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Bacterial Filtration Efficiency of Different Masks

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Face coverings, such as surgical masks and respirators, have an important role in preventing bacterial and viral transmission, especially during a global pandemic like COVID-19. Therefore, to secure their availability, new manufacturers and the use of novel materials must be encouraged. However, masks and their materials must first be properly tested for safety and efficiency, as required by the relevant standard, valid in a specific region. All standards prescribe determination of the bacterial filtration efficiency (BFE) of masks. In this study, we report the establishment of a test method for the BFE of face masks in accordance with European standard EN 14683:2019, by which we tested 52 samples, each composed of 3 to 5 subsamples, of surgical and cloth masks, respirators, filters, and mask materials. Forty-seven out of the 52 samples reached a BFE above 75 %. Of these, 16 samples had a BFE of 75 % to 95 %, 3 had a BFE of 95 % to 98 %, while 28 reached a filtration efficiency above 98 %. Our findings show that all tested samples provided some level of protection, most of which met the requirements for the national or European market.

Keywords: bacterial filtration efficiency, face coverings, masks, respirators, Andersen Cascade Impactor, EN 14683:2019

Highlights

- *A method for the determination of BFE testing was established according to the European standard EN 14683:2019, using bacteria Staphylococcus aureus subsp. aureus (ATCC® 6538™).*
- *The method was shown to be robust and provided high quality and well reproducible results.*
- *A total of 52 experiments were conducted and 245 mask subsamples were tested, including 153 surgical and cloth masks, 20 respirators, 25 filters, and 47 mask materials.*
- *Tested masks and materials were evaluated based on their BFE values and grouped according to the national and European standard.*
- *The majority of the tested samples, i.e., 90.38 %, reached the BFE ≥ 75 %, while 5.77 % and 53.85 % had even higher filtration efficiencies of 95 % to 98 % and ≥ 98 %, respectively.*

0 INTRODUCTION

In December 2019, several cases of pneumonia-like disease of an unknown cause were reported in Wuhan, China. This disease was later named COVID-19 and its novel viral agent, severe acute respiratory syndrome coronavirus (SARS-CoV-2), was identified [1]. The virus is transmitted mainly by respiratory secretions that include both larger and smaller droplets (the latter are also known as aerosols). These are expelled during coughing, sneezing, talking, and singing, and may continue to linger in the air for longer periods of time depending on their size. Such mode of transmission is highly efficient, and resulted in a surge of number of cases, soon leading to a worldwide pandemic [2].

To reduce the number of active cases of COVID-19 and prevent further transmission of SARS-CoV-2, certain government-issued measures and restrictions were set in place. These differed slightly from country to country, but most included social distancing, vaccination, regular testing, proper hand hygiene, and the use of masks as personal protective equipment (PPE) [3] and [4]. As a result, the use of face masks become mandatory not only in the public health sector, but also in people's everyday life. Due to the sudden increase in demand and the disruption of global supply chains, countless countries faced a shortage of aforementioned items [3] to [5]. To combat these problems and secure the general availability of PPE, certain strategies have been proposed. These included decontamination of respirators, reuse of disposable masks and their extended use, and the use of expired masks. The appearance of new manufacturers of face masks on the market was also noted, as was the number of novel materials being tested for their safety and filtration efficiency. In addition, the introduction of disposable mask replacements, such as machine-washable cloth masks, was encouraged, resulting in a high occurrence and availability of handmade cloth masks [3] and [4].

Many types of face masks with a broad range of filtration efficiencies are currently available and include surgical masks, cloth masks, and respirators, with surgical masks being the most widespread [6]. A standard surgical mask consists of three layers (Fig. 1). The outermost layer is waterproof and repels external fluids, such as salivary droplets produced by breathing, speaking, coughing, and sneezing. The middle layer is a filter, composed of tightly interwoven thin synthetic fibres. This prevents particles, including

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microorganisms, above a certain size from penetrating the mask from either side. Lastly, the innermost layer absorbs salivary droplets from the user, and moisture from the exhaled air, aiding in the comfort and wearability of the mask. Such masks are typically made of polypropylene, although polystyrene, polycarbonate, polyethylene, and polyester can also be used [6]. Similarly, respirators consist of four layers of materials. The outermost and innermost are made from hydrophobic non-woven polypropylene. This prevents external moisture and liquid particles, exhaled during the user's breathing, from being absorbed and penetrating the mask, respectively. The second outer layer is a melt-blown non-woven polypropylene layer, which captures oil and non-oil-based particles. The third layer is made from modacrylic. It provides rigidity and thickness to the mask, giving it more structure and aiding in comfort [7].

