs.

Conclusion: Based on the results of this study, the new kit can be used to isolate the SAS-COV2 virus in an infected person instead of a nasopharyngeal swab.

Keywords: *COVID-19, Cough, Sputum, Mask Kit.*

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Introduction

The new coronavirus SARS-COV2 is considered one of the advanced viral types of the Coronavirus family, which causes acute respiratory infection, from which its name is derived. The disease appeared in the Chinese city of Wuhan and was then recorded in many countries of the world until the World Health Organization (1) declared it a global epidemic on 3/11/2020. 121,564 cases of infection were recorded in more than 110 countries in the world, and 4,373 cases of death were recorded on that date (2).

The health protocols of the World Health Organization have been adopted for taking samples from the pharynx or nasopharynx of infected people. Many studies showed that isolating the virus from sputum samples was more effective than isolating it from the nasal area (3). The virus was effectively isolated from saliva using passive drooling method (4). The sputum and oropharyngeal secretions are considered the basic samples for examining the presence of the virus using RT-PCR technology (5). Saliva particles are also considered the primary source of transmission of the Coronavirus from one person to another (6). It has also been scientifically proven that all viruses that infect the respiratory system are abundant in saliva and respiratory droplets and can be detected directly from them (7).

Cellulose filters with 0.45 micrometer holes were used to isolate the 200 nm varicella-zoster virus from the droplets of infected people with viral pneumonia, and to isolate respiratory syncytial virus with a size of 150 nm (8). Other types of filters have been produced, such as polycarbonate filters and polytetrafluoroethylene (PTFE) filters (9), all of which aim to isolate viruses present in the respiratory tract that can come out with droplets from a person carrying the virus. Recently, a gelatin filter was used to isolate the coronavirus. This filter was used to ensure that the air surrounding infected patients was free, and it was found that the virus was not present in the air of isolation rooms (10).

A kit consisting of a paper filter with a polyester swab inside it was produced at the University of Basrah to collect the droplets from the infected person's cough. The swab was then placed in a medium conveyor tube to be transported to the laboratory for viral detection by RT-PCR technique examination.

Because all countries faced a major challenge in providing resources to isolate the new coronavirus due to lack of materials and distressingly, a new cough kit was developed, which is more convenient than a nasopharyngeal or pharyngeal swab.

The purpose of this study is to evaluate and validate a new sample collection kit based on RT-PCR for detection of coronavirus (SARS-COV-2).

Methods

This cross-sectional study was approved by the ethics committee of the Al-Zahraa College of Medicine, University of Basrah under the code E/T 36.

The entire kit and all raw materials were manufactured during the study to cover the needs of the health departments to confront the coronavirus crisis.

Samples: 40 coronavirus patients at different disease stages were hospitalized in the Basrah Teaching Hospital in the Epidemiological department, and they were asked to use the coughing kit. 20 healthy people were also examined as a negative control group.

Coughing kit design: A cone shape paper filter was used with dimensions (base 52 mm, longest arm 68 mm, smallest arm 62 mm) as shown in image 1. It was opened to a distance of 120 mm to cover the nose and mouth together, as shown in image 2. This filter contained holes ranging from 60-70 micrometers at the bottom to serve as an entrance for the swab stick, as shown in image 3. A swab made of polyester polymer (0.0150 grams) for medical use with a 70 mm length of plastic stick and a plastic mask were used. The

rolled polyester was passed on a Hot Plate at a temperature of 150°C between two pieces of aluminum cellophane sterilized with alcohol to avoid adhesion of the polyester due to the heat. A plastic stick was placed inside the cone and its handle exited from the pierce, covering the polyester over a distance of 40 mm, as shown in image 4.

Two methods of dry heat sterilization with ultraviolet radiation were adopted according to Bae et al. method (11). An Ethylene Oxide (ETO) gas cabin was also used as an alternative to ultraviolet sterilization.

Study of the possibility of isolating cough particles: The isolation of cough droplets inside the filter was confirmed, as cough particles were photographed on the surface of the swab inside the filter using an optical microscope (Olympus cx 23, Germany).

Study of the efficiency of the kit in isolating the new coronavirus: The kit was tested by using it to isolate samples from 20 healthy and 40 patients hospitalized in Basra Teaching Hospital. The filter was given to people diagnosed with the virus, and they coughed several times into the filter, then the swab was placed in the carrier medium and transferred to the central laboratory for RT-PCR testing. A questionnaire about their opinion on the new method was used to see if it were more comfortable and to evaluate its speed.

