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Short Report

Efficacy of SARS-CoV-2 detection from used surgical masks compared with standard detection method

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ABSTRACT

The real-time reverse transcription-polymerase chain reaction (RT-PCR) test is the gold standard for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) detection. Proper specimen collection and obtaining a sufficient specimen are the most essential steps for laboratory diagnosis. The nasopharyngeal (NP) swab is recommended as the reference collection method. However, NP swab collection is invasive and uncomfortable for patients and poses some risk to healthcare workers. This study aimed to compare the efficacy of SARS-CoV-2 RNA detection from surgical masks with the NP swab method using RT-PCR testing. Of 269 patients, RT-PCR RNA from NP swabs was detected among 82 patients (30.5%) and was undetected among 187 patients (69.5%). All patients were tested for SARS-CoV-2 RNA from surgical masks. SARS-CoV-2 RNA was detected in 25/82 (30.5%) surgical mask filters, while undetected among 57 (69.5%). For the surgical mask with an average use time of 7.05 h, the sensitivity was 30.5%, the specificity was 100.0%, with positive predictive value of 100.0% and negative predictive value of 76.2%. Therefore, surgical masks could be an alternative non-invasive specimen source for SARS-CoV-2 RT-PCR testing. The results of our study suggest that the test could be employed after wearing surgical masks for at least 8-12 h, with increased sensitivity when used for more than 12 h.

1. Introduction

An outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported in Wuhan, Hubei Province, China, in late December 2019 [1], and the disease caused by SARS-CoV-2 was subsequently named coronavirus disease 2019 (COVID-19). The World Health Organization (WHO) then declared COVID-19 as a global pandemic, which is considered a public health concern to the entire world. It disrupted people's living and working conditions and affected public health, economy, and society [2]. Multiple public health prevention and control measures have been implemented worldwide, such as wearing masks, social distancing, lockdowns, early screening, and quarantine of exposed individuals [1,3]. Rapid and accurate diagnosis is vital to detect affected individuals, especially those without symptoms, and to control the spread of COVID-19. Real-time reverse transcription-polymerase chain reaction (RT-PCR) is the gold standard technique for detecting SARS-CoV-2 RNA in patient specimens. The assay is based on multiple primer and probe sets that target different regions of the SARS-CoV-2 gene.

Proper specimen collection and obtaining sufficient specimens are the most critical steps in the laboratory diagnosis of an infectious disease. Improper collection may lead to false or inconclusive test results. During the early stages of the COVID-19 outbreak, the Centers for Disease Control and Prevention in The US recommended collecting and testing an upper respiratory specimen [4]. Nasopharyngeal (NP) and oropharyngeal (OP) swabs are preferred for initial SARS-CoV-2 diagnostic testing. The specimens must be collected by trained medical personnel and are not suitable for self-detection. In lower respiratory tract infections, bronchoalveolar lavage, tracheal aspirate, pleural fluid, and lung biopsy specimens should be collected and tested only when clinically required [5]. The NP swab RT-PCR testing is the primary diagnostic tool for COVID-19 because it yields early results with high specificity and moderate sensitivity. However, it is invasive and can be uncomfortable for patients, causing pain or bleeding, leading to

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physical resistance and compromising specimen quality for virus detection [6,7]. Risks to healthcare workers exist as it may induce patients to sneeze or cough and expel viral particles. Sampling requires the proper use of personal protective equipment (PPE) to minimize this risk. When global shortages of swabs and PPE occurred, the procedure proved unconducive to large-scale testing [4,6–8].

Given the limitations of NP swabs, interest in non-invasive and self-sample collection methods has increased. Saliva is an alternative to the NP swab because it is easy to self-collect and require less PPE and fewer specimen sampling tools. However, limitations include false negative results due to decreased sensitivity compared with NP specimens [9,10]. With the increase in the demand for both speedy diagnostic tests and a decentralized healthcare system at the regional level, the introduction of point-of-care testing (POCT) kits that can be used in remote settings is essential. Antigen test kits (ATK) are extensively used for screening and diagnosis of COVID-19 due to their ease of use. However, ATKs exhibit poor sensitivity and cannot detect low concentrations of SARS-CoV-2 [11,12].

