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Validation of a method to elute viruses from different types of face masks

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Autoclaving

ABSTRACT

Due to the SARS-CoV-2 pandemic, it is crucial to study the efficiency of face masks in retaining viruses for the upcoming years. The first objective of this study was to validate a method to elute viruses from polyester and cotton face masks. We observed that deionized water followed by 3% beef glycine (pH 9.5 or pH 7.2) was significantly more efficient ($p < 0.05$) in eluting the bacteriophage phiX174 virus from polyester (4.73% ± 0.25% to 28.67% ± 1.89%), polyester/cotton (3% ± 0.33%), and cotton (1.7% ± 0.21%) face masks than 3% beef glycine only (pH 9.5 or pH 7.2) as a single eluent (3.4% ± 0.16% to 21.33% ± 0.94% for polyester, 1.91% ± 0.08% for polyester/cotton, and 1.47% ± 0.12% for cotton face masks). Also, deionized water was significantly less efficient as a single eluent for eluting bacteriophage phiX174 from all the studied face mask types. The polyethylene glycol (PEG) precipitation method was substantially more efficient ($p < 0.05$) as a second step concentration method for the viruses in the eluates than the organic flocculation (OF) method. Higher viral loads were eluted from polyester face masks than cotton ones. We also found varying viral loads in the eluate solutions from different commercial polyester face masks, with the highest percentage seen for the N95 face mask. The second objective was to apply the validated method to study the effect of autoclaving on the different face mask materials. Results of the study did not show any significant differences in the viral loads eluted from the

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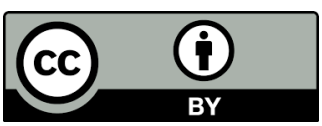


studied face masks before and after one and five autoclaving cycles. Moreover, a scanning electron microscope (SEM) analysis revealed no changes in the yarns, elongation, tensile strength, and contact angle measurements of the polyester or cotton materials after one or five autoclaving cycles.

1 Introduction

Coughing, talking, and breathing are different transmission routes for respiratory viruses, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which spreads through microdroplets expelled from the human respiratory tract into the air. The airborne transmission of these microdroplets depends on their sizes, which usually are in the µm to mm range, while some larger ones can settle on surfaces. Several viral diseases can be transmitted through airborne routes, such as influenza, SARS, respiratory syncytial virus, adenoviruses types 4 and 7, porcine coronavirus, and foot and mouth disease virus. However, they can also be transmitted *via* direct contact with infected persons (Yu et al. 2004; Atkinson and Wein 2008; Kuo et al. 2009; Gloster et al. 2010; Lindsley et al. 2010; Verreault et al. 2010).

Bacteriophages are believed to represent good surrogates for studying airborne viruses as they are safe for laboratory workers, relatively easy to produce on a large scale, and can be purified using several available techniques (Gill and Hyman 2010). Due to their high genetic and morphological diversity, they can be easily distinguished from a large pool of viruses (Ackermann and Prangishvili 2012). Some phages display structural features similar to eukaryotic viruses (Krupovic and Bamford 2008). Double-stranded DNA-tailed phages (Caudovirales order) were the most studied among bacterial viruses and were used in a wide range of fields, including aerosol studies (Verreault et al. 2008). However, since eukaryotic viruses are tail-less, members of tailed phages (Caudovirales order) such as coliphages T4 and T7 might not be the most suitable models. So, tail-less phages like MS2, Φ6, and ΦX174 may be better to be used as viral aerosol models (Verreault et al. 2008).

The Center for Disease Control (CDC) guidelines regarding COVID-19 recommend that even fully vaccinated individuals should wear masks in public (https://www.news4jax.com/news/2021/07/25/fauci-cdc-may-back-wearing-face-masks-more/). The detection of new SARS-CoV 2 variants, including the Delta (https://www.sciencemediacentre.org/expert-reaction-to-cases-of-variant-b-1-617-the-indian-variant-being-investigated-in-the-uk) and Omicron (https://www.who.int/news/item/26-11-2021-classification-of-omicron-(b.1.1.529)-sars-cov-2-variant-of-concern) variants might warrant the usage of face masks for the next months or years. Wearing face masks might be strongly recommended until most of the global population is vaccinated against SARS-CoV-2. Moreover, frequent usage of face masks worldwide for the following years might protect people from respiratory infections. Masks are physical coverings that prevent the spread of respiratory droplets and, therefore, protect the wearer (https://www.cnbc.com/2021/01/25/dr-fauci-double-mask-during-covid-makes-common-sense-more effective.html). Although the CDC and World Health Organization (WHO 2021) guidelines strongly recommend the usage of face masks to prevent the spread of SARS-CoV 2, the information about their protective efficiency against the airborne transmission of infectious SARS-CoV-2 and other respiratory viruses through droplets/aerosols is insufficient (Ueki et al. 2020). The ability of masks to filter particles depends on the particle size and trajectory, as smaller floating aerosols are more challenging to filter than larger particles with momentum. Speech produces more SARS-CoV-2 virus particles and the asymptomatic transmission of SARS-CoV-2 is associated with upper respiratory tract shedding, where the virus-laden particles are formed during vocalization (Howard et al. 2021). Ueki et al. (2020) developed an airborne transmission simulator for infectious SARS-CoV-2-containing droplets/aerosols produced by human respiration and cough. They also assessed the transmissibility of the infectious droplets/aerosols and the ability of various types of face masks to block the transmission. They found that cotton, surgical, and N95 masks can prevent the transmission of infective SARS-CoV-2 droplets/aerosol and this protective efficiency was higher when masks were worn by infected individuals. However, the masks could not completely block viral transmission through droplets or aerosols even when sealed. Also, Ueki et al. (2020) did not estimate the efficiency of different face mask types by quantifying the viruses collected in the solution passed through these face masks. Therefore, taking into account the viruses that are already adsorbed and/or absorbed on face masks is important. It is also necessary to evaluate various parameters about the efficiency of the different viral elution methods, such as comparing different types of face masks and the effect of autoclaving on the efficiency of face masks. Moreover, to estimate the efficiency of the face masks in retaining viruses for several applications, the methods for eluting viruses from different textile types need to be validated. This will help in distinguishing between the elution efficiency for viruses from each type and the capability of the same material to retain viruses. It is also essential to validate the viral concentration method for eluting viruses from different face mask types and then re-concentrating the viruses from the eluate.