Statistical analysis: Statistical analysis was performed by SPSS version 26, the data were presented as frequencies and percentage, and the association was measured by Chi-square test when the expectation frequencies were less than 5. P-value less than 0.05 was considered as significant.



Image 1. Dimensions of the cone paper filter in millimeters

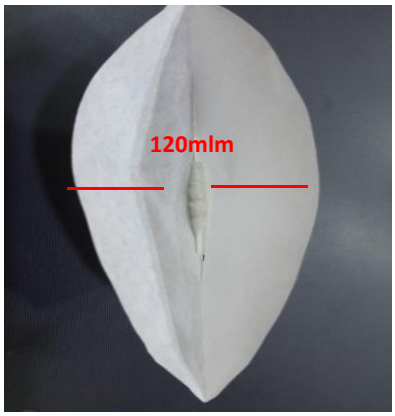


Image 2. The diameter of the filter opening to cover the nose and mouth

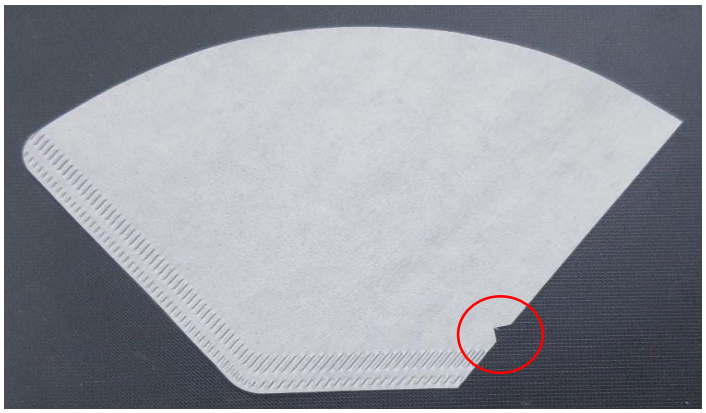


Image 3. Side opening to insert the swab

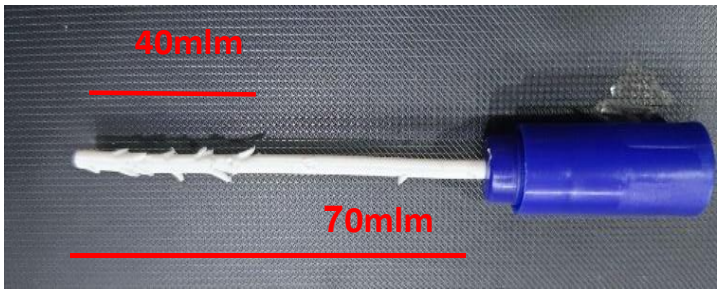


Image 4. The protrusions of the stick and the dimensions of the swab with polyester

Results

Observation of droplet particles from patients' cough: It is clear from images 5 and 6 that the kit is able to isolate flying droplets from the coughs of infected people. Cough particles are evident in their liquid form when examined immediately after coughing (image 5), and their effect is also evident in samples after they dry (image 6).

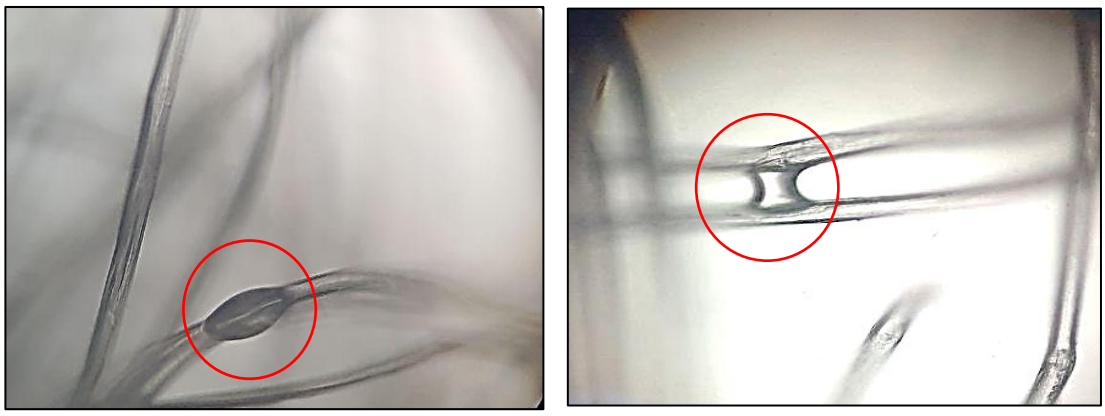


Image 5. The spray falling on the polyester threads from which the swab was made, 20X