COVID-19 remains a global health challenge. The virus is transmitted by airborne droplets and aerosols containing the virus. In addition to respiratory droplets, SARS-CoV-2 can spread indirectly through contaminated objects and airborne transmission [13]. Face masks in a positive individual significantly reduce the detection of influenza virus RNA in respiratory droplets and seasonal coronavirus RNA in aerosols [14]. Therefore, collecting respiratory specimens from facemasks with the highest pathogen densities offers a potential advantage [15,16].

This study aimed to compare the efficacy of SARS-CoV-2 RNA detection from used surgical masks employing standard detection methods. We tested for SARS-CoV-2 RNA in masks from patients with COVID-19 that had been worn for 8 h and compared this with NP swabs using RT-PCR testing. Hospitalized patients with laboratory-confirmed COVID-19 were recruited to provide surgical masks. Collection of used surgical mask specimens by patients is simple and convenient, requires no sampling equipment, and reduces the risk of healthcare personnel exposure to COVID-19. If SARS-CoV-2 RNA can be detected on used surgical mask specimens, this may encourage patients to provide specimens for testing and aid in early detection and prevention of transmission. As an alternative method to NP swabs, it may facilitate timely diagnosis and treatment.

2. Materials and methods

2.1. Sample size and collection

This study was conducted between May and August 2021. We recruited patients attending the Acute Respiratory Illness Clinic (ARI Clinic) with common cold or fever symptoms, and clinical and demographic data were collected. NP swab specimens were collected, and face masks with a high efficiency particulate air (HEPA) filter attached to the inner side were provided to the patients. They were instructed to wear them for at least 8 h except during mealtime. The NP swab-negative patients were allowed to wear the mask while isolating at home. The facemasks of all patients were then returned to the laboratory for SARS-CoV-2 RT-PCR.

2.2. Sample size calculation

A pilot study of SARS-CoV-2 RNA detection in used mask filters from 20 positive patients in our COVID ward showed that the sensitivity was 80.0% (positive cases 16/20 = 80.0%), significance level = 0.05, allowable error = 0.1, and the prevalence of COVID-19 detection among high-risk exposed groups was 25%. We calculated the sample size for our study to be 248 (STATA, Ver. 14.0, sample size calculation), as shown below.

$$n = \frac{Z^2_{\alpha/2} p(1-p)}{d^2} \quad (1)$$

Define $\alpha = 0.05$, $Z_{\alpha/2} = 1.96$
p = sensitivity = 0.8 from a pilot study
d is allowable error = 0.1
n = 62

$$n_s = \frac{n \times 100}{Prevalence} \quad (2)$$

Prevalence = 25.0%
n_s = (62 × 100) / 25 = 248

Add 10% for the possible patients drop off, then n = 268.

Study participants must have obtained permission, not require oxygen intubation or any other breathing support device, and should be able to wear a mask for 8 h, according to medical advice (Supplementary Fig. 1).

All participants provided written informed consent. After the participants had worn the masks with the HEPA filter attached horizontally to the inner surface of the face mask with thin tape (Supplementary Fig. 1) for 8 h, no restrictions were made on activities such as talking, coughing, sneezing, or sleeping. The HEPA filters were then removed from the face mask by personnel dressed in full PPE and each placed into a viral transport medium (VTM) tube (Supplementary Fig. 2). This was placed in a clean plastic bag, sprayed with alcohol, placed in an icebox and sent to the biomolecular laboratory for real-time RT-PCR SARS-CoV-2 testing. Additionally, the patient's clinical data, biological sex, and age were collected.

2.3. Detection of SARS-CoV-2

Laboratory staff were blinded to the participant details. The VTM with the filter was processed with vortex homogenization using a centrifuge for two minutes. Two mL of the VTM samples were transferred from the mixture using a Pasteur pipette to extract the prototype genetic material (Template). The SARS-CoV-2 RNA was extracted using the magnetic bead method. Total RNA was extracted from a 200 μL volume of the samples using the Zybio nucleic acid extraction kit (Zybio, Chongqing, China) on the EXM3000 nucleic acid isolation system (Zybio Inc.) according to the manufacturer's instructions.

