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RESEARCH ARTICLE **AJHSE-CONF-2023-8**

ASSESSMENT OF USED NOSE MASKS FOR THE PRESENCE OF SARS-CoV-2

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ABSTRACT

The science around the use of masks by the public to impede COVID-19 transmission is advancing rapidly. A primary route of transmission of COVID-19 is via respiratory particles, and it is known to be transmissible from pre-symptomatic, pauci-symptomatic and asymptomatic individuals. This study was carried with the aim of evaluating the presence of SARS-CoV-2 in used nose masks in Benin City. Three different locations were chosen; University of Benin Teaching Hospital, Department of Microbiology, Faculty of Life Sciences and Ekae market at Sapele Road. New packs of sealed nose masks were purchased and used in the course of the study. The study was conducted within the dry and wet seasons. Nose masks were distributed to different volunteers in the various study locations at day 1, day 2 and day 3. The nose masks were retrieved at the time lapse from the volunteers and taken to the laboratory for analysis. Samples of nose masks retrieved from the volunteers were subjected to SARS-CoV-2 identification test was carried out on the nosemask samples. Also used and unused nose masks were subjected to viral detection technique to determine the possible presence of SARS-COV 2. Viral detection techniques revealed no presence of SARS-CoV-2 in nose masks samples analysed. This study provided early evidence for the microbial contamination of nose masks which should be a basis for improved hygienic practices by nose mask wearers.

Keywords: *Asymptomatic, COVID-19, Pauci-symptomatic Pre-symptomatic, SARS-COV 2*

INTRODUCTION

Used nose mask usually harbor microorganisms both from the body's normal flora as well as transient microbes contracted from the environment (Burton *et al*., 2011). One common way by which organisms non-resident in the used nose mask are picked up is by contact with surfaces such as skin, air and the environment. Microorganisms are ubiquitous in nature, therefore, exposure to pathogens on surfaces may take place either by direct contact with contaminated objects or indirectly through airborne particles (Prescott *et al*., 1999).

A fomite is any inanimate object, that when contaminated with or exposed to infectious agents, such as pathogenic bacteria, viruses or fungi, can transfer disease to a new host. For humans, skin cells, hair, clothing, and bedding are common sources of contamination of fomites (Abad *et al*., 1994). Inanimate objects are known to be a reservoir for the transmission of pathogens in the environment directly, by surface contact with the mouth or abraded skin, or indirectly by contamination of fingers and subsequent hand-to-mouth, hand-to-eye, or hand-to-nose contact (Haas *et al*., 1999).

The occurrence and spread of pathogens have also been studied to better understand the role of used nose mask in pathogen exposure and acquired infections (Medrano *et al*., 2011). The role of used nose mask in the transmission of infections has long been established, however, current evidence shows that environmental surfaces harbor microorganisms and can be transmitted (Andargie *et al*., 2008).

Studies have demonstrated that pathogens can be transmitted from surfaces to personnel and patients, and that these pathogens are not adequately removed by routine room cleaning. This has led to an increased focus on the importance of cleaning and disinfecting surfaces and equipment and efforts to assess and improve the effectiveness of these practices (Trail, 2007; Pringle *et al*., 2005). The study of microorganisms on nose mask is important to understand the dissemination of microbes particularly the pathogenic one (Jaffa *et al*., 2007). The number and type of surface microorganisms can be used to determine the degree of cleanliness (Ekhaise *et al*., 2010).

During the COVID19 pandemic outbreaks in 2019, there were few published reports on the practical monitoring of airborne microorganisms in public facilities. To protect the populace, numerous unspecific protective measures were applied. The protective measure were use of nosemask (medicated or locally made), frequent washing of hand with soap and water, use of hand sanitizers, nosegloves and nose shield. The aim of this study was to investigate by viral detection, possible presence of SARS-CoV-2 on used nose mask surfaces.

MATERIALS AND METHODS

Study Location

This study was conducted in Benin-City metropolis. A total of three (3) sampling locations were chosen. The sampling locations were Uniben Ugbowo campus, University of Benin Teaching Hospital and Ekae market at Sapele Road.

Purchase of Samples

Two (2) new packs of surgical nose mask (Dr. Brown Nose Mask) and Ziploc bags were purchased.