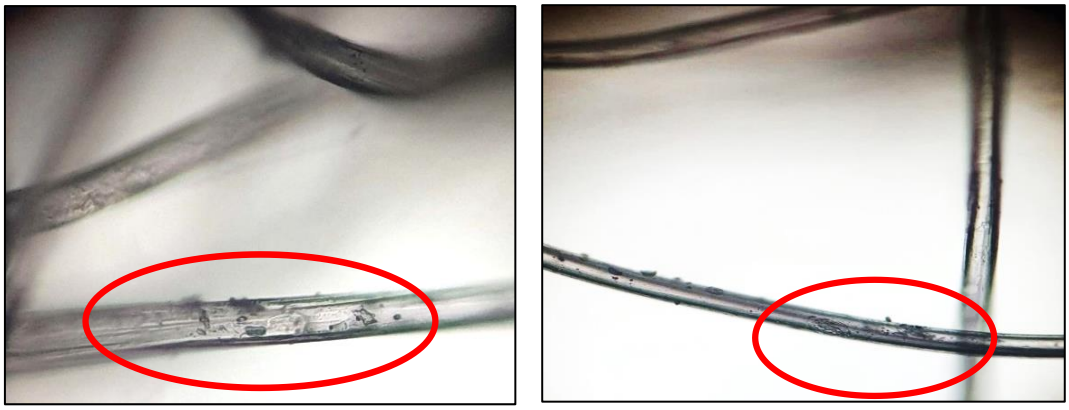


Image 6. Traces of spray on the polyester threads from which the swab was made after they dry, 20X

Isolation of coronavirus: The results showed highly significant differences (p-value≤0.05) in isolating the virus from infected people, as shown in Table (1). It was found that the number of patients who were positive based on examination of nasopharyngeal swabs was 21 samples out of 40 (52.5%). However, the number of positive samples using the cough kit was 32 out of 40 (80%). The number of samples that were positive using both techniques was 16 (40%). The number of samples that were negative by examining nasopharyngeal swabs and appeared positive using a cough swab was 16 (40%). This means that cough swabs were 40% more accurate than nasopharyngeal swabs. Five samples out of the total samples appeared positive in the nasopharyngeal swab examination and negative in the cough examination. After re-examination, four of the samples showed negative results, while one result was positive in two nasopharyngeal swabs tests, while its result was negative in the cough test (Table 1, Figure 1). Although one sample was negative using a cough kit, it was positive in two tests using nasopharyngeal swabs. We must point out the possibility of incorrect results due to laboratory procedures.

Table 1. Numbers of samples of nasal swabs compared to the results of cough swabs

Number of sample	Nasal swab result First time	Nasal swab result Second time	Result of Coughing Kit
1	+	+	+
2	-	-	+
3	-	-	+
4	+	+	+
5	+	+	+
6	+	-	-
7	+	-	-
8	+	+	+
9	-	-	+
10	+	+	+
11	-	-	+
12	+	+	+
13	-	-	+
14	+	-	-
15	-	-	+
16	+	+	+

17	-	-	+
18	+	+	+
19	-	-	+
20	+	+	+
21	+	+	-
22	+	+	+
23	-	-	+
24	-	-	+
25	-	-	+
26	-	-	+
28	+	+	+
29	-	-	-
30	-	-	+
31	-	-	-
32	+	+	+
33	+	+	+
34	-	-	+
35	-	-	+
36	+	+	+
37	+	+	+
38	+	-	-
39	+	+	+
40	-	-	+
Total, Number(%)	21(52.5)	5(12.5)	32(80)