For amplification, 5 μL of extracted RNA samples were processed on an RT-PCR SANSURE MA6000 thermal cycler (Sansure Biotech Inc., China). Positive and negative controls were added to each run. The two target genes included the nucleocapsid protein (*N*) and a non-structural region specific for the SARS-CoV-2 (*ORF1ab*) gene on the SARS-CoV-2 genome. According to the instructions, a cycle threshold (Ct) value of ≤ 36 was considered detectable for the SARS-CoV-2. Ct values between 36 and 40 were considered inconclusive, and those over Ct 40 were considered undetectable.

2.4. Statistical analysis

Mean and standard deviation (SD) or median and interquartile range for continuous variables and frequencies and percentages for categorical data depend on the normality of data. The performance of the diagnostic tests was determined by the sensitivity, specificity, and positive and negative predictive values with 95% confidence intervals (CI). We considered 2-sided *P* values less than 0.05 to be statistically significant. The association between sex and symptom duration was tested using the Chi-square test. These analyses were performed using the STATA Program (V14.0 StataCorp LLC College Station, TX, USA).

3. Results

We enrolled 350 patients from the Respiratory Infectious Disease Clinic (ARI Clinic), Vajira Hospital, in the study. A total of 81 patients refused to participate in the study. The remaining 269 patients comprised 110 males and 159 females. The mean age was 48.65 ± 19.87 years among positive patients and 57.7 ± 25.22 years among negative patients (Table 1). Of these, 109 patients were symptomatic, 149 participants were high-risk contacts, and 19 cases were undergoing presurgical screening. The most frequent underlying diseases included diabetes mellitus, hypertension, and dyslipidemia (Table 1).

Five of the 269 patients did not return masks for the test, and the remaining 264 were investigated. RT-PCR RNA was detected from NP swabs in 82 patients (30.5%) while undetected in 187 patients (69.5%). All 264 patients were tested for SARS-CoV-2 RNA with face masks and, for comparison, confirmed with NP swabs using RT-PCR. Therefore, each patient will have the swab once from the NP (confirm test) and once from the mask. The RT-PCR results showed that SARS-CoV-2 RNA was detected in 82/82 (100.0%) of all NP swab specimens, of which 25/82 (30.5%) were detected from surgical mask filters, while all 57 (69.5%) in the NP negative cohort had negative face masks results. The positive and negative predictive values of the face masks were 100 and 76.2% (95% CI, 70.2% - 81.4%), respectively. The sensitivity and specificity of the face masks were 30.5% (95% CI, 20.8% - 41.6%) and 100% (95% CI, 100.0% - 100.0%), respectively. The overall accuracy was 78.4%. Accuracy of the new test determined by sensitivity and specificity: The breath mask showed poor discriminatory performance in identifying positive COVID-19 with a sensitivity of 30.5% (95% CI, 24.9% - 36.0%)), but a high negative predictive value with specificity of 100.0% (95% CI, 100.0%-100.0%). The positive predictive value (PPV) to diagnose COVID-19 cases was 100.0% (95% CI, 100.0% - 100.0%), while the patients who had negative COVID-19 results will be diagnosed with non-COVID-19 (NPV) were 76.2% (95% CI, 71.0% - 81.3%). Surgical masks exhibited an average use time of 7.05 h, as shown in Table 2.

Table 1
Characteristics of 269 participants with SARS-CoV-2 testing.

Characteristics	COVID-19 detected patients (NP RT-PCR positive, n = 82)	COVID-19 undetected patients (NP RT-PCR negative, n = 187)	*P*-value
Sex, n (%)			
Male	35 (43.0)	75 (40.0)	0.069
Female	47 (57.0)	112 (60.0)	
Age (years)*	48.65 ± 19.87	57.7 ± 25.22	0.005
BMI (kg/m^2)*	24.27 ± 5.00	23.08 ± 3.56	0.027
Diseases, n (%)			
Diabetes	12 (14.6)	30 (16.0)	0.770
Hypertension	15 (18.3)	21 (11.2)	0.115
Dyslipidemia	14 (17.1)	19 (10.5)	0.132
Coronary artery	4 (4.8)	11 (5.8)	0.740
Asthma or emphysema	4 (4.8)	2 (1.1)	0.058
Cancer	3 (3.6)	6 (3.2)	0.866
Cerebrovascular	6 (7.3)	5 (2.7)	0.790
Chronic kidney disease failure	1 (1.2)	3 (1.6)	0.807
Reason for analysis, n (%)			<0.001
With symptoms	64 (78.0)	45 (24.2)	
High-risk exposure	12 (19.6)	129 (68.9)	
Screening before surgery	6 (7.9)	13 (6.9)	