Materials Used

The following materials were employed in the experiment (i). New and used nose mask, (ii). Ziploc bags, (iii). Nutrient broth, (iv). Agar (Nutrient agar, Potato dextrose agar, MacConkey and mannitol salt agar), (v). Other materials include alcohol, cotton wool, inoculating loop, hand gloves, Bunsen burner.

Sample Distribution and Collection

Subjects/volunteers were chosen from the various sampling locations. The study was conducted within two separate seasons (i.e. dry and rainy season). Samples of clean unused nose masks were distributed to a total of twenty five different selected volunteers in the sampling locations. The samples of nose masks were retrieved from the volunteers at day 1, and later presented with a fresh nose masks which was retrieved after day 2 of usage. Another set of fresh nose masks were presented to all volunteers and retrieved after day 3 of usage. Each nose mask was placed in a clean Ziploc bag and labeled. Samples were collected seasonally during the dry season between November to January 2021 and the wet season within the months of March to May, 2021.

Experimental Precautions

Precautions taken to ensure safety while collecting used nose mask include; (i). Pair of clean hand gloves were worn while collecting the nose mask, (ii). Collected used nose mask were carefully placed in labeled zip lock bags and labeled appropriately, (iii). The ziploc bags were kept in a polythene bag, sealed and taken to the laboratory, (iv). Hands were washed with antiseptic soaps and dried afterwards. After washing hands, an alcohol based hand sanitizer were applied to hands to kill any possible pathogens obtained in the course of sample collection.

SARS- CoV-2 Viral Level Determination

The viral level determination of SARS-CoV-2 was carried out in molecular virology research laboratory University of Benin Teaching Hospital Benin City, Nigeria.

RNA extraction or purification reagents:

The procedure for RNA extraction was obtained from the manufacturer's manual of instruction. A measure of 560 µL of prepared Qiagen Buffer containing carrier RNA was pipetted into a 1.5 ml micro-centrifuge tube. For sample volumes larger than 140 µL, the amount of Qiagen Buffer-carrier RNA was increased proportionally. A 280 µL sample required 1120 µL of Qiagen Buffer-carrier RNA, and a larger tube was used.

After that, 140 µL of cell-free body fluid was added to the Qiagen Buffer-carrier RNA in the microcentrifuge tube. It was then mixed by pulse-vortexing for 15 seconds. To ensure efficient lysis, the sample was mixed thoroughly with Qiagen Buffer to yield a homogeneous solution. Frozen samples that had only been thawed once were used. Samples was then incubated at room temperature for 10 minutes. Viral particle lysis was completed after 10 minutes of lysis at room temperature, and longer incubation times had no effect on the yield or quality of the purified RNA.

Following the incubation, the tube was centrifuged to remove drops from the inside of the lid. Then, 560 µL of ethanol (96-100%) was added to the sample and mixed by pulse-vortexing for 15 seconds. Only ethanol was used since other alcohols might result in reduced RNA yield and purity. Denatured alcohol was not used, as it contained other substances such as methanol or methylethylketone. For sample volumes greater than 140 µL, the amount of

ethanol was increased proportionally. A 280 µL sample would require 1120 µL of ethanol. To ensure efficient binding, the sample was mixed thoroughly with the ethanol to yield a homogeneous solution.

Subsequently, 630 µL of the solution from step 5 was applied to the QIAamp Mini column (in a 2 ml collection tube) without wetting the rim. The cap was closed, and it was centrifuged at 6000 x g (8000 rpm) for 1 minute. The QIAamp Mini column was then placed into a clean 2 ml collection tube, and the tube containing the filtrate was discarded. Each spin column was closed to avoid cross-contamination during centrifugation. Centrifugation was performed at 6000 x g (8000 rpm) to limit microcentrifuge noise, and centrifugation at full speed had no effect on the yield or purity of the viral RNA. Solution that did not completely pass through the membrane, was centrifuged again at a higher speed until all of the solution had passed through. This step was repeated carefully for sample volume greater than 140 µL.

The QIAamp Mini column was carefully opened, and 500 µL of Buffer AW1 was added. The cap was closed, and it was centrifuged at 6000 x g (8000 rpm) for 1 minute. The QIAamp Mini column was placed in a clean 2 ml collection tube, and the tube containing the filtrate was discarded. The volume of Buffer AW1 was increased once original sample volume was larger than 140 µL.