The number of layers and the properties of the materials used contribute to the high efficiency and safety of surgical masks and respirators [8]. Therefore, masks made of other materials (e.g., cloth masks) are of different quality and efficiency. The most common fabrics used in the production of cloth masks are cotton and different cotton blends, silk, linen, and certain synthetic fabrics [8]. Multi-layer cloth masks made of a combination of fabrics, such as cotton and chiffon or cotton and silk, are most efficient, but still do not provide the same protection as surgical masks or respirators [9] to [11].

When it comes to producing new types of masks, the most common strategy is to use new materials and materials with specialized functions (i.e., functionalized materials and masks). The latter enhances the protective properties and longevity of masks by incorporating graphenes, alkyl silanes, and metal nanoparticles into otherwise standard materials used for production of face coverings [6], [12] and [13].

With the increase of newly available face masks and materials, the number of inadequate products also increased. In Europe, medical face masks must comply with the standard EN 14683 [14], which prescribes testing requirements and relevant methods. Masks must be tested for their general construction and design, cleanliness of materials used, splash resistance, breathability, microbial cleanliness, biocompatibility, and bacterial filtration efficiency (BFE). The latter is determined based on filtration efficiency of bacterial droplets and aerosols, carried by ambient air, mimicking air exhaled into the environment. Therefore, this parameter defines the tested masks' ability to prevent further spread of bacteria. As of October 25, 2021, the European co-operation for Accreditation (EA) [15] reports 52 accredited laboratories for face mask testing, of which only 40 are accredited for the scope EN 14683. Due to the limited number of available accredited testing laboratories, many newly available face masks or materials have not been properly tested, rendering them unsuitable as part of effective PPE [15].

In this study, we report the process of establishing and validating a protocol for determining BFE of face masks, compliant with the European standard EN 14683:2019. Using the six-stage Andersen Cascade Impactor (ACI), we determined BFE of 52 different face coverings and materials. This resulted in a total of 245 items tested (3 to 5 subsamples of each face covering or material were tested). We evaluated the reproducibility of the used method, and its suitability for testing different masks and materials.

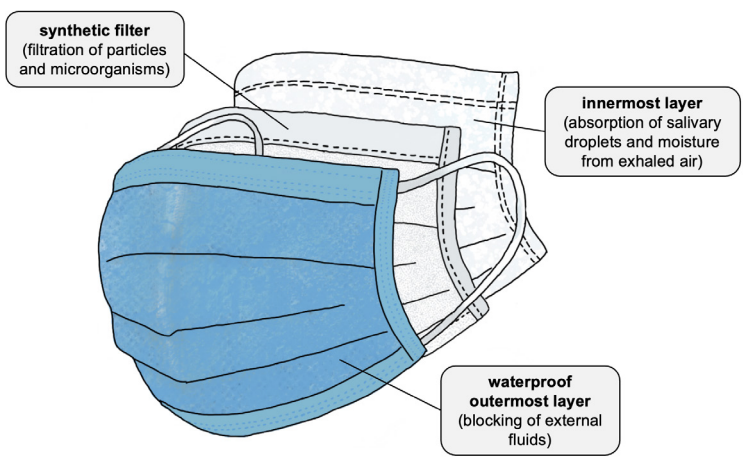

Fig. 1. *Layers of a standard surgical mask*

1 TEST METHOD

The test method for determining the BFE of masks and materials was performed as required by the European standard for face mask testing EN 14683:2019. This included the construction of a test apparatus and its validation. The standard defines testing methods and requirements for medical face masks only, although filters, respirators, and mask materials were tested using the same procedure.