Abbreviations: BMI, body mass index; COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; NP, nasopharyngeal; RT-PCR, real-time reverse transcription-polymerase chain reaction.
* Mean and standard deviation (SD) for continuous variables.

In the group of 82 patients with COVID-19 who tested positive by NP swab, we found that mask use time, symptom onset, and duration of symptoms ($P < 0.001$, $P < 0.001$, $P = 0.009$, respectively) affected the detection of COVID-19 from the filter, as shown in Table 3. Twelve (14.6%) of 82 patients with confirmation test for COVID-19 by NP swab were asymptomatic and 70 (85.4%) had COVID-19 symptoms at the onset of infection. In the group of 70 symptomatic participants, cough was the most common, followed by nasal congestion, fever, sore throat, rhinorrhea, headache, dyspnea, sputum, loss of taste or smell, and diarrhea. The duration of symptoms ranged from 0 to 14 days for participants with symptoms, with the highest prevalence in the first 2-3 days after testing.

We found that an RT-PCR Ct value of approximately 25.23 ± 6.78 was detected from the NP swab and surgical mask filter samples. However, patients with NP Ct values ≥ 38.33 ± 1.25 did not have SARS-CoV-2 detected on face masks ($P = 0.05$). Of these 82 patients, those who had tested positive with face masks had significantly lower NP Ct values. The mean Ct ratio of the mask filter was 36.18, while that of the NP swab was 28.97, which was significantly different ($P = 0.05$).

4. Discussion

The above study was the first to detect SARS-CoV-2 RNA based on the exhaled breath of inpatients with COVID-19, high-risk contacts, and those screened before surgery. This non-invasive method provides an additional option to the standard examination by NP swab. Breath contains relatively little viral RNA. Therefore, the sensitivity for pathogen detection is low compared with gold standard methods. Depending on their size and origin, droplet nuclei can remain suspended in the air and travel long distances in the airflow. Importantly, bioaerosols contain infectious SARS-CoV-2, which can be deposited on surfaces, survive for a few days, and differ significantly ($P = 0.05$) [17]. Therefore, methods to detect and quantify the virus in bioaerosols can help preventive measures, manage the disinfection of contaminated areas more efficiently, and estimate infection risk. The finding was consistent with the research of P. Fabian et al. [18], who measured influenza virus fragments in exhaled air through a condensation system with steam and cooling. Through various virus filters, such as Teflon, gelatin, and polyurethane, the sensitivity ranged from 7 to 22-a meta-analysis by BL Guillaume et al. [19] in 2021 compared saliva and NP swabs.

Saliva showed a sensitivity of 83.2% and a specificity of 99.2%, which is higher than that of the breath test in volunteers. Another meta-analysis by Jessica Tsao et al. [20] in 2022 using the rapid antigen test showed a sensitivity of 63.0% and a specificity of 99.8% among symptomatic subjects, increasing the diagnostic sensitivity to 77.8%. Gallichotte et al. [21] showed the efficient recovery of RNA and infectious viruses from virus-spiked polyvinyl alcohol (PVA) with detection limits comparable with nasal swab samples. They detected both human and SARS-CoV-2 RNA on PVA strips, and the levels did not correlate with the duration of mask-wearing, the rate of coughing or sneezing, or the level of viruses in the nasal swab specimens. C.M. Williams et al. [22] demonstrated that high levels of facemask sampling and shorter intervals between sampling and symptom onset were associated with higher mortality, while NP viral load showed no significant association. However, in our study, the sensitivity and specificity of subjects with negative rapid antigen test results but positive RT-PCR results correlated with a Ct value of 38.33, and the duration of mask-wearing affected the virus detection. The sensitivity in the pilot study was higher than that in the main result (80% vs. 30%), which may be due to the selection of the patients. In the pilot study, we selected only the COVID-19 positive cases with moderate symptoms. The Ct values were low, and the tendency to test positive was higher than in the main study results.