Finally, the QIAamp Mini column was carefully opened, and 500 µL of Buffer AW2 was added. The cap was closed, and it was centrifuged at full speed (20,000 x g; 14,000 rpm) for 3 minutes.

Recommended: The QIAamp Mini column was positioned inside a new 2 ml collection tube (which was not provided), and the old collection tube containing the filtrate got discarded. Following this step, the centrifuge was employed at full speed for 1 minute. The QIAamp Mini column was then relocated into a clean 1.5 ml microcentrifuge tube (also not provided), and the previous collection tube with the filtrate was discarded once more. With care, the QIAamp Mini column was opened, and 60µl Buffer AVE was allowed to equilibrate to room temperature. The closure of the cap was followed by an incubation period at room temperature for 1 minute. Subsequently, the centrifuge was used at 6000 x g (8000 rpm) for 1 minute. For elution, it was indicated that the use of a single 60 ul Buffer AVE was adequate to elute at least 90% of the viral RNA from the QIAamp Mini column. It was also noted that employing a double elution using 2 x 40 uL Qiagen Buffer could increase the yield by up to 10%. Elution with volumes less than 30 µL yielded lower results and did not elevate the final RNA concentration in the eluate. Regarding the PCR amplification in the amplification detection area, the reaction tube was carefully placed in the sample sink of the instrument. The probe detection modes were set as: Repoiterl: FAM, Quencher 1: NONE; Reporter2: VIC, Quencher2: NONE; Reporter3: Cy5, Quencher3: NONE; Passive Reference: NONE0 4.2.2 Open the "Instrument" window and set the cycle conditions as follows:

Stage Reps Target (°C) Running Time Data

Collection 1

1 5000:15:00

21 9500:15:00

3459400:00:15

55 00:00:45

After setting, the file was saved and the program was run to adjust them.

RESULTS

Table 1 shows results for SARS-COV-2 using polymerase chain reaction (PCR) technique. All analyzed samples revealed negative except for the positive control that was used alongside with the samples.

Figure 1 is a graphical representation of the table 1. The figure shows sigma curve which is an indication of positive control that was used alongside tested samples. During the process of amplification and detection, the RNA attaches to the primer and annealing occurs. Usually when the RNA is detected, there is a response which is in the form of a sigma curve indicating a positive result. All samples analysed for this research were negative fir SARS-COV-2 hence no curve, no viral particle was detected.

Figure 2 shows a cycling analysis for the internal control. It is not suggestive of positive or negative results but a quality control for the process.

Table 1: Confirmation of SARS-CoV2 using the PCR Technique

Well	Sample ID		Cq	Efficiency	R^2	Result
1	1	-1	-1			NEGATIVE
2	2	-1	-1			NEGATIVE
3	3	-1	-1			NEGATIVE
4	4	-1	-1			NEGATIVE
5	5	-1	-1			NEGATIVE
6	6	-1	-1			NEGATIVE
7	7	-1	-1			NEGATIVE
8	8	-1	-1			NEGATIVE
9	9	-1	-1			NEGATIVE
10	10	-1	-1			NEGATIVE
11	11	-1	-1			NEGATIVE
12	12	-1	-1			NEGATIVE
13	13	-1	-1			NEGATIVE
14	14	-1	-1			NEGATIVE
15	15	-1	-1			NEGATIVE
16	16	-1	-1			NEGATIVE
17	17	-1	-1			NEGATIVE
18	18	-1	-1			NEGATIVE
19	19	-1	-1			NEGATIVE
20	21	-1	-1			NEGATIVE
21	22	-1	-1			NEGATIVE
22	23	-1	-1			NEGATIVE
23	24	-1	-1			NEGATIVE
24	25	-1	-1			NEGATIVE
31	POS PCR	21.23438965		1.012233295	0.99842	POSITIVE
32	NEG PCR	-1	-1			NEGATIVE