The test apparatus consists of multiple parts with different functions (Fig. 2). The nebulizer is used to produce droplets and aerosols with an average diameter of 3.0 µm ± 0.3 µm (Fig. 2b) from a bacterial suspension (Fig. 2c). These are then mixed with ambient air and travel through a cylindrical glass chamber (Fig. 2d), which ends with a smaller and narrower cylindrical glass outlet connected to a two-piece metal clamp (Fig. 2e), between which mask samples are attached during testing of BFE (masks were not used for the positive controls, as discussed below). Droplets and aerosols are then collected using the ACI (Fig. 2f), which contains six agar plates (i.e., one per stage). Each stage has 400 holes that differ in diameter, which decreases from top to bottom stage. These dictate the size of particles (i.e., aerosolized bacterial suspension) that can pass through a certain stage and settle on the plate [16]. The bottom part of the ACI is attached to a vacuum pump that controls the airflow in the system (Fig. 2h). In between is a HEPA filter (Fig. 2g), which prevents aerosolized bacterial particles from leaving the test system. The entire setup is located in a Class 2 biological safety cabinet to minimize the risk for infection with bacteria *S. aureus*.

2 EXPERIMENTAL PROCEDURE

We tested several types of face masks and materials from different manufacturers. We grouped these samples into four types, which included face masks, respirators, filters, and mask materials (henceforth referred to as mask samples). Note that the group of face masks included not only surgical masks but cloth masks as well, while the group of filters included filters for hospital ventilators (aiding patients in breathing) and standalone filters, usually attached to respirators.

The experimental setup was the same for all experiments and followed the principles described in EN 14683:2019, with minor optimizations [14]. Suspension of the bacterium *Staphylococcus aureus subsp. aureus* (ATCC® 6538™) was used in all BFE tests. It was prepared by inoculating 30 ml of tryptic soy agar (TSB) prepared from 30 g/l TSB (BD), with the frozen Microbank bead containing the bacteria. This was incubated for 24±2 h at 37±2 °C with shaking at 240 rpm. The appropriate dilution of the bacteria was then prepared in peptone water [10 g/l peptone (BD) and 5 g/l NaCl (Merck)]. So prepared bacterial suspension was aerosolized in the nebulizer and the concentration was maintained at 1.28×10^3 to 3.07×10^3 colony forming units (CFU)/test. The generated droplets and aerosols were then mixed with high-pressure ambient air at a flow rate of 28.3 l/min, mimicking the respiratory flow rate. After the

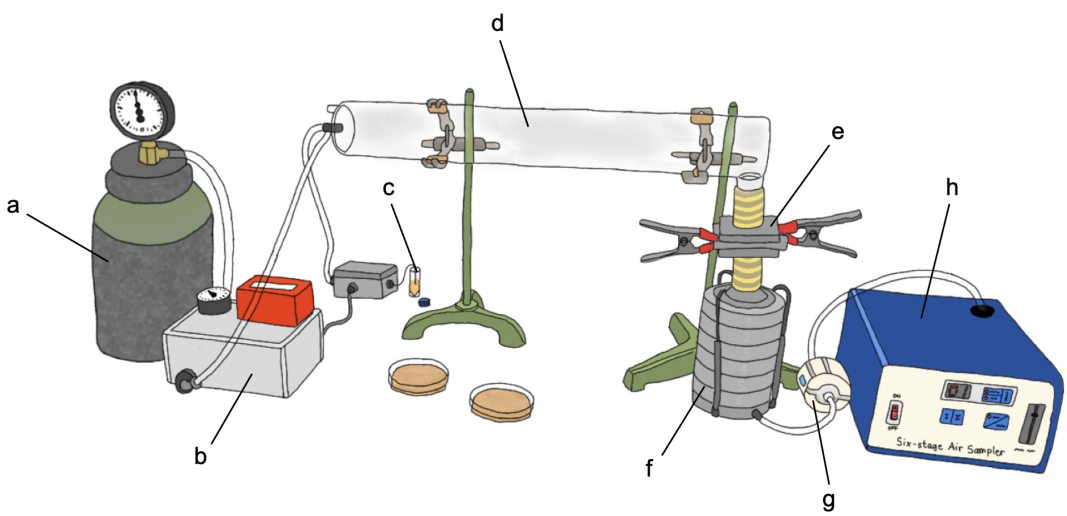

Fig. 2. *Scheme of the test apparatus; a) high-pressure air source, b) nebulizer, c) suspension of S. aureus, d) cylindrical glass chamber, e) two-piece metal clamp, f) Andersen Cascade Impactor (ACI), g) HEPA filter, and h) vacuum pump*

1-minute aerosolization, airflow through the system was maintained for an additional minute, so that the total test time was 2 minutes. The air carried the generated droplets and aerosols through a cylindrical glass chamber and on through a metal clamp, with or without (in the case of positive controls) a mask sample. The mask sample was firmly attached with the innermost layer facing upward and the area of (10×10) cm^2 was exposed to droplets and aerosols. In the case of filters and respirators, modified clamps were used to enable their proper fitting on the test apparatus (the area tested was smaller than for the surgical or cloth masks). Particles, which were not intercepted by the attached face mask, passed on to enter the ACI and were collected on agar plates.