Table 2
Comparison of SARS-CoV-2 RT-PCR values obtained from mask and NP swab.

Gold standard	Mask positive	Mask negative	Sum	Accuracy, % (95% CI)	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	Yuden index*	LR+	LR− (95% CI)
Positive	25	57	82	78.4 (68.9-99.7)	30.5 (20.8-41.6)	100.0 (98.0-100.0)	100.0 (86.3-100.0)	76.2 (70.2-81.4)	NA	NA	0.7 (0.6, 0.8)
Negative	0	182	182								
Sum	25	239	264								

Notes: Sensitivity = (25/82) × 100% = 30.5%; Specificity = (182/182) × 100% = 100%; PPV = (25/25) × 100% = 100%; NPV = (57/182) × 100% = 76.2%.
*Yuden index can not be performed because the outcomes are both binary (positive and negative LR+ can not be calculated because specificity = 100.00%.
Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; NA, not available; NP, nasopharyngeal; RT-PCR, real-time reverse transcription-polymerase chain reaction; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ration; LR−, negative likelihood ration.

Table 3
Factors affecting COVID-19 detection from the surgical mask filter.

Factors	Patients with COVID-19 (n = 82)		*P* - value
	Mask filter (+) (n = 25)	Mask filter (−) (n = 57)	
Sex			0.356
Male	10	29	
Female	15	28	
Duration of symptoms (days)			0.009
0–1	5	5	
2–3	15	50	
>4	5	2	
Symptoms			<0.001
Asymptomatic	0	12	
Symptoms	25	45	
Time of mask use (hours)			<0.001
4–6	1	22	
7–8	6	25	
9–12	12	8	
>12	6	2	

Categorial data by using the Chi-square test. Abbreviation: COVID-19, coronavirus disease 2019.

This study encountered some limitations concerning analyzing factors affecting the detection of SARS-CoV-2 in air filters. First, with regard to the onset of symptoms and the duration of the treatment process, the virus was less likely to be detected among recently developed symptoms. The peak of detection occurred after 48 h, which is consistent with the study of R. Gillent et al. [23], who revealed that more than 1 million viral particles/minute were found after 48 h of infection, while viral fragments were detected at <7,000 particles/minute at the end of the infection. The virus was more easily detected in patients with sputum and cough symptoms than in others, which is consistent with the study of Yang et al.[24], who reported more sputum viruses than nasal swabs.

Second, the virus was more likely to be detected on used surgical mask filters from patients with symptoms than from those without, which was confirmed in one study indicating that face mask positivity was significantly associated with time from onset of fever or fever and ARI symptoms [12]. Third, the average time of surgical mask use by the participants was 8.10 h in one day, but patients with COVID-19-positive NP swabs showed an average time of 7.05 h. Shorter surgical mask use in patients with COVID-19 may have affected the sensitivity of detection. Finally, the method of specimen collection and handling prior to genomic detection may have affected the results of SARS-CoV-2 detection. Among the various steps, RNA isolation may be a critical factor as, to our knowledge, no standard method is available for isolating RNA from air samples. In one study, influenza virus bioaerosol was collected using a Teflon and gelatin filter [18].

5. Conclusion

This study confirmed the feasibility of detecting the SARS-CoV-2 virus from used surgical masks among patients with COVID-19. Therefore, surgical masks could be an alternative source of non-invasive specimens for SARS-CoV-2 RT-PCR testing. The results of our study suggest that the test could be employed among patients who have worn surgical masks for at least 8-12 h, with increased sensitivity if the masks were used for more than 12 h.

Ethics statement

The Institutional Review Board approved this study at Faculty of Medicine Vajira Hospital Navamindradhiraj University (No. 138/2564). Each participant provides signed written informed consent.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Author contributions

Uraporn Phumisantiphong: Conceptualization, Data curation, Validation, Formal analysis, Visualization, Writing - Original draft. **Anan Manomaipiboon:** Conceptualization, Project administration. **Yuttana Apichatbutr:** Conceptualization, Data curation, Validation. **Kittisak Pholtawornkulchai:** Resources, Formal analysis. **Chunlanee Sangketchon:** Data curation, Validation. **Busaba Supawattanabodee:** Validation, Formal analysis. **Thananda Trakarnvanich:** Validation, Formal analysis, Writing - review & editing, Supervision.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bsheal.2023.12.004.