The first objective of this work was to validate a method to elute viruses from polyester and cotton face masks. Secondly, we applied this method to study the effect of autoclaving on different

face mask materials by testing the viral loads eluted from these masks. This indicates the effect of autoclaving on the efficiency of the face masks to adsorb or absorb viruses.

2 Materials and Methods

2.1 Description of examined face masks

Eight different commercial face masks were purchased from markets (A, B, C, D, E, F, G, and H). One of them (F) is the N95 face mask, while face masks A, B, C, D, and E are surgical face masks, and masks G and H are made of cloth. They were analyzed in our labs for their internal and external composition, tensile strength, elongation, air permeability assessment, and contact angle measurements.

2.2 Validation of a method to elute phiX174 bacteriophage virus from the studied face masks

Different types of face masks were put on the mannequin face which simulates the human face. One milliliter (ml) of three different doses of bacteriophage phiX174 virus ($5x10^8$, $5x10^6$, and $5x10^5$ PFU/ml) were dispersed using a plastic aerosol sprayer perpendicularly from 20 cm distance on the different types of face masks that the mannequin wears on his face. The diameter of the droplets dispersed on the face masks was measured using the immersion sampling method as will be described later. A plastic container was put below for the dispersing process to receive the rejected droplets from the face mask and re-disperse them on the face mask. One mask from each type was used for each experiment and the experiment of each type of face mask was performed separately with different concentrations of bacteriophage phiX174 virus. The experiments were performed for different types of unautoclaved masks, masks autoclaved for one cycle, and masks autoclaved for five cycles in an experimental cabin with sterile air. After 15 minutes of virus dispersion on face masks, the masks were put separately in nylon bags filled with 100 ml of the different eluents [deionized water in 2 bags and 3% beef in 0.05M glycine (at either pH 9.5 or pH 7.2) in one bag]. For bags of deionized water and after 24 hours at 4°C, rubbing of masks with hands for 2 minutes and then squeezing of the masks was performed. Then bacteriophage phiX174 in one of the two bags of deionized water was quantified. The masks in the other bag which contains deionized water were exposed to re-elution using 100 ml [3% beef-glycine (at either pH 9.5 or pH 7.2)] for 30 minutes contact time and then rubbing with hands for 2 minutes and then squeezing the masks performed. Virus in the beef extract was quantified directly (neutralization of beef-glycine 3% at either pH 9.5 or 7.2 was performed by adjusting the pH to 7 before inoculation) or after the re-concentration processes using organic flocculation according to Katzenelson et al. (1976) and PEG precipitation method according to Lewis and Metcalf (1988). For PEG concentration, 0.25 volumes of a 5x PEG 8000/1.5 M NaCl solution were added to the eluates and incubated with rocking at 60 rpm at 4°C for 60 min. After centrifugation at 10,000 ×*g* for 30 min at 4°C, the pellets were suspended in 500 µl of PBS and stored at −70°C. For OF reconcentration method, the eluates were adjusted to pH 3.5 and kept with rocking at 60 rpm at 4°C for 30 min. After centrifugation at 3000 rpm for 15 min at 4°C, the pellets were suspended in 500 µl of PBS and stored at −70°C. Each experiment was repeated three times and calculations of mean and standard deviation were done.

2.3 The concentration of bacteriophage phiX174 from aerosols

The area of the experimental cabin was 1.8 m^3 and viruses in aerosols were concentrated in parallel to each experiment according to Harstad (1965). Briefly, glass impingers were used with a flow rate of 12 liters per minute with 25 ml of 0.1% tryptone nutrient broth (Bio-Basic Canada). The glass impingers were put inside the experimental cabin during the dispersion of the different doses of bacteriophage phiX174 virus on the different types of face masks. The same experiment which was explained above was repeated with the addition of using the glass impingers containing 25 ml of 0.1% tryptone nutrient broth and with continuous suction using a suction pump with a flow rate of 12 liters per minute. Briefly, different types of face masks were put on the mannequin face which simulates the human face. One milliliter (ml) of three different doses of bacteriophage phiX174 virus ($5x10^8$, $5x10^6$, and $5x10^5$ PFU/ml) were dispersed using a plastic aerosol sprayer perpendicularly from 20 cm distance on the different types of face masks that the mannequin wears on his face. The diameter of the droplets dispersed on the face masks was measured using the immersion sampling method as will be explained later. A plastic container was put below for the dispersing process to receive the rejected droplets from the face mask and re-disperse them on the face mask. After 15 minutes of virus dispersion on face masks, suction was stopped and the 0.1% tryptone nutrient broth was collected to quantify the bacteriophage phiX174 virus which was collected in it.

2.4 Quantification of infectious bacteriophage phiX174 virus either eluted from face masks or concentrated from aerosols

Quantification of infectious bacteriophage phiX174 was performed according to the standard methods for the examination of water and wastewater, 23rd edition (APHA 2017). Bacteriophage phiX174 strain (ATCC 13706B1) and *Escherichia coli* strain C (ATCC 13706) ATCC as a viral host were used in this study. Briefly, Three mL of melted tryptone top agar were held at 44.5 °C in each of ten 16x150 mm test tubes for sample assay and in each of two additional test tubes to serve as negative and positive controls. Test tubes were held in a water bath to avoid premature solidifying of agar. Then, 0.1 mL of host culture was

added to each of the 12 test tubes. One ml tryptone broth was added to the test tube serving as the negative control and one ml phiX174 preparation (30 to 80 PFU/ml) was added to the test tube serving as the positive control. To each of the remaining 10 tubes, one ml sample (direct and after serial ten-fold dilutions) was added. For each tube, mixing and immediately pouring contents over the bottom agar of a Petri dish that has been suitably labeled was performed. Tilting and rotating the dish to spread suspension evenly and placing it on a level surface to let agar solidify were performed. Then, incubation at 36.5 °C overnight and examination for plaques the following day, and counting of the total number of plaques on the ten dishes receiving the sample (for each direct or diluted sample) were performed. Calculation of the somatic coliphage concentration according to the formula: Ca = (P÷10) X D was done, where: Ca: is the somatic coliphage concentration, PFU/ml, P: is the total number of plaques from the 10 dishes, D: is the reciprocal of dilution made on the inoculum before plating (D =1 for undiluted samples).

2.5 Sterilization of different types of face masks

Different types of examined face masks were sterilized using an autoclave at 126°C for 30 minutes and at 0.15 Mpa to examine the effect of autoclaving on the viral loads that could be eluted from different types of face masks. All types of face masks were put inside thermal bags before being put inside the autoclave to maintain the uniformity of the face masks after the autoclaving process. Normal bags which are not suitable for high temperatures and pressure will affect the form of face masks after the autoclaving process. Elution of bacteriophage phiX174 virus from face masks exposed to one or five autoclaving cycles was performed using the same methods explained above and each cycle was performed at 126°C for 30 minutes and 0.15 Mpa.

2.6 Electron microscope examination

SEM was used according to the manufacturer's instructions to examine if there are changes in the yarns of the studied face masks before autoclaving and after five autoclaving cycles. Briefly, a piece of the mask (1 cm X 1 cm) was cut, placed on copper tape, stuck with double-face carbon tape, coated with a thin gold layer using a sputter coater (S 150A, Edwards, England), and examined using SEM (Quanta 250 FEG, Holland).

2.7 Mechanical properties of studied face masks

2.7.1 Tensile strength and elongation

Tensile strength and elongation at a break were reported by (ASTM D1388-14e1 1994) using a cantilever bending test instrument. All reported values were the average of three readings.

2.7.2 Air-permeability assessment

The air permeability of both treated and pristine cotton samples was recorded according to (ASTM D737 1996) standard method employing TEXTEST FX-3300 at a pressure gradient of 100 Pa. For each sample, an average of five measurements recorded at five different locations was reported.

2.7.3 Contact angle measurements

The contact angle is defined as the angle between the drop's outline tangent at the three-phase contact point, and the substrate. Contact angle measurements were performed using Theta Optical Tensiometer (Dataphysics, Model OAC 13EC, Dataphysics Instrument GmbH, Germany) and according to the manufacturer's instructions. An automatic single-liquid dispenser was used to automatically dispense a precise volume of 1µl liquid drop and then descended until the drop was contacted with the paper surface. It was raised again until the water drop stayed at the sample surface. The water drop image was taken by the camera and analyzed by Dataphysics software using Young fitting mode to obtain the contact angles.

2.8 Immersion sampling method for measuring the diameter of the droplets containing viruses

Droplets are collected on a glass plate coated with silicone oil and they are immediately photographed at high magnification for subsequent scanning. In this method, the collected droplets quickly settle in the silicone oil and do not evaporate even underneath the strong light while being photographed. Stuck in silicone oil, they are measured as perfect spheres (Hurlburt and Hanratty 2002).

2.9 Statistics

A paired Student's t-test was applied to ascertain the significance at $p < 0.05$ of differences in the mean of the virus recovery after direct beef-glycine quantification, organic flocculation, and PEG precipitation methods. To ascertain the significance at $p < 0.05$ between elongation, tensile strength, air permeability, and contact angles before and after autoclaving, the measurement results obtained from the tests were analyzed and evaluated with the help of the SPSS 24 Statistical Analysis Package Program. In this context, after determining the normal distribution of the data, analysis of variance (ANOVA) was performed to determine the relationship between the groups, and correlation analysis was performed to determine the strength and direction of the relationship. The one-way analysis of variance (ANOVA) test was used to determine whether there was a significant difference ($p < 0.05$) in the virus recovery by comparing the mean values of the viral loads eluted from the different types of face masks before and after the autoclaving process. On the other hand, each

experiment was repeated three times and both mean and standard deviation (SD) were calculated.

3 Results and Discussion

We obtained six types of commercial face masks (A, B, C, D, E, and F) with 100% polyester in both internal and external parts, including N95 masks (F). Face mask H was composed of 100% cotton, both internally and externally. No changes were observed in the internal and external compositions of these face masks after one or five autoclaving cycles. Face mask G was a polyester and cotton blend composed of 91.75% polyester + 8.25% cotton externally and 67.95% polyester + 32.05% cotton internally (before autoclaving). The external and internal composition changed to 93.95% polyester + 6.05% cotton and 68% polyester + 32% cotton, respectively, after one autoclaving cycle, and to 94.77% polyester + 5.23% cotton and 68% polyester and 32% cotton after five autoclaving cycles, respectively.

The bacteriophage phiX174 was eluted from all the studied face masks using deionized water, 3% beef glycine (pH 9.5 or pH 7.2), and deionized water followed by 3% beef glycine (pH 9.5 or pH 7.2) (Figure 1). No significant differences were observed between the viral loads in the eluate solutions when either pH 9.5 or pH 7.2 was used. The highest elution efficiency was observed using deionized water followed by 3% beef glycine (pH 9.5 or pH 7.2) with the studied face masks. Direct elution with only 3% beef glycine (pH 9.5 or pH 7.2) was less efficient than deionized water, followed by 3% beef glycine (pH 9.5 or pH 7.2), but more efficient than deionized water as a single eluent. Deionized water followed by 3% beef glycine (pH 9.5 or pH 7.2) was significantly more efficient ($p < 0.05$) in eluting the bacteriophage phiX174 from polyester (from 4.73% ± 0.25% to 28.67% ± 1.89%), polyester/cotton (3% ± 0.33%) and cotton (1.7% ± 0.21%) face masks than 3% beef glycine (pH 9.5 or pH 7.2) as a single eluent (3.4% ± 0.16% to 21.33% ± 0.94% for polyester, 1.91% ± 0.08% for polyester/cotton, and 1.47% ± 0.12% for cotton face masks). Deionized water also was significantly less efficient as a single eluent for the bacteriophage phiX174 from all the face masks (2.2% ± 0.16% to 17.87% ± 0.41% for polyester, 1.62% ± 0.24% for polyester/cotton, and 1.41% ± 0.17% for cotton face masks). However, the elution was significantly better when N95 face masks were used, followed by other commercial polyester face masks. Lower elution percentages were observed with the polyester/cotton face masks, with the lowest being for cotton face masks. No significant differences in the viral elution percentages were observed with all the viral concentrations examined ($5x10^8$, $5x10^6$, and $5x10^5$ PFU/ml).

No significant differences ($p > 0.05$) were observed in the viral load eluted from face masks that were non-autoclaved and autoclaved for one and five cycles with different eluents. After a single autoclaving cycle, 2.16% ± 0.12% to 17.81% ± 0.63% of sprayed bacteriophage phiX174 could be eluted from polyester,

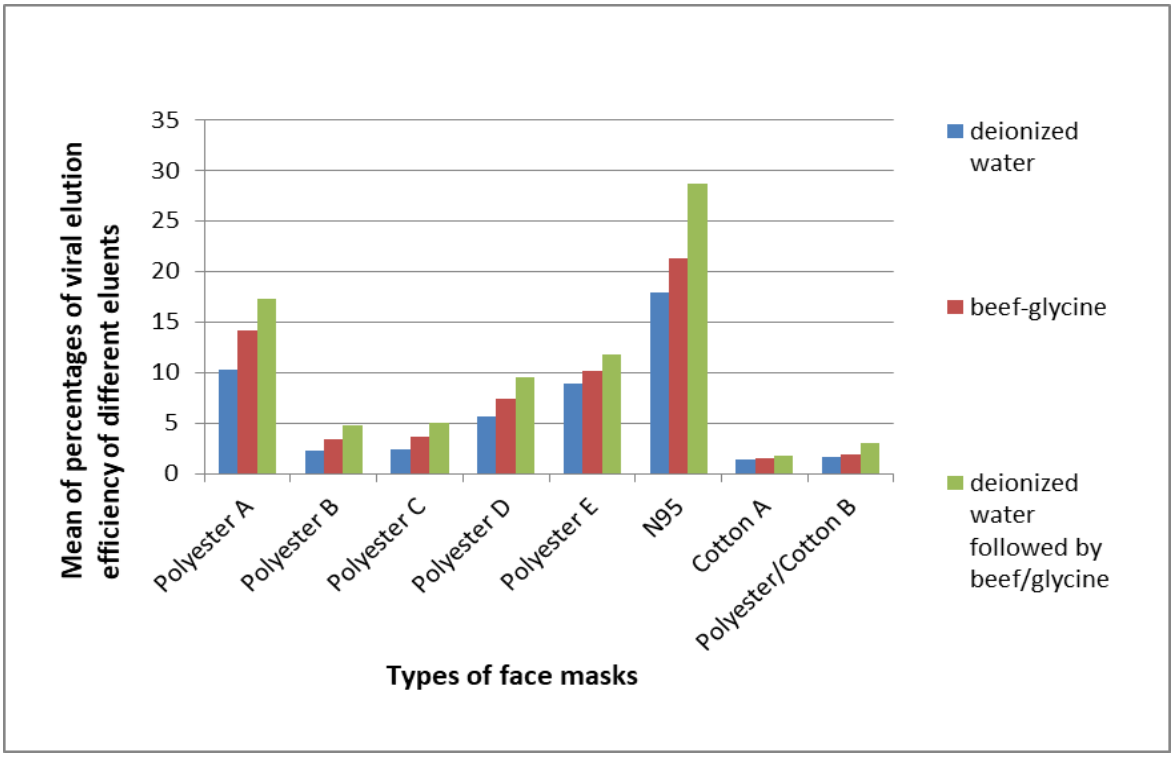
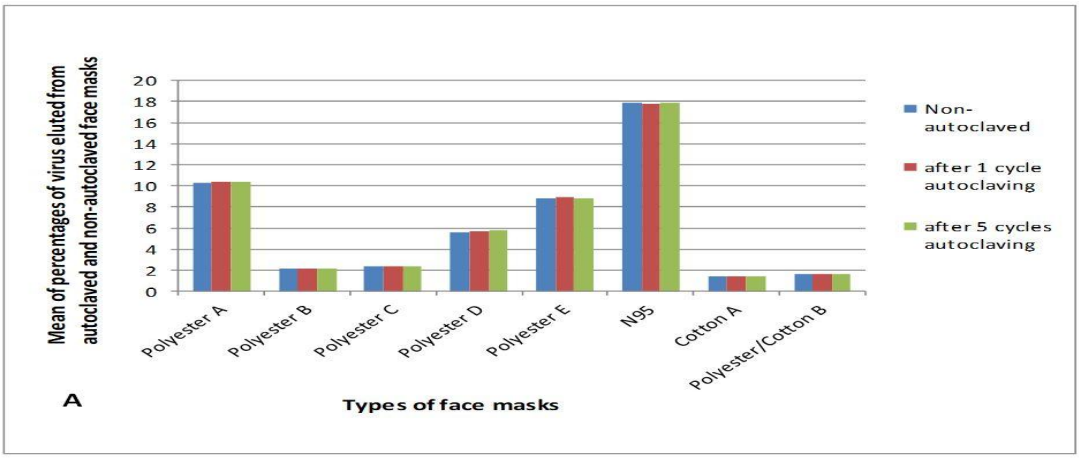
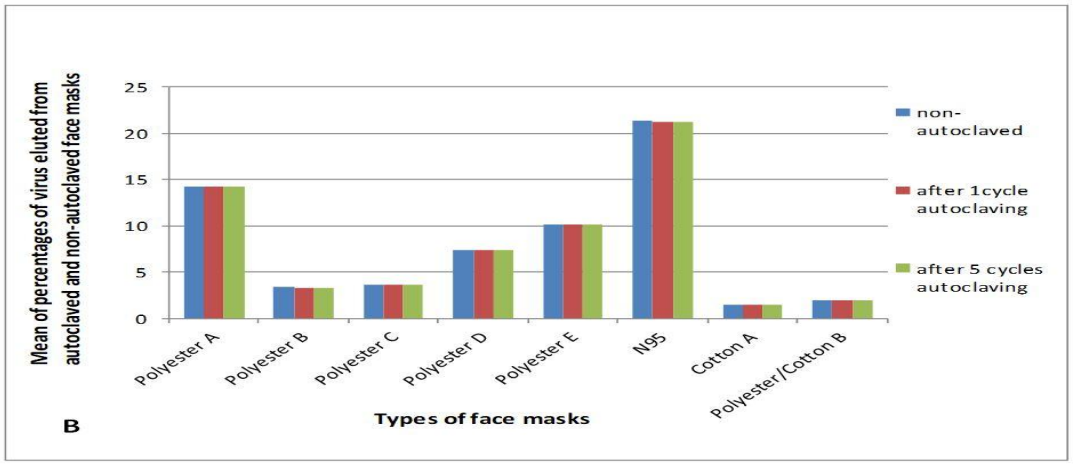


Figure 1 Validation of the elution of bacteriophage phiX174 from non-autoclaved polyester and cotton face masks using different eluents.

1.64% ± 0.11% from polyester/cotton, and 1.4% ± 0.15% from cotton face masks using deionized water as eluent. When 3% beef glycine was used as eluent, 3.33% ± 0.24% to 21.29% ± 0.82% of sprayed bacteriophage phiX174 virus could be eluted from polyester, 1.94% ± 0.14% from polyester/cotton, and 1.49% ± 0.09% from cotton face masks. Further, 4.58% ± 0.27% to 28.81% ± 0.71% of sprayed bacteriophage phiX174 virus could be eluted from polyester, 2.91% ± 0.11% from polyester/cotton, and 1.73%





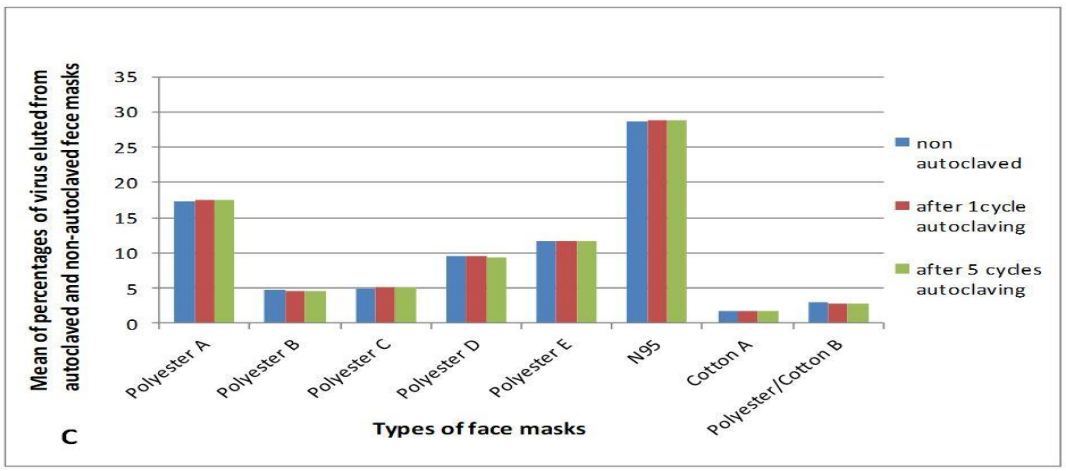


Figure 2 (A, B, and C) Validation of elution of bacteriophage phiX174 from non-autoclaved and autoclaved polyester and cotton face masks using different eluents. A: deionized water. B: Beef glycine. C: deionized water followed by beef glycine

± 0.14% from cotton face masks using deionized water followed by 3% beef glycine as eluent. After five autoclaving cycles, 2.13% ± 0.16% to 17.9% ± 0.36% of sprayed bacteriophage phiX174 virus could be eluted from polyester, 1.6% ± 0.12% from polyester/cotton, and 1.44% ± 0.08% from cotton face masks using deionized water as eluent. Similarly, 3.3% ± 0.23% to 21.315 ± 0.93% of sprayed bacteriophage phiX174 virus could be eluted from polyester, 1.91% ± 0.18% from polyester/cotton, and 1.5% ± 0.12% from cotton face masks using 3% beef glycine as eluent. Then, 4.62% ± 0.32% to 28.84% ± 0.94% of sprayed bacteriophage phiX174 virus could be eluted from polyester, 2.89% ± 0.18% from polyester/cotton, and 1.77% ± 0.13% from cotton face masks using deionized water followed by 3% beef glycine as eluent (Figure 2).

No significant differences ($p > 0.05$) were observed between the results of the viral loads in the 3% beef-glycine eluate or after using organic flocculation as a secondary concentration method. However, significantly higher efficiency ($p < 0.05$) was observed using PEG compared to organic flocculation as a secondary concentration method. The efficiencies for polyester, polyester/cotton, and cotton face masks were 7.8% ± 2.16% to 39.33% ± 2.49%, 5.53% ± 0.5%, and 4.2% ± 0.43%, respectively when PEG was used in the second concentration step. While we observed that the efficiencies for polyester, polyester/cotton, and cotton face masks were 3.2% ± 0.59% to 22.47% ± 2.17%, 1.97% ± 0.16%, and 1.46% ± 0.11%, respectively when organic flocculation was used as a second viral concentration step (Figure 3).

We simultaneously measured the viral loads in the aerosols in the experimental cabin along with the dispersion of viruses on the different face masks and during the 15 mins contact period. We found lower viral loads in aerosols with higher viral elution rates from different face masks. The viral load was highest in the aerosols when cotton face masks were used, which also showed the lowest elution efficiency from their surface and threads (16.53% ± 0.09%, 16.73% ± 0.09%, and 16.8% ± 0.28% when using deionized water followed by beef/glycine, beef/glycine, and deionized water as different eluents respectively). However, the bacteriophage phiX174 load was lowest in the aerosols when N95 face masks were used, which also demonstrated the highest virus elution efficiency from their surface and threads (0.64% ± 0.03%, 0.97% ± 0.02%, and 1.85% ± 0.11% when using deionized water followed by beef/glycine, beef/glycine, and deionized water as different eluents respectively). No significant differences ($p > 0.05$) were observed in the percentages of viruses in the aerosols when the face masks were autoclaved for one or five cycles. After one cycle of autoclaving, the bacteriophage phiX174 was highest in the aerosols with cotton face masks (16.56% ± 0.1%, 16.7% ± 0.18%, and 16.72% ± 0.24% when using deionized water followed by beef/glycine, beef/glycine, and deionized water as different eluents respectively) and was lowest when N95 face masks were used (0.61% ± 0.05%, 0.94% ± 0.03%, and 1.89% ± 0.08% when using deionized water, followed by beef/glycine, beef/glycine, and deionized water as different eluents respectively). After five cycles of autoclaving, the viral load was highest in the aerosols when cotton face masks were used (16.49% ± 0.07%, 16.7% ± 0.05%,

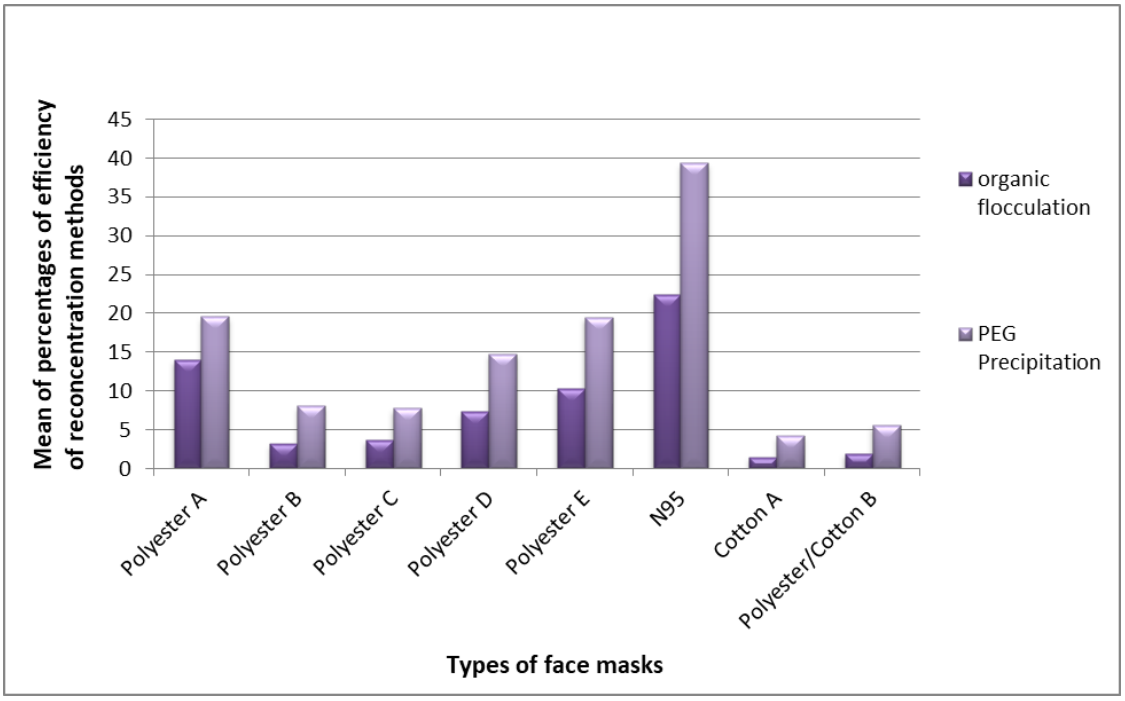
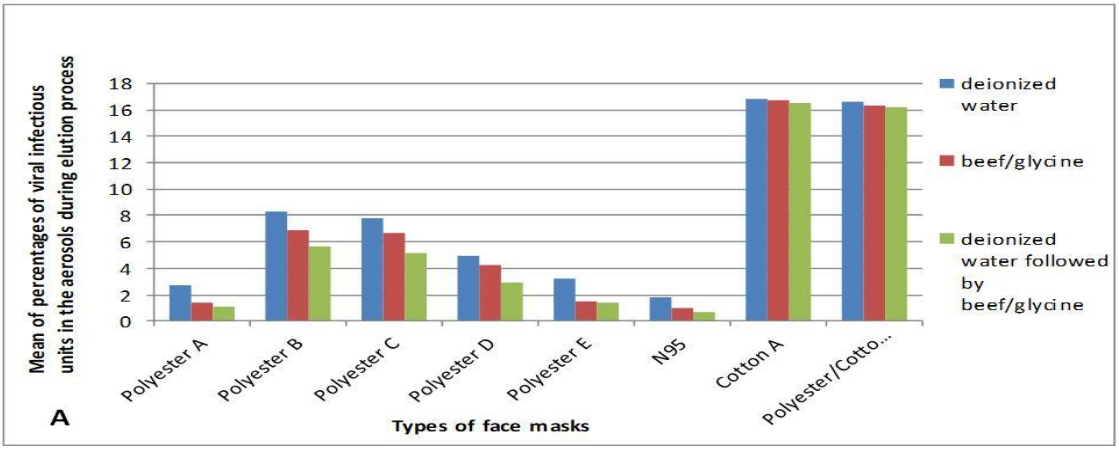
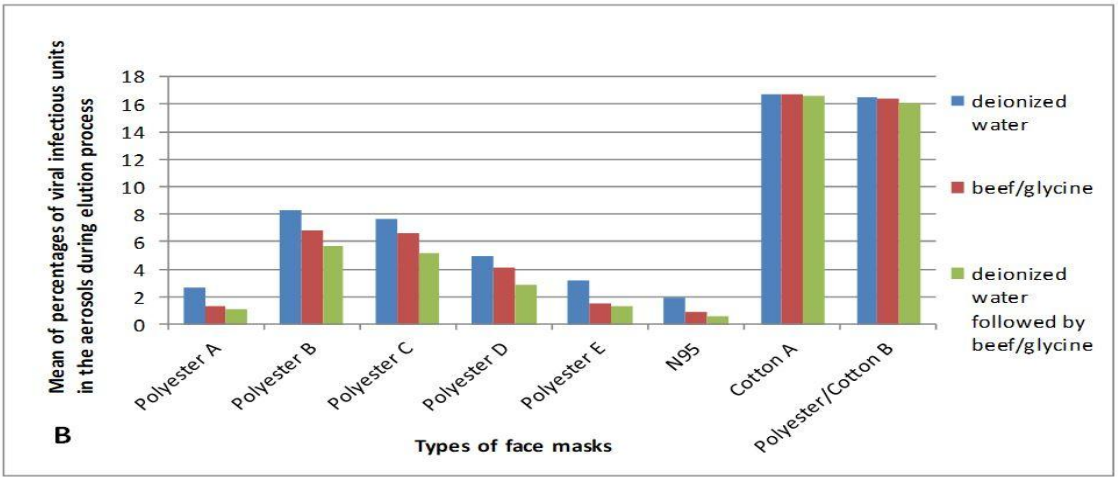


Figure 3 Percentages of bacteriophage phiX174 virus re-concentrated using either organic flocculation and/or PEG precipitation methods.

and 16.74% ± 0.09% when using deionized water, followed by beef/glycine, beef/glycine, and deionized water as different eluents respectively) and lowest when using N95 face masks (0.58% ± 0.04%, 0.95% ± 0.06%, and 1.88% ± 0.03% when using deionized water, followed by beef/glycine, beef/glycine, and deionized water as different eluents respectively) (Figure 4).





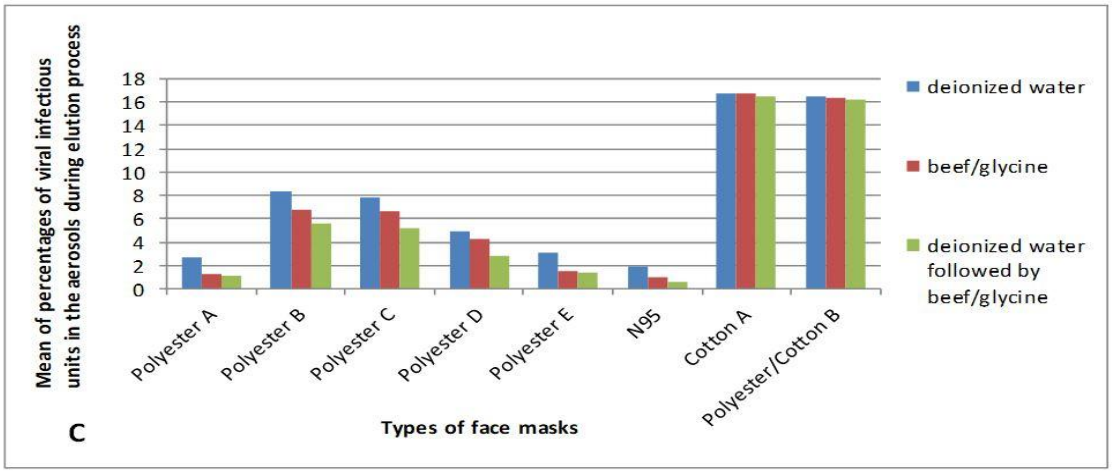


Figure 4 (A, B, and C) Number of bacteriophage phiX174 infectious units in the aerosols in the experimental cabin after using non-autoclaved and autoclaved face masks with different eluents. A) non-autoclaved and autoclaved for B) one cycle and C) five cycles

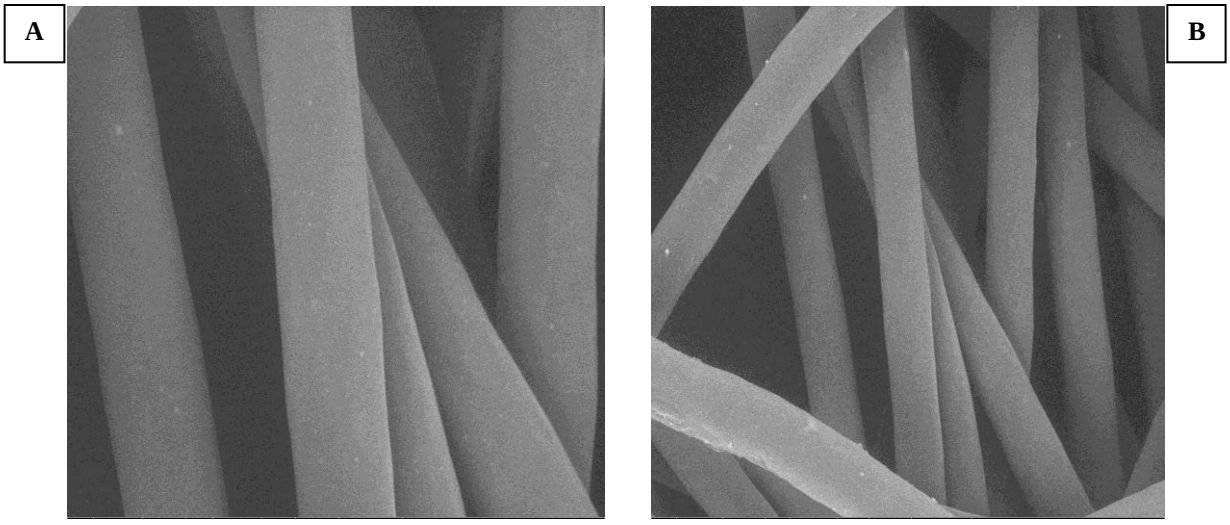


Figure 5 The threads (yarn) of a polyester face mask. A) Before and B) after autoclaving for five cycles

Table 1 Tensile strength, elongation, and air permeability of different sterile and non-sterile face masks

Types of face masks	before sterilization			After sterilization once			After sterilization five times		
	tensile strength (Kgm)	elongation	air permeability ($Cm^3/Cm^2/S$)*	tensile strength (Kgm)	elongation	air permeability ($Cm^3/Cm^2/S$)*	tensile strength (Kgm)	elongation	air permeability ($Cm^3/Cm^2/S$)*
Polyester A	17	15	333	12	13	ND	10	9	339.5
Polyester B	40	25	238	39.79	24.86	ND	39.65	24.43	239.5
Polyester C	27	30	124	27	29.89	ND	26.88	29.05	137.6
Polyester D	32	60	306	31.96	59.89	ND	31.99	59.81	357
Polyester E	25	35	70.4	24.89	34.79	ND	24.97	34.77	80.8
N95	37	20	ND	36.56	19.91	ND	35.99	19.95	ND
Cotton A	107	110	ND	106.02	108.54	ND	105.98	108.34	ND
Polyester/ Cotton B	45	80	ND	44.74	79.82	ND	44.03	79.05	ND

* Cubic centimeter of air per square centimeter of face mask per second; ND: Not done

Figure 5 shows the effect of five autoclaving cycles on polyester face masks evaluated using SEM, indicating no difference in the textile threads (yarns) before and after autoclaving. There were no changes in the yarn, such as cutting, grooves, breaks, and defects. Similar results were observed for all the studied face masks.

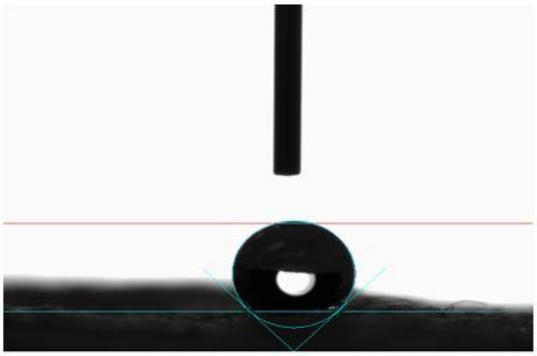
The diameter of the droplets containing bacteriophage phiX174 dispersed on the studied face masks ranged from 1 to 10 µm. Except for the polyester face mask A, there were no significant differences in both the tensile strength and elongation before and after autoclaving (for one or five cycles) in all the studied face masks (Table 1). The results of air permeability for the five polyester face masks tested before and after five autoclaving cycles indicate a slight increase in the air permeability of the masks autoclaved for five cycles compared to the non-autoclaved ones (Table 1).

Figures 6 A and B illustrate the right and left contact angles between 1 µl water droplets and the surface of different face masks, which were 0 values in the case of the cotton masks. The right and left contact angle values of the polyester/cotton masks were lower than those with polyester masks. The highest values were observed with the N95 mask.

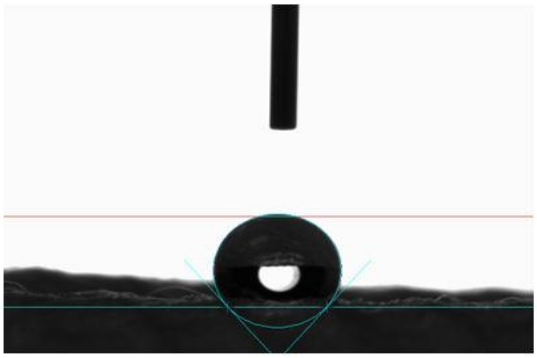
No significant differences were observed in both right and left angle values before and after five cycles of autoclaving for all the studied face masks. Previous studies have shown that using masks and social distancing can potentially reduce SARS-CoV-2 transmission and the number of associated cases (Choi and Ki 2020; Chu et al. 2020). Ma et al. (2020) reported that the virus-blocking rates of surgical and homemade masks were approximately 97% and 95%, respectively, by using an automated system that mimicked human breathing. Morais et al. (2021) reported that similar

Fig. 6 A

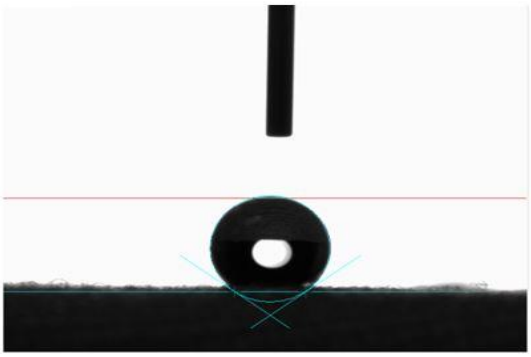
6 AA



6 AB



6 AC



6 AD

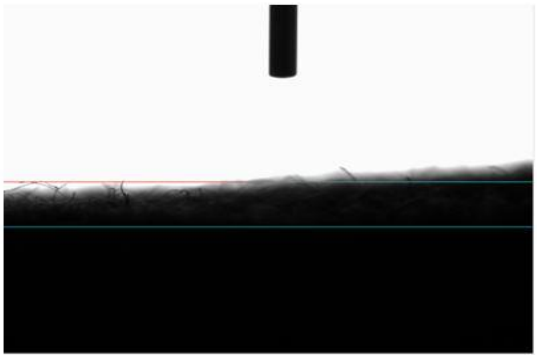


Fig. 6 B