The ACI is designed to mimic the flow of inhaled particles through the human respiratory system. During nasal breathing, larger particles (5 µm and larger) get caught in the nasal cavity and esophagus, while smaller droplets and aerosols enter the lower respiratory system. In the first stage of the ACI, particles larger than 7 µm in diameter are captured and represent droplets that remain in the nasal area. Particles with a diameter of 4.7 µm to 7 µm are collected in the second stage and can enter the lower levels of respiratory system, the pharynx. Droplets with a diameter of 3.3 µm to 4.7 µm, collected in the third stage, remain in the trachea and primary bronchi. Particles (2.1 µm to 3.3 µm) that can reach the secondary bronchi are collected in the fourth stage, whereas those with a size of 1.1 µm to 2.1 µm reach the terminal bronchioles and are collected in the fifth stage. The smallest aerosols (up to 0.65 µm to 1 µm) can penetrate the lowest parts of the respiratory system, the alveoli, and reach the sixth and final stage of the ACI [16] and [17]. Particles that reach the lower respiratory organs (i.e., those with a size of 5 µm or less) pose the highest risk of infection, as they can carry harmful bacteria and viruses into the lungs [17].

As the ACI used consisted of six stages, six plates were used for each positive control, mask sample, or negative control (henceforth referred to as subsamples). The plates were prepared from 40 g/l tryptic soy agar (Fluka). After collection of the aerosolized particles, the plates were left to dry and were then incubated overnight at 37 °C. The following morning, bacterial colonies were counted (Fig. 3) and CFU of each subsample was determined. Colonies on plates 1 and 2 are scattered, and therefore counted normally, while colonies on plates 3 through 6 form a pattern similar to the layout of holes on each corresponding plate, increasing the likelihood that multiple colonies are growing in the same location. Therefore, when counting these colonies, the positive hole correction must be taken into account [16]. The number of CFU on each plate is used to calculate the BFE of each mask, as described below.

The experimental design is as follows: the experiment started with a positive control (PC), in which no mask was used to intercept the generated droplets and aerosols. Then, testing of mask subsamples was performed, where three to five mask subsamples were tested, followed by another PC. Lastly, a negative control (NC) was carried out, where air without addition of the bacterial suspension was passed through the system for 2 minutes. At the end of each experiment, the system was first cleaned by the aerosolization of 70 % ethanol (Merck) for 30 minutes, and then MilliQ water for 10 minutes. Efficiency of cleaning was monitored regularly by placing agar plates into the ACI during the 10-minute aerosolization of water. To minimize the possibilities

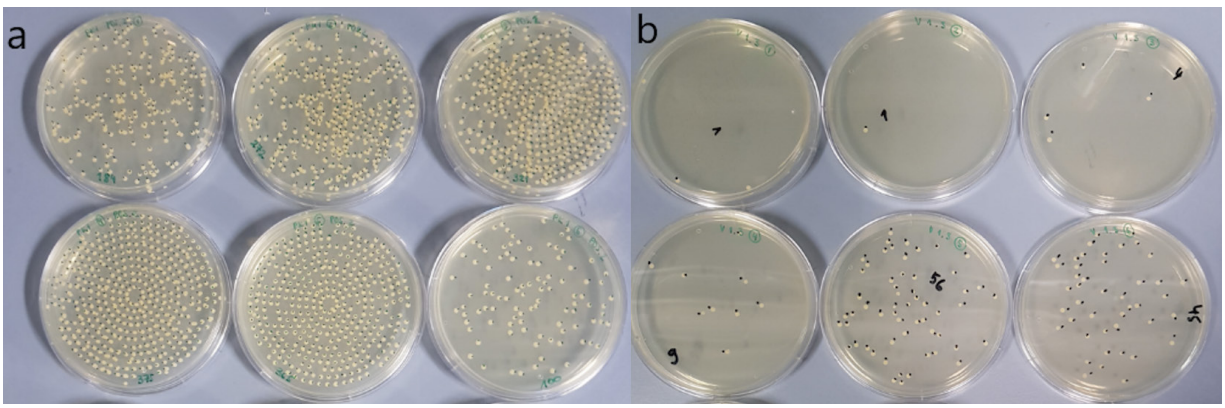

Fig. 3. *Agar plates with colonies of S. aureus used to calculate bacterial filtration efficiency (BFE) of a face mask; a) positive control run plates (one of two runs), where no mask was used during testing, and b) mask subsample run plates; the BFE of the tested mask was 83 %*

for contamination of the system, heat-resistant parts of the apparatus were autoclaved strictly after each experiment.