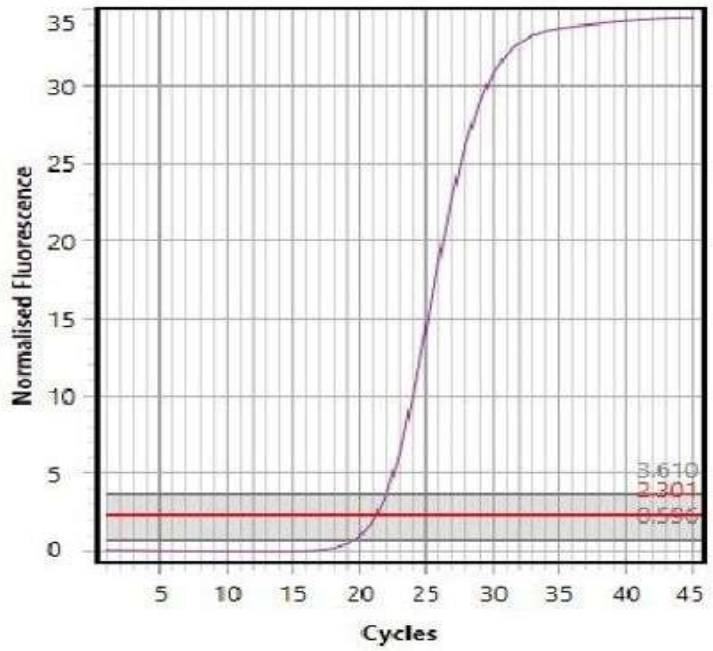

Figure 1: Cycling analysis for SARS-COV2 using Gene finder machine

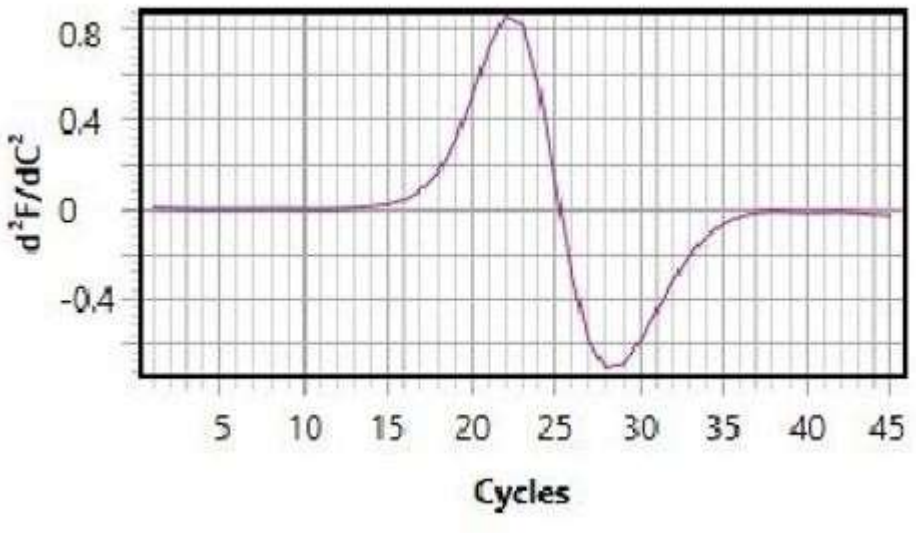

Figure 2: Cycling analysis for Quality control

DISCUSSION

Findings in this study showed that viral detection techniques indicated that the RNA was not detected in the samples of face masks worn by volunteers. Similar case study was conducted by Williams *et al*. (2021) revealed detection and quantification of SAR-COV-2 genomes in nose mask of hospitalized patients with COVID-19 especially those who were asymptomatic.

This study was limited by population size, testing of volunteers prior to time face mask was distributed. Also, samples could have been affected by environmental factors during the course of transporting them from collection site to the

laboratory. Findings in this study has created room for more future investigative approach to understand the pattern and quantity of exudates from skin of COVID-19 positive and negative individuals in the community and work places.

CONCLUSION

The absence of SARS-CoV-2 on nosemask reafffirms that surgical nosemask can be used as protection against COVID-19 as prescribed by the World Health Organization. Despite the absence of SARS-CoV-2 on nosemasks analysed in this study, there is need for thorough cleaning of hands and conscientious contact control procedures to minimize the spread of these pathogens.

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COMPETING INTERESTS

The authors declare that they have no competing interests

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