

# Filtration efficiency of medical and community face masks against particles carrying SARS-CoV-2

# Sara Dalvand<sup>1</sup>, Rasul Nasiri<sup>2</sup>, Rasoul Yarahmadi<sup>2,3,\*</sup>, Farah Bokharaei Salim<sup>4</sup>, Ali Asghar Farshad<sup>3</sup>, Somayeh Soleimani Alyar<sup>1</sup>

<sup>1</sup> Department of Occupational Health Engineering, School of Public Health, Iran University of Medical Sciences, Tehran, Iran

<sup>2</sup> Air Pollution Research Center, Iran University of Medical Sciences, Tehran, Iran

<sup>3</sup> Department of Occupational Health and Safety Engineering, School of Public Health, Iran University of Medical Sciences, Tehran, Iran <sup>4</sup> Department of Medical Virology School of Medicale Iran University of Medical Sciences, Tehran, Iran

<sup>4</sup> Department of Medical Virology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

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#### **CORRESPONDING AUTHOR:**

yarahmadi.r@Iums.ac.ir Tel: (+98 21) 86704625 Fax: (+98 21) 86704625

#### ABSTRACT

**Introduction:** Airborne bioaerosols like SARS-CoV-2 can pose a significant threat to the respiratory system of humans. Airborne bioaerosols, such as SARS-CoV-2, pose significant respiratory risks. Wearing respiratory masks is a preventive measure to reduce exposure and control the transmission of airborne diseases. Hence, this study aims to assess the effectiveness of the masks in filtering airborne particulates, specifically those that carry SARS-CoV-2.

**Materials and methods:** The filtration efficiency of three types of face masks was investigated for particulate matters in a laboratory setup using a custom-designed system, including a human head mannequin and controlled aerosol injection. Air samples were also collected from the breathing zone of COVID-19 patients in hospital settings, both with and without masks. Data analysis used Python tools, including Seaborn and Matplotlib, to generate visual insights.

**Results:** The study findings revealed variations in particle penetration and filtration efficiency of the tested masks for particles and SARS-CoV-2 based on mask types. The particles smaller than 700 nm penetrated N95 masks by 4.61%, with efficiency reaching 99.2% as particle size increased. Particle filtration efficiency for other masks, including surgical masks, ranged from 31%-68%, and for cloth masks, it was between 28%-86%.

**Conclusion:** The effectiveness of respiratory masks in preventing the transmission of airborne particles and viruses, like SARS-CoV-2, into the human respiratory system and regular use of suitable respiratory masks can help control disease transmission, especially in high-risk environments such as hospitals. In summary, using respiratory masks is essential in reducing the spread of airborne viruses and improving public health.

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#### Introduction

Bioaerosols, due to their small size and mass, easily disperse in the surrounding air [1]. Exhalation activities produce respirable particles of varying sizes, with larger particles expelled during sneezing and coughing, and smaller ones released during speech [2]. Exhalation is a primary mechanism for transmitting bioaerosols and pathogens, which can lead to dangerous pandemics [3, 4]. During exhalation, respiratory droplets are formed when a fast airflow passes through the moist surfaces inside the nose or mouth. This airflow causes liquid bands to lift from the boundaries and break apart, eventually forming droplets. The size and distribution of these droplets depend on several factors, including the velocity of the liquid, the topology of the airflow pathways, the wetting properties of the surfaces, and the characteristics of phlegm [5]. Particle size is very effective in the possibility of human respiratory system infection by pathogenic agents. Fine particles less than 2.5 µm can penetrate the lungs; particles of 2.5–10 µm settle in the airways; and larger particles deposit in the upper parts of the respiratory system [6]. The penetration of fine respiratory droplets into the tracheobronchial and alveolar depends on environmental factors such as airflow speed, relative humidity, and temperature [7]. Exposure to bioaerosol has adverse effects on human health, and the most common side effects are problems in the human respiratory system [8]. Asthma and mucous membrane irritation are local effects, and mycotoxicosis and infectious diseases are systemic effects of bioaerosol on humans. Therefore, the occurrence of diseases such as influenza, rhinoviruses, and coronaviruses are the most common causes of respiratory tract infections [9], which are the main cause of epidemics in the world and lead to the death of people [10]. Acute respiratory syndrome coronavirus2 (SARS-CoV-2) was discovered in China in December 2019 and has been recognized as a significant challenge in the world's health systems. This disease introduces a virus in the human respiratory system that leads to severe

acute respiratory syndrome and pneumonia [11-13]. The World Health Organization (WHO), On March 11, 2020, declared the prevalence of the COVID-19 virus as a public health crisis, which indicated the extent of its spread. SARS-CoV-2 is transmitted through straight contact, indirect contact with contaminated areas, and inhalation of droplets from sneezing and coughing. The most important way to spread the droplets of this virus is through the air and airborne particles [12].