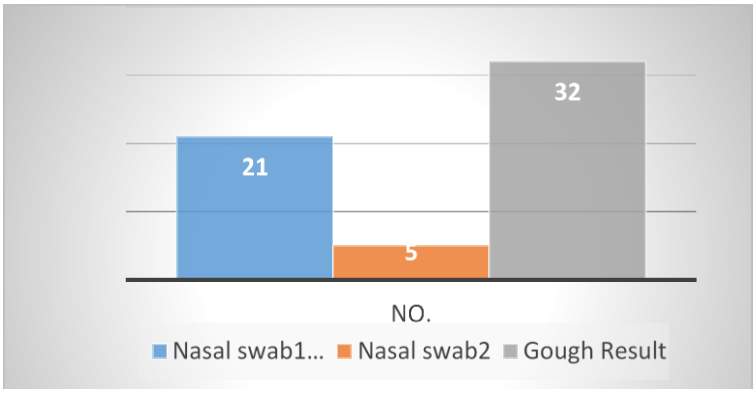


Figure 1. The difference between the RT-PCR results of samples taken from nasal swab and the result of samples taken from coughing kit

Negative control samples: The cough suppressant was used on 20 samples from healthy people who did not suffer from any symptoms of the new Coronavirus, and the RT-PCR results were negative for all of them. The results of patients' opinion based on the questionnaire showed that about 96% of them believed that the coughing kit was more comfortable and easier than nasal swab (Figure 2).

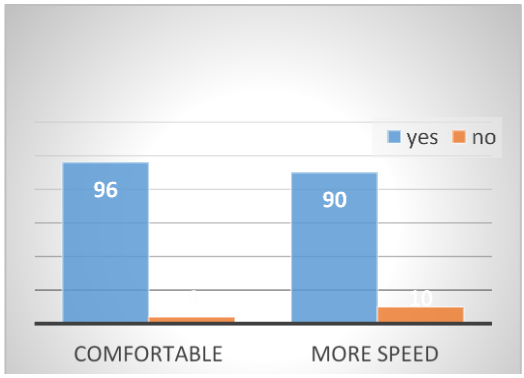


Figure 2. Questionnaire about the usage of the coughing kit

Discussion

This study indicated that there is a high rate of error in nasal and nasopharyngeal samples. Winichakoon et al. (12) stated that few cases show negative results using nasal or nasopharyngeal samples, and therefore they recommended that clinical analysis has a greater role in detecting the new coronavirus. However, another study recommends using bronchial samples with a bronchoscope over using nasopharyngeal swabs, which give some negative results (13). Bullis et al. (14) warned that some clinical cases showed negative results based on nasal and nasopharyngeal swabs while the results of cough samples were positive; as a results, their study recommends using cough samples instead of nasopharyngeal samples.

The negative samples using the Cough kit that were positive may be due to the fact that they were taken during the recovery period of the infected people, and this is supported by the fact that the results of the second examination using nasopharyngeal swabs were negative.

The negative results of nasal and nasopharyngeal samples may be due to several reasons, the most important of which is inaccuracy, as the process of taking samples may be carried out by a non-specialized staff when taking these samples, which lack of sufficient time for training due to large number of patients in health departments. Therefore, in delivering the swab to the nasopharyngeal area, it is sufficient to extend it to the middle of the nose, which makes obtaining the virus minimal. There are also indications that the virus leaves the nasopharyngeal area after 5-7 days to the lower respiratory tract area of the pharynx and bronchi, which makes finding it in the nose and nasopharyngeal area rare.

The principles of using cough samples for detecting virus agreed with many researchers who used the same principles, such as the studies by Hatagishi et al and Lindsley et al (15, 16). They used cough samples, which included the use of a device containing a gelatin filter (which was used to isolate the Influenza A virus from the coughs of patients). The result was also accepted with Huynh et al. who developed a mask to collect virus samples from the respiratory system and this mask covers half of the face, including the nose and mouth, and is made by hand from impermeable PVC rubber material with a central hole in which a filter is placed to catch viruses. Then, the filter is taken and placed in an RNA lysis buffer solution and examined using PCR machine (17).

The kit showed that the SARS-COV2 virus, concentrated in the nasopharyngeal region, could be isolated by coughing instead of swabs. This fact was used to produce a kit to easily isolate the virus from an infected person. The kit was shown to be successful in isolating viruses from several individuals who had negative swab results. Therefore, the new kit can be used to isolate the SAS-COV2 virus in an infected person instead of a nasopharyngeal swab.

Acknowledgment

We would like to thank the members of Basrah health directorate and the people who managed COVID-19 in a social group called “Together to fight COVID-19”, which is managed by Al-Zahraa College of Medicine, University of Basrah, and kindly contacted the patients to get the samples. In addition, we would like to thank all study participants.

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