References

[1] F. Wu, S. Zhao, B. Yu, Y.M. Chen, W. Wang, et al., A new coronavirus associated with human respiratory disease in China, Nature 579 (2020) 265–269, https://doi.org/10.1038/s41586-020-2008-3.

[2] WHO, WHO coronavirus disease (COVID-19) dashboard. https://covid19whoint/, 2021 (accessed 10 January 2023).

[3] H. Esakandari, M. Nabi-Afjadi, J. Fakkari-Afjadi, N. Farahmandian, S.M. Miresmaeili, E. Bahreini, A comprehensive review of COVID-19 characteristics, Biol. Proced. Online 22 (2020) 19, https://doi.org/10.1186/s12575-020-00128-2.

[4] National Center for Immunization and Respiratory Diseases (U.S.), Division of viral Diseases, interim guidelines for collecting, handling, and testing clinical specimens for COVID-19: Interim guidelines for collecting, handling, and testing clinical specimens from persons for coronavirus disease 2019 (COVID-19). https://stacks.cdc.gov/view/cdc/104491, 2021 (accessed 10 January 2023).

[5] J. Phua, L. Weng, L. Ling, M. Egi, C.M. Lim, J.V. Divatia, et al., & Asian Critical Care Clinical Trials Group, Intensive care management of coronavirus disease 2019 (COVID-19): challenges and recommendations, Lancet Respi. Med. 8 (2020) 506–517, https://doi.org/10.1016/S2213-2600(20)30161-2.

[6] Q. Gao, L. Bao, H. Mao, L. Wang, K. Xu, M. Yang, et al., Development of an inactivated vaccine candidate for SARS-CoV-2, Science 369 (2020) 77–81, https://doi.org/10.1126/science.abc1932.

[7] Y. Qian, T. Zeng, H. Wang, M. Xu, J. Chen, N. Hu, D. Chen, Y. Liu, Safety management of nasopharyngeal specimen collection from suspected cases of coronavirus disease 2019, Int. J. Nurs. Sci. 7 (2020) 153–156, https://doi.org/10.1016/j.ijnss.2020.03.012.

[8] L. Comber, K.A. Walsh, K. Jordan, K.K. O'Brien, B. Clyne, C. Teljeur, et al., Alternative clinical specimens for the detection of SARS-CoV-2: a rapid review, Rev. Med. Virol. 31 (4) (2021) e2185, https://doi.org/10.1002/rmv.2185.

[9] E. Williams, K. Bond, B. Zhang, M. Putland, D.A. Williamson, Saliva as a non-invasive specimen for detection of SARS-CoV-2, J. Clin. Microbiol. (2020) e00776–20, https://doi.org/10.1128/JCM.00776-20.

[10] D. Becker, E. Sandoval, A. Amin, P. De Hoff, A. Diets, N. Leonetti, et al., Saliva is less sensitive than nasopharyngeal swabs for COVID-19 detection in the community setting [Preprint], medRxiv 20.05.11.20092338, https://doi.org/10.1101/2020.05.11.20092338.

[11] W. Deenin, A. Yakoh, U. Pimpitak, E. Pasomsub, S. Rengpipat, G.A. Crespo, S. Chaiyo, Electrochemical lateral-flow device for rapid COVID-19 antigen-diagnostic testing, Bioelectrochemistry 152 (2023) 108438, https://doi.org/10.1016/j.bioelechem.2023.108438.

[12] N. Sayabovorn, P. Phisalprapa, W. Srivanichakorn, C. Washirasaksiri, C. Auesomwang, T. Sitasuwan, et al., Early diagnosis by antigen test kit and early treatment by antiviral therapy: an ambulatory management strategy during COVID-19 crisis in Thailand, Medicine 101 (2022) e29888, https://doi.org/10.1097/MD.0000000000029888.