The average positive hole value of PCs was used to calculate the mean particle size (*MPS*), using Eq. (1). The values of P_1 through P_6 are 7.00 µm, 4.70 µm, 3.30 µm, 2.10 µm, 1.10 µm, and 0.65 µm, while C_1 through C_6 represent the number of colonies on plates 1 through 6 with the positive hole correction taken into account.

$$MPS = \frac{(P_1 \times C_1) + \ldots + (P_6 \times C_6)}{C_1 + \ldots + C_6}. \quad (1)$$

Using the average bacterial concentrations of both PCs (C_{PC}), and the bacterial concentration in a mask subsample (C_{SB}), the BFE for mask subsample ($BFE\,(SB)$) was calculated according to the Eq. (2).

$$BFE(SB)(\%) = \frac{(C_{PC} - C_{SB})}{C_{PC}} \times 100. \quad (2)$$

The final (i.e., average) *BFE* was then calculated with the Eq. (3) as the average value of all mask subsamples of the same experiment:

$$BFE(\%) = average\left[BFE(SBs)\right]. \quad (3)$$

The validity of the results was monitored with two parameters, i.e., MPS and the concentration of bacteria in the airflow, which must be within their determined range in each experiment. MPS values must be maintained at 3.0 ± 0.3 µm, while the concentration of bacteria between 1.7×10^3 and 3.0×10^3 CFU/test **[14]**.

3 RESULTS AND DISCUSSION

Overall, we conducted 52 BFE tests (Fig. 4). This included a total of 245 mask subsamples or 153 surgical and cloth masks, 20 respirators, 25 filters, and 47 mask materials. In addition, each experiment included two positive and one negative control. In parallel, cleaning of the system was monitored to prove the sterility of equipment and thus the adequacy of the whole testing procedure.

The most frequently tested sample type were cloth and surgical masks, as they were tested in 32 out of 52 experiments. Their average BFE was 94.23 %, with the minimum and maximum of 72.64 % and 100 %, respectively. The lower filtration efficiencies are from the cloth masks, which generally have lower filtration efficiencies than surgical masks **[10]** and **[11]**. Filters had the lowest average BFE of 74.27 % (with a range of 64.44 % to 83.51 %), which may be due to the fact that they are not meant to be used as PPE (filters for ventilators) or are less effective in bacterial filtration when used on their own (i.e., not as a part of a respirator). It is also important to mention that EN 14683:2019 does not define the testing methods and requirements for filters, which could affect their determined BFE values **[14]**. The average BFE of mask materials was 93.84 %, while the average BFE value of respirators was 96.18 %, which is similar to that of surgical and cloth masks. While respirators generally offer more protection than surgical masks, this is due to their better fit (when fitting is possible) and not necessarily to their higher filtration efficiency **[18]**, as discussed below. However, as with filters, the testing methods and requirements for respirators are defined by a different standard, EN 149:2001 **[19]**.