Ambient air sampling provides more reliable evidence for better identification of the bioaerosol and the risk of their existence, which can used to break the chain of disease transmission [14]. There are different physical mechanisms to part of viral, bacterial, and fungal pollutant particles from the airflow [15]. Most technologies of bioaerosol sampling affiliate with factors such as the average equivalent diameter of particles, adhesion of particles in air, thermal gradient, inertia of suspended particles, and their Brownian motion [16]. Aerosol and bioaerosol particles stick to the surfaces they hit through Vander Waals forces, electrostatic forces, and surface tension. The most common devices for bioaerosol sampling are solid and liquid impactors, electrostatic precipitators, and filters. This equipment has a lot of variety, which is selected based on the purpose and sampling protocol [17]. Solid impactors, such as Andersen samplers, slit samplers, and cyclone samplers, are usually more efficient at capturing large particles [18, 19]. All-Glass Impingers (AGIs) similar samplers are the most often used for airborne virus capture [15]. To sample air bioaerosol, wet bed samplers are used, which include devices such as (AGI-30, GCS, Bio sampler, NIOSH Onstage, and Two-stage, CIP 10M) [8, 18]. This sampler isn't suitable for hydrophobic pollutants. Also, it is used mainly to determine the size distribution of infectious particles [20, 21]. Because of the inefficiency of most samplers in capturing particles with a size of 500 nm aerodynamically, filters are often used for airborne viruses [16]. Electrostatic precipitation is used to sample air with new technology. This device can attract more particles by increasing high-voltage corona to 1000 L/min [18].

In purpose control exposure to bioaerosol, it is necessary to use personal protective equipment as the last occupational health control method. Additionally, CDC guidelines recommend wearing face masks against the Covid-19 epidemic for all individuals [22, 23]. Also, amid the COVID-19 pandemic, people didn't use a wide variety of face masks. To address this issue, this study was conducted on three common types of face masks with different levels of respiratory protection found in society. For example, healthcare and industrial employees mainly use N95 masks to reduce exposure to airborne particles and microorganisms. Many people use masks to protect against PM in highly polluted areas [24]. The other common masks used during the outbreak of the COVID-19 pandemic were surgical masks and cloth ones. Surgical masks are recommended to control the source of transmission of viral agents and infections through exhalation activities such as sneezing, coughing, and talking [25]. The main objective of this study is to evaluate the effectiveness of different types of face masks in filtering bioaerosols, specifically SARS-CoV-2 particles, during exhalation activities. While previous studies have examined bioaerosol sampling methods, there is a lack of comprehensive research on the efficiency of masks in controlling the transmission of airborne viruses. This study aims to fill this knowledge gap by investigating the filtration efficiency of N95, surgical, and cloth masks and providing insights into their role in reducing the spread of respiratory infections during pandemics. Therefore, the present study evaluates the effectiveness of different face masks in filtering particles carrying the viral genomes, specifically SARS-CoV-2, generalizing the laboratory and field study results.

#### Materials and methods

In this study, we investigated the filtration effectiveness of three different types of face masks: surgical masks, N95 respirators, and

cloth masks. The specifications of these masks, including materials, layers, dimensions, and filtration efficiencies, are summarized in Table 1. First, we evaluated the filtration capacity of these masks for particulate matter in a laboratory setting. Subsequently, we examined the filtration efficiency of particles carrying SARS-CoV-2 in a hospital environment involving three COVID-19 patients.

#### **Respirator selecting**

The three tested types of masks were including; a) N95 face piece respirator (N95 FFR) made in Iran, b) surgical mask made in Iran, and c) Cloth or fabric mask made in Iran.