[13] J.L. Santarpia, D.N. Rivera, V.L. Herrera, M.J. Morwitzer, H.M. Creager, E.R. Schnaubelt, M.J. Broadhurst, J.V. Lawler, S.P. Reid, J.J. Lowe, et al., Aerosol and surface contamination of SARS-CoV-2 observed in quarantine and isolation care, Sci. Rep. 10 (1) (2020) 12732, https://doi.org/10.1038/s41598-020-69286-3.

[14] S. Asadi, C.D. Cappa, S. Barreda, et al., Efficacy of masks and face coverings in controlling outward aerosol particle emission from expiratory activities, Sci. Rep. 10 (2020) 15665, https://doi.org/10.1038/s41598-020-72798-7.

[15] K.P. Fennelly, J.W. Martyny, K.E. Fulton, I.M. Orme, D.M. Cave, L.B. Heifets, Cough-generated aerosols of Mycobacterium tuberculosis: a new method to study infectiousness, Am. J. Respir. Crit. Care. Med. 169 (2004) 604–609, https://doi.org/10.1164/rccm.200308-1101OC.

[16] K.N. Huynh, B.G. Oliver, S. Stelzer, W.D. Rawlinson, E.R. Tovey, A new method for sampling and detection of exhaled respiratory virus aerosols, Clin. Infect. Dis. 46 (2008) 93–95, https://doi.org/10.1086/523000.

[17] Y. Liu, Z. Ning, Y. Chen, M. Guo, Y. Liu, et al., Aerodynamic analysis of SARS-CoV-2 in two Wuhan hospitals, Nature 582 (2020) 557–560, https://doi.org/10.1038/s41586-020-2271-3.

[18] P. Fabian, J.J. McDevitt, E.A. Houseman, D.K. Milton, Airborne influenza virus detection with four aerosol samplers using molecular and infectivity assays: considerations for a new infectious virus aerosol sampler, Indoor. Air. 19 (2009) 433–441, https://doi.org/10.1111/j.1600-0668.2009.00609.x.

[19] G. Butler-Laporte, A. Lawandi, I. Schiller, M. Yao, N. Dendukuri, E.G. McDonald, T. C. Lee, Comparison of saliva and nasopharyngeal swab nucleic acid amplification testing for detection of SARS-CoV-2: a systematic review and meta-analysis, JAMA Intern. Med. 181 (2021) 353–360, https://doi.org/10.1001/jamainternmed.2020.8876.

[20] J. Tsao, A.L. Kussman, C. Costales, B.A. Pinsky, G.D. Abrams, C.E. Hwang, Accuracy of rapid antigen vs reverse transcriptase-polymerase chain reaction testing for SARS-CoV-2 infection in college athletes during prevalence of the omicron variant, JAMA Netw. Open. 5 (6) (2022) e2217234, https://doi.org/10.1001/jamanetworkopen.2022.17234.

[21] E.N. Gallichotte, W. Windsor, S. Watts, N. Sexton, C. Henry, T. Jaenisch, et al., Detection of SARS-CoV-2 in exhaled air using non-invasive embedded strips in masks, Am. J. Infect. Control. 50 (2022) 890–897, https://doi.org/10.1016/j.ajic.2022.01.010.

[22] C.M. Williams, D. Pan, J. Decker, A. Wisniewska, E. Fletcher, S. Sze, et al., Exhaled SARS-CoV-2 quantified by facemask sampling in hospitalised patients with COVID-19, J. Infect. 82 (2021) 253–259, https://doi.org/10.1016/j.jinf.2021.03.018.

[23] R. Gilbert, J.H. Auchincloss Jr, J. Brodsky, W. Boden, Changes in tidal volume, frequency, and ventilation induced by their measurement, J. Appl. Physiol. 33 (1972) 252–254, https://doi.org/10.1152/jappl.1972.33.2.252.

[24] Y. Yang, M. Yang, J. Yuan, F. Wang, Z. Wang, J. Li, et al., Laboratory diagnosis and monitoring the viral shedding of SARS-CoV-2 infection, Innovation 1 (3) (2020) 100061, https://doi.org/10.1016/j.xinn.2020.100061.