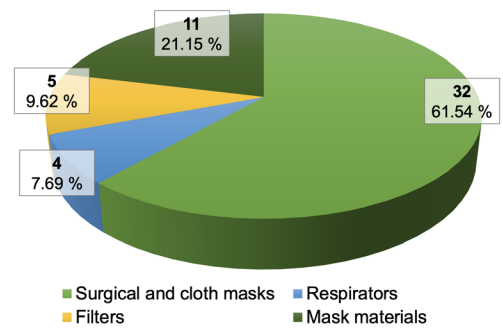

Fig. 4. *Number and percentage of mask samples tested*

At the beginning of the epidemic, as a response to the shortage in mask availability in Slovenia and EU, Slovenian Institute of Standardization prepared the Slovenian Specification for personal half masks SIST-TS 1200:2020 **[20]**, which prescribes a minimal BFE of 75 %. The European standard EN 14683:2019 sets the criteria even higher, i.e., for Type I masks at a BFE of 95 % and for Type II masks at a BFE of 98 % **[14]**. Forty-seven of the 52 (90.38 %) tested mask samples had a BFE of at least 75 %, meeting the criteria of SIST-TS 1200:2020. These included 30 surgical and cloth masks, 4 respirators, 3 filters, and 10 mask materials. Of these, 16 mask samples had a BFE in the range of 75 % to 95 %, 3 had a BFE of 95 % to 98 %, and 28 had a BFE above 98 % (Table 1) (Fig. 5). These findings show that the majority of tested mask samples are suitable for general use and sale at the national (i.e., Slovenian) level, while more than half meet the demands of the European market, either as Type I or Type II.

The BFE of mask samples is dependent on the conditions in the test system during the experiment, and the characteristics of the mask samples themselves. The former are defined by MPS (i.e., the range and average size of droplets and aerosols

in the airflow), and the concentration of bacteria in the particle-carrying droplets and aerosols, both of which are determined in PCs when masks are not used. These parameters must be maintained during all experiments [14]. As shown in Tables 2 and 3, the MPS for 52 experiments was maintained at 2.802 ± 0.004 µm, while the average bacterial concentration was $2.96 \times 10^3 \pm 1.86 \times 10^1$ CFU/test (i.e., the average error rate was 0.63 %). Both values are within their required ranges and have a small margin of error. While the MPS and concentration values are not expected to depend on the mask samples, both values are consistent regardless of the mask samples used, making the results highly reproducible. As such, the test system and method are stable and provide consistent results. They can be reliably used to test different kinds of cloth and surgical masks, respirators, filters, and mask materials.

As the test conditions were consistent, the differences in BFE between the four groups of samples and between multiple experiments within each group can be attributed to the differences in materials. As previously stated, surgical and cloth masks were grouped together, resulting in the broadest range of BFE (the minimum BFE value in this group was 72.64 % and belonged to a machine-washable cloth mask). The filtration efficiency of masks depends on the characteristics of the materials, such as chemical structure, number of layers, pore size, fibre organization, thickness, and diameter, packing density, charge, and hydrophilicity. Masks, woven from fibres with a small diameter (and therefore a large surface area), which form small pores, display the highest efficiency in particle trapping. Electrostatic properties are also critical – charged materials enable attraction between fibres and air droplets or aerosols, that may carry bacteria, preventing the latter from penetrating the mask. Therefore, polypropylene, polyethylene, polyacrylonitrile, and other polymeric materials are the best choice for production of face masks. Highly

Table 1. *Number and percentage of mask samples meeting different bacterial filtration efficiency (BFE) requirements and classification of the masks into Type I and II (EN 14683:2019)*

	BFE < 75 %		BFE 75 % to 95 %		BFE 95 % to 98 % (Type I)		BFE ≥ 98 % (Type II)	
Sample type	Number of masks	% of masks	Number of masks	% of masks	Number of masks	% of masks	Number of masks	% of masks
Surgical and cloth masks	2	6.25	9	28.13	2	6.25	19	59.38
Respirators	0	-	1	25.00	0	-	3	75.00
Filters	2	40.00	3	60.00	0	-	0	-
Mask materials	1	9.09	3	27.27	1	9.09	6	54.55
Total	5	9.62	16	30.77	3	5.77	28	53.85