The most common control method to reduce individual exposure to airborne particles is by using an N95 filtering face piece respirator that fits extremely close to the face and effectively filters tiny particles (0.3 mm), stopping at least 95% of them [24]. N95 FFR masks have at least four layers: an inner layer, a support layer, a mask filter layer, and an outer layer. N95 FFR masks are divided into two categories: Standard N95 and Surgical N95/FFP2, which masks Surgical N95/FFP2, have greater filtration power and applicability [26]. In the present study, the N95 type was studied. Although the N95 respirator mask is primarily used by industrial workers, it protects the wearer from environmental pathogens more effectively than surgical masks. As a result, the US Centers for Disease Control and Prevention (CDC) has approved it to protect healthcare workers from clinical respiratory diseases such as coronavirus disease 2019 (COVID-19), severe acute respiratory syndrome (SARS-CoV-2) and Mycobacterium tuberculosis [27].

Medical or surgical masks are prepared according to the European Union health and safety standard EN 14683:2019, which is usually used by healthcare workers [25]. Surgical masks have a three-layer structure, with the middle layer serving as a filter, known as the melt-blown layer, absorbing water, and the two outer layers serving as support [28].

Mask type	Materials	Layers	Dimensions (cm)	Filtration efficiency	Main applications
Surgical Mask	Polypropylene (two hydrophilic inner layers, one melt-blown layer)	3	17.5 × 9.5	70-80% for particles $\ge 0.3$ $\mu m$	Medical, general use
N95 Mask	Four layers of spunbond + SMS + two layers of melt-blown	6	Standard	95% for particles $\geq 0.3 \ \mu m$	Medical, dentistry, hospital use
Cloth Mask	Two layers of melt-blown, two layers of spunbond, one layer of activated carbon	5	Standard	Variable (depends on fabric structure)	General use, air pollution protection

Table 1. Specifications of the selected masks

#### **Experimental** setup

Fig. 1 shows the experimental setup used in this study. The testing is based on the use of head forms mannequins and Cube boxes with dimensions 32\*28\*28 cm3. A 6 inch-diameter hole was made in the mannequin's mouth to inhale the airflow with the pollutant to simulate the human respiratory system. The upstream and downstream channels of the system were connected to this hole and the test chamber. A silicon sealant was used to avoid any leakage at the connection of the mask on the mannequin and in the test box. A disperser device is used to generate an aerosol from talcum powder. In the setup shown, clean air (without airborne particles) is first passed through a HEPA filter and then flowed through the system by a vacuum pump. The aerosol generated upstream of the system exited from the downstream of the system through the outlet pipe connected to the box after coming into contact with the mask and passing through the respiratory system of the mannequin. An airflow rate across the respirator was evaluated as a function of the inlet flow rate at 18 LPM and 42 LPM. These values represent adult respiration

rates at rest and during exercise [29, 30]. The test temperature and relative humidity were kept at  $25\pm3^{\circ}$ C and %30±4 by silica gel. Two probes, one placed upstream of the PPE and one placed downstream, measured the concentration of the aerosols. Particle size concentrations inside (C<sub>in</sub>) and outside (C<sub>out</sub>) of the FFR/SM (Face filtering respirator/Surgical mask) were measured using a Sideway light-scattering device (Particle Counter TES-5110, Taiwan) operating within a range of d<sub>p</sub> = 500–10<sup>4</sup> nm at a sampling flow rate of 2.83 L/min. Experimental data were analyzed and interpreted based on the theoretical framework describing the main paths followed by the exhaled air.

The particle penetration as an effective parameter through the respirator filter was determined as the ratio of the downstream concentration ( $C_{down}$ ) to the upstream concentration ( $C_{up}$ ) at each tested particle size ( $d_p$ ), which is presented as follows in Eq. 1 [31] :

$$P(dp) = \frac{c \operatorname{down(dp)}}{c \operatorname{up(dp)}} \times 100\%$$
<sup>(1)</sup>

(1)



- 3: Differential pressure probes
- 4: vacuum pump

- 7: Test chamber
- 8: HEPA filter

Fig. 1. The schematics of the study laboratory setup

#### Statistical methods

Data were analyzed using ANCOVA (Analysis of Covariance) to assess the effects of mask type on filtration efficiency and penetration rates, controlling for potential covariates such as airflow rate and humidity. Post-hoc comparisons were performed using the Tukey test to determine significant differences between the mask types. A significance level of p<0.05 was considered statistically significant.

#### The field sampling

The study was conducted in the emergency department of the Firouzgar Medical Research Training Center, a center for accepting and treating patients with COVID-19 in Tehran. Only patients with a positive RT-PCR test result or a Cycle Threshold (CT) with respiratory symptoms, such as cough, participated in the sampling process.

#### **Bioaerosol sampling method**

Because of viruses' structure that have genomes DNA or RNA enclosed in a fatty liquid membrane and need the host cell to multiply and cause infection, they aren't able to gather in sampling environments [32]. A liquid contact medium (salt solution with anti-foam agents and proteins) is used to collect the microorganisms in the air. Entrapping of air through liquid makes dispersion of particles in it, followed by quantitative measurement through serial dilution. All Glass Impinger samplers exhibit high velocity, a typical example of this category. These methods are inexpensive and highly useful for viable cell collection [33, 34].

In the present study, glass impingers with liquid absorbents (GILA) were chosen for sampling from the patients' respiratory zone because of their small sampling area and ability to capture the desired virus. The liquid impinger (liquidphase sampler) expanded as a highly efficient technique to capture airborne viruses in bioaerosol sampling [35].

In a hospital's isolated room, the sampling setup was positioned 1.5 meters above the surface to collect samples from the patient's respiratory area. The experimental arrangement included a vacuum pump, connecting tubes, and a standard impinger. During a 20-minute pumping session, 10 ml of Hank's Balanced Salts Solution (HBSS) was used as the impingement medium. Sampling was conducted on the breathing zone of three SARS-CoV-2 infected patients. Each patient wore a mask for 20 minutes and exhaled during the sampling. Six samples were collected from each patient (three samples with a mask and three without a mask for respiratory protection). Additionally, the control samples were taken from three healthy individuals at the hospital. Following each 20-minute sampling period, the samples were prepared and dispatched to a clinical virology laboratory for RT-PCR testing [36]. Fig. 2 shows the sampling schematic.



Fig. 2. The schematic of the air sampling experiment setup in the COVID-19 isolation room

# *RNA extraction and SARS CoV-2 genome amplification by real-time PCR*

The viral RNA was isolated from 500 µl of the specimens using a QIAamp DSP Virus Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's protocols. The quality and quantity of the extracted RNA were tested by a NanoDrop spectrophotometer instrument (Thermo Scientific, Wilmington, MA), and then the isolated RNA was stored at -20°C until the experiment. The real-time polymerase chain reaction (RT-PCR) with specific primers and TaqMan probes was used to determine the presence of the RNA of SARS-CoV-2 in the specimens. The first complementary DNA (cDNA) was synthesized to detection of the virus genome, as described in detail earlier [37]. In the current survey, a conserved region of RNAdependent RNA polymerase (RdRp) gene [38], Nucleocapsid (N) gene [39] of SARS-CoV-2, and RNase P gene as an internal control [40] were amplified as mentioned previously in detail [39-41].

#### **Results and discussion**

#### Evaluation of filtration efficiency of N95, surgical, and fabric masks across particle sizes from 0.5 to 10 $\mu$ m at various humidity levels and inlet velocities

Several tests were conducted at different surface levels to determine the inflow rate and filtration efficiency of the N95 mask. Fig. 3 illustrates the filtration efficiency results for particles of various sizes by N95, fabric, and surgical masks. These findings indicate that as the input speed and the number of tests increases, the filtration efficiency of N95 and fabric masks decreases for particles smaller than 5 and 2.5 microns, respectively. However, for particles larger than 5 and 2.5 microns, there is no significant change in the efficiency of N95 and cloth masks with increased humidity and input speed. As for surgical masks, the results show that with the rise in input speed and number of tests, the filtration efficiency of the surgical mask decreases as the particle size increases. It is worth noting that for small particles  $(0.5\mu)$ , the efficiency of the surgical mask reaches a minimum of 30% with increased input speed and number of tests.

The ANCOVA results indicated that the two variables of speed and humidity had a notable impact on the filtration efficiency of all three mask types. Further analysis using Tukey's post hoc test revealed that the N95 mask outperforms all three facemasks in filtering particles (P<0.05).

In investigating the effect of particle size on the filtration efficiency of masks, the results showed that particle size affects the filtration efficiency of N95 and cloth masks, while this effect is not significant for surgical masks (P=0.612). Tukey's post hoc test also reconfirmed that the N95 mask has the highest filtration efficiency.

To investigate the simultaneous effect of different levels of speed and humidity on the filtration efficiency of masks when exposed to the tested aerosols, the results of the ANCOVA test confirmed that simultaneous changes in the levels of speed and humidity significantly affect the filtration efficiency of pollutants in three types of masks. Tukey's post hoc test indicated that the N95 mask had a better filtering performance for particles (P < 0.05).









# Comparison of permeability of N95, surgical, and cloth masks across particle sizes from 0.5 to 10 $\mu$ m at various humidity levels and inlet speeds

Several tests were conducted at various humidity levels to determine the relationship between the inlet flow rate and the permeability of the N95 mask.

The N95 mask's average particle permeability results are shown in Fig. 4. These results indicate that increasing input speed and humidity during tests can lead to higher permeability of N95 and fabric masks for particle sizes below 5  $\mu$ m and 2.5  $\mu$ m, respectively. However, there is no significant impact on permeability for larger particle sizes with humidity and input speed adjustments. Regarding the surgical masks, the study revealed that as input speed and humidity levels rose during testing, pollutant penetration through the masks increased, especially with larger particles, peaking at 70% with particles as small as 0.5  $\mu$ .

The penetrations of particles through respirators for monodisperse aerosols in the 500–104 nm range were measured at flow rates of 18 and 42 L/min are presented in Fig. 4. Each point represents the penetration mean value determined for respirators, and the corresponding error bars represent the standard deviation.

The results showed that the maximum mean initial penetration level for N95 masks in particle size less than 700 nm was equal to 4.61%. However, this percentage decreased significantly to 0.8% when the particle size was increased to 10 µ; When compared to the particle size range of 500 nm to 10  $\mu$ , the results indicated lower mean initial penetrations for particle sizes greater than 5 µ. Furthermore, as illustrated in diagram 3, as the flow rate and face velocity increase, there is a noticeable shift in penetration towards smaller particles. This increase in airflow also results in a decrease in the retention time of these smaller particles, subsequently leading to a higher level of pollutant penetration in N95 masks. These factors collectively decrease the diffusion mechanisms and electrostatic properties of the masks, resulting in less effective removal of smaller particles [42].

The aerosol penetration characteristics of the surgical masks were different, as seen in Fig. 4. This mask has passed more than 60% of pollutants and had high airflow dependence for filtration of particles in the sub-micrometer size range. The penetration of particles into the filter was dependent on the airflow speed; A boost in airflow from 18 to 42 LPM resulted in roughly a 19% rise in the pollutants penetrating the masks. Surgical masks are equipped with a filter layer that effectively removes particles, enabling them to exhibit superior efficacy against respiratory droplets [43].

In fabric masks, according to Fig. 4, the penetration values in masks at the airflow rate of 18 l/min at particle size less than 2.5 $\mu$  were equal to 64%, 51%, 43%, and 40%. Increasing the airflow rate to 42 LPM, the permeability of the fabric mask has reached a high level, i.e., up to more than 60% in the size of small particles. Penetration of aerosol with large particle size 10 $\mu$  has reached its lowest level of 14 percent. Increasing the number of layers in a cloth mask generally improves its performance. For instance, a tested mask with six layers of different materials performed better [44].

The ANCOVA analysis showed that the speed and humidity levels significantly affect the penetration of pollutants in all three types of masks. Tukey's post hoc test further showed that the N95 mask outperformed in particle absorption with consistent speed and humidity conditions (P<0.05).

In examining the relationship between particle size and the permeability of masks, the results showed that particle size is one of the factors affecting aerosol penetration in N95 and fabric masks. Tukey's post hoc test confirmed that at the same particle size, the N95 mask performed best in absorbing particles, while the permeability of pollutants to the surgical mask was not significantly affected by particle size (P=0.545).

In general, the results of the ANCOVA test confirmed that the simultaneous changes in the speed and humidity levels significantly affect the penetration of pollutants in all three types of masks. Tukey's post hoc test also revealed that the N95 mask has a better absorbing performance for particles (P<0.05).







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#### Comparison of pressure lrop in masks

The pressure drop was measured directly during the tests to determine the relationship between the speed of the incoming flow and the pressure drop in the tested masks. The results of pressure drop variations at different inlet velocities are shown in Fig. 5. These results indicate that as the inlet velocity rises, the pressure drop also increases, making it harder for the user to breathe.

The ANCOVA results showed that among the factors of speed, humidity, and particle size, speed is the most influential on pressure drop across all three types of masks. Moreover, Tukey's post hoc

test revealed that using an N95 mask significantly hinders breathing ease (P<0.05).

When examining the combined effect of speed and humidity on mask breathability, the results demonstrated that simultaneous changes in these two parameters significantly affect the breathability of all three mask types. Tukey's post hoc indicated that the surgical mask provides the best breathing ease (P<0.05). Overall, the study's findings demonstrated that humidity and aerosol particle size do not significantly impact the breathability of masks. In simpler terms, the pressure drop in masks does not depend on changes in particle size or humidity.



Fig. 5. Pressure drop across masks at inlet velocities of 30, 40, 62, and 72.5 ft/min

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Fig. 5. Continued

#### Quality factor of masks

Fig. 6 illustrates the quality factor for various particles under different humidity and velocity conditions for surgical masks, cloth masks, and N95 respirators. As depicted in the figure, the quality factor reaches its maximum for all particles under low humidity and low-velocity conditions, decreasing to its minimum under high humidity and high-velocity conditions. In all experimental conditions, the quality factor for surgical masks was higher than that for cloth masks and N95 respirators. In general, surgical masks effectively filter particles across various humidity and velocity conditions. The improved performance of surgical masks is likely attributable to their structure and the materials used in their construction. Under low humidity and velocity conditions, filtration is more efficient, probably due to the decreased concentration of particles and increased contact time with the filtration material. Conversely, under high humidity and velocity conditions, the reduction in the quality factor may be due to increased particle concentration and decreased contact time with the filter, thereby reducing the mask's efficiency. These findings suggest that further research and development in mask materials and design are necessary to enhance various performance across conditions. For instance, developing masks with novel materials that maintain high efficiency in high humidity and velocity conditions could significantly improve personal protection and reduce the transmission of respiratory diseases. The effectiveness of surgical masks can be assessed from various angles. Firstly, their enhanced filtration efficiency across various conditions suggests that they are more effective in trapping particles. Secondly, the materials and design of surgical masks likely contribute to a more consistent and reliable performance. Finally, the ability of surgical masks to maintain higher quality factors under varying conditions indicates their robustness and suitability for a wider range of applications compared to cloth masks and N95 respirators.



Fig. 6. Quality factor across masks at inlet velocities of 30, 40, 62, and 72.5 ft/min

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Fig. 6. Continued

Table 2. Sampling and clinical characteristics of persons at risk of exposure to SARS-CoV-2

Patients	Clinical symptoms	sex	Average age (Year)	Relative humidity (RH)	Temperature	Air- conditioning (On/Off)
А	Headache-Fever-Anorexia- Cough	male	35	40.6%	24.6°C	off
В	Headache-Bodypain- Backache-Insomnia-Severe coughs	female	50	34.2%	26.7°C	off
С	Fever and chills- Bodypain- anorexia-Cough-Loss of sense of taste and smell	female	64	37.1%	28.4°C	off

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Sample code	Face mask type				
	N95	Surgical	Fabric		
Patient A	Negative	Positive	Positive		
Patient B	Negative	Negative	Negative		
Patient C	Negative	Positive	Negative		
Filtration Rate of Viral Particles	100%	33%	66%		

Table 3. The results of analysis for samples from patients' breathing zones of SARS-CoV-2

# Filtration efficacy against COVID-19 bioaerosols (field sampling)

In this study, the respiratory area of the patient was sampled as a source of contamination.

It was necessary to consider the patient's clinical symptoms, including severe symptoms at the sampling time, such as fever, severe cough, body ache, and sneezing, and their medication history. It ensures that a significant amount of exhalation droplets are collected, which can be easily absorbed by the sampling solution.

A total of 18 samples were collected from three COVID-19 patients to test the effectiveness of respiratory protective masks against SARS-CoV-2 (The samples were taken using three types of face masks and in two different situations - before and after sampling). The results of the sample analysis are given in Table 3. As presented in Table 3, the results of RT-PCR analysis confirmed the existence of SARS-CoV-2 in patients' breathing zones. The RT-PCR analysis results of all samples taken while the patient was wearing an N95 mask were negative, indicating its ability to filter smallsized virus particles. Surgical masks cannot filter virus particles; In other words, this mask isn't suitable for use in high concentrations of COVID-19 virus. In the second patient, the

results were all negative, which means the virus is weak in the patient's body.

Based on the results of surgical masks, 33% of patients' RT-PCR results were negative. That means the type of mask has not been effective as a parameter in the filtration rate of viral particles.

In the present study, we tested the effectiveness of respiratory protection masks in SARS-CoV-2 pandemic conditions on a laboratory scale using by Setup and SARS-CoV-2 in the inhalation samples of COVID-19 patients from the emergency department. Due to intense exhalation activities, coronavirus aerosols spread from the patient's respiratory area into the air, resulting in a high concentration of particles in the samples collected. The study results show that reducing the risk of spreading viral particles and Non-viral particles has been approved for respiratory protective masks for all people in hazardous conditions [45, 46].

Particle size is the most important factor for identifying pollutant behavior in the mechanisms of separation and filtration of aerosol particles, such as direct interception, inertial deposition, diffusion, and electrostatic [47]. For this purpose, it is recommended to use respiratory protection masks to control the risk of exposure to particles, which serve different functions depending on the size of the inhaled particles [48].

To prove the results of the studies, we can mention other side factors that are effective in the sampling process. Humidity and temperature are among the factors affecting the spread and stability of biological agents in the airborne. RH is classified into three levels: high levels of humidity (more than 70%), medium humidity (30% to 70%), and low humidity (less than 30%) [17]. For example, enveloped viruses such as Influenza [49] and Newcastle disease virus [50] are stable at low humidity, while nonenveloped viruses such as rhinoviruses [51] and poliovirus [49] remain stable at high humidity. On the other hand, Coronaviruses [52] and rotaviruses [53] are constants in intermediate RH. The stability or instability of the viruses in the air doesn't depend on the enveloping genomic RNA, but the structure of the virus is the determining factor [54]. Temperature also affects the stability of viruses, which means decreasing the temperature leads to improving the persistence of the virus in the air [49, 55, 56].

Also, airflow is a significant factor in the effectiveness of face masks. At low velocities, pollutants stay longer on the filter bed, which enables electrostatic diffusion and absorption mechanisms to work effectively for pollutant removal. As the flow rate increases, the interception mechanism becomes dominant [57].

Exhalation events are divided into two categories: breathing, as a continuous and gentle event, and other random events, which are talking, sneezing, and coughing. Each event contains an airflow full of suspended particles that spread due to their size distribution, angle of emission, and initial velocity. Depending on the state of the emitted contamination, only a portion of the particles can be considered infectious particles, and the probability that a respiratory droplet contains the virus depends on its initial volume and the above factors [58]. As pointed out above, a patient can release simultaneously infectious and non-infectious particles. Sneezing and coughing release more molecules, but breathing has less emission pollution [59]. The study found that using a respiratory protection mask can effectively prevent the spread of COVID-19 droplets from the air and the patient's respiratory area.

In the situation where sampling of the inhaled air of the patient is done, due to exhalation activities such as talking, coughing, and sneezing, concentrations of pollutants are detected in the samples, thus confirming the hypothesis that SARS-CoV-2 is released through exhaled respiratory droplets, increasing the likelihood of exposure. These small pollutant particles pass through the airflow and enter the sampling solution [60, 61]. As a result, the release rate of viral aerosols from a patient with COVID-19 depends on the strength of the release source [62].

According to Yarahmadi et al.'s study, all samples taken from the respiratory zone of COVID-19 patients (the source of infection spread), were positive. The sampling method is similar to our study and was done by impinging method via HBSS culture medium at high osmotic pressure. In our study, the results of RT-PCR of samples taken from patients were positive in the condition of not wearing a respiratory protection mask. Therefore, the closest point to the highest pollution concentration is the patient's respiratory area, which is aggravated by performing respiratory activities such as sneezing, coughing, and talking [63].

Regarding the effect of speed on changing the filtration efficiency of masks, Konda et al. investigated the filtration efficiency of fabric masks at two-speed levels of 19.6 ft/min and 51 ft/min (3.2 CFM and 1.2 CFM), which are equivalent to rest and light physical activity in the use of a breathing mask. They showed increasing the inlet speed at higher flow rates led to the filtration efficiency of the masks

weakening [44]. In the research of Eninger et al., the permeability of N95 respirator masks was investigated at flow rates of 30 L/min, 85 L/min, and 150 L/min. The maximum pollutant penetration in the tested N95 masks occurred at high flow rates related to particle sizes 0.02 nm to 0.5 nm. The maximum pollutant penetration is 8.1% at an airflow rate of 150 L/min for particles less than 0.1 nm. While the permeability reached less than 0.5% as the pollutant particles became larger [64].

In contrast to the results of our study, the study by Cheng et al. in 2020, in the conditions with surgical masks and without it, did not observe the SARS-CoV-2 in the air samples that were taken from a 10 cm distance of the patient's chin [45].

Also, in the study of Ong et al. in 2020, the samples Collected in the respiratory area of the patients were found to be negative [65]. This result contradicts our study, despite the samplers were placed inside a shelter covered around COVID-19 patients at a distance of 10cm from patients' chin to increase the collection efficacy of exhaled virus and avoid the effect of environmental airflow but all samples tested negative for SARS-CoV-2.

The reason for the contradictory findings in the studies is the lack of a standard method for collecting air samples in virology studies. In this research, sampling of SARS-CoV-2 was done by a midget impinger, while other studies have used a G-II bioaerosol collecting device, Andersen sampler, bio sampler, and others [17]. However, the fabric masks have little filtration compared to other masks [26], The present study shows that wearing fabric masks multilayered by patients with coronaviruses infected could filtrate bioaerosols ≤5 µm. Surgical masks weren't as effective as respiratory protection devices against COVID-19. The surgical mask provides little protection against aerosols and bioaerosols [28]. Also, all values of the particle's penetration through respirator N95 are below 5% because this is a certified N95 respirator. Between the three mask models, penetration of all N95 model FFRs was lower and as expected for FFRs containing electrostatic filter media It may be this fact that the ability to remove aerosol particles less than 0.3  $\mu$ m, low breathing resistance to make breathing comfortable in the user, and tight fitting on the user's face so that the air passing around doesn't enter the mask are the main features for an N95 mask [66]. Also, particle penetration significantly increased as the flow rate increased in all three types of masks, consistent with previous studies [42, 67-69].

On the other hand, a possible limitation of studies could be that the volume of air collected for each sample influenced the ability to detect a PCR peak. Therefore, the large volume of sampled air leads to more negative results. Most of the collected samples were positive because there was no window in the sampling room, and the ventilation system was turned off, which shows the significant role of air ventilation in removing or diluting virus-laden aerosols exhaled by infected patients [60, 70]. It's necessary to mention we didn't investigate the prevalence of SARS-CoV-2 in other conditions, including in ambient room air. However, in other studies, the presence of SARS-CoV-2 in the air of the hospital environment has been proven [63]. In the following sampling room, due to the isolation of the room and low usability, the room ventilation system, which was in the form of ceiling vents, was turned off during all sampling hours. Also, the room lacked a window to allow air circulation within. The lack of a window and the shutdown of the room's ventilation system (Table 2) led to airborne transmission, which helped spread further infection. Studies have shown that insufficient ventilation increases the risk of infection [71].

This research was a cross-sectional study on respiratory protection masks against aerosols and bioaerosols particles. In this study, the performance of the mask was estimated by testing their effectiveness in laboratory conditions with aerosol challenge and the field environment with virus particles during the COVID-19 pandemic. There were several limitations in the study. First, due to the COVID-19 pandemic outbreak and the complexity of treating patients, the study suffered from low cooperation and participation. Second, the total air sampling process, including patient preparation and instructions, took at least 2 hours per patient; Some patients declined to participate in the study. Third, the infectious virus dose calculation did not consider factors such as the patient's viral load fluctuations, the particle-generating procedures efficiency, and the immunity of sensitive individuals. Fourth, the experiment didn't measure the impact of varying humidity levels on the masks.

## Conclusion

Exposure to bioaerosols poses significant risks to human health, particularly affecting the respiratory system. The study findings highlight the importance of selecting appropriate respiratory masks to mitigate these risks. Variations in particle penetration and filtration efficiency were observed among different mask types. N95 masks showed exceptional performance, with only 4.61% penetration for particles smaller than 700 nm and efficiency increasing to 99.2% for larger particles. In contrast, surgical masks demonstrated filtration efficiencies ranging from 31% to 68%, while cloth masks varied from 28% to 86%.

The effectiveness of respiratory masks in preventing the transmission of airborne particles and viruses, such as SARS-CoV-2, underscores their critical role in controlling disease spread, particularly in high-risk environments like hospitals. Regular and proper use of masks, especially those with proven efficacy, can significantly reduce the risk of airborne virus transmission and improve public health outcomes. Efforts should focus on public education about mask selection and usage and ensuring access to high-quality masks for both healthcare workers and the general public to better manage and control future epidemics.

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## **Competing interests**

The authors declare no competing interests.

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## Ethical considerations

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