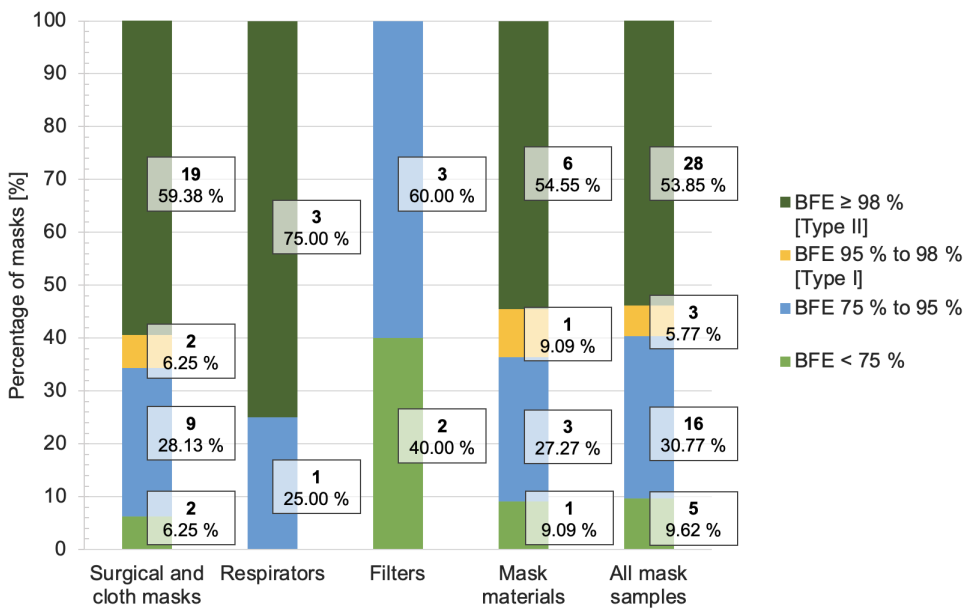

Fig. 5. *Number of samples meeting different bacterial filtration efficiency (BFE) requirements*

Table 2. *Average mean particle size (MPS) values*

Sample type	Number of experiments	Average MPS [μm]			
		Minimum	Maximum	Average	Standard error
Surgical and cloth masks	32	2.35	3.14	2.76	0.01
Respirators	4	2.54	3.05	2.74	0.05
Filters	5	2.42	2.95	2.77	0.03
Mask materials	11	2.35	3.39	2.83	0.02
Total	52	2.35	3.39	2.802	0.004

Table 3. *Average bacterial concentrations*

Sample type	Average concentration [CFU/test]				Error rate* [%]
	Minimum	Maximum	Average	Standard error	
Surgical and cloth masks	1.51×10^3	5.16×10^3	2.78×10^3	77.57	1.29
Respirators	1.51×10^3	4.45×10^3	2.94×10^3	37.79	11.69
Filters	2.14×10^3	6.62×10^3	3.70×10^3	432.22	4.43
Mask materials	2.04×10^3	4.68×10^3	3.18×10^3	140.75	2.79
Total	1.51×10^3	6.62×10^3	2.96×10^3	18.69	0.63

*The error rate was calculated as standard error divided by average concentration.

hydrophilic materials are also efficient in trapping and filtration of liquid particles [8]. This is also related to the number of layers of a mask, as each layer acts as an obstruction for droplets and aerosols, carried by the airflow. This further explains the three and four-ply structure of standard surgical masks and respirators, respectively [6] and [7]. Although it does not affect the BFE of face masks, proper fit further improves their protective properties [21]. Fit is especially relevant when comparing the filtration efficiency of surgical masks and respirators. As shown above, these often have a similar BFE, however due to their design and fit (if properly fitted on each individual), respirators seem to offer better protection [18]. This, however, is not evident in the proposed results, as we only tested the filtration efficiency or the material, irrespective of shape and fit.

4 CONCLUSIONS

Our study offers valuable results and information regarding the establishment of a test method for the BFE of masks, as required by EN 14683, especially considering lack of recent studies on this topic. Since the process parameters, i.e., MPS and bacterial concentration, were similar in all experiments, regardless of the mask sample tested, this indicates that used method is stable and reproducible, and thus can be used for determining BFE of different types of face masks. This is especially valuable in situations with limited availability of PPE, as is the case with the COVID-19 pandemic.

Based on the determined BFE values of the tested samples, a number of face masks provided adequate protection against aerosolized bacteria, with 90.38 % having BFE of more than 75 %. Therefore, they are suitable as PPE, and prevent or lower the possibility of spread of pathogenic bacteria and other microorganisms through exhaled air. However, other properties of the face masks need to be tested in addition to the BFE, highlighting the need for more EA-accredited testing laboratories.

By proper and widespread testing of masks, additional masks could be available on the market, making such protective equipment more accessible, especially in times of need. This would aid in prevention of further dissemination of pathogens, as face coverings protect not only the user, but others as well.

Although this study focused on the BFE of different mask samples, they also play an important role in the defense against viral pathogens. However, to properly determine their protection against viruses such as SARS-CoV-2, they must be tested for viral filtration efficiency (VFE). We have also established a protocol for VFE and tested a number of masks using it. The results, which will be published in an upcoming paper, will give insight into the protective abilities of face masks against virus spread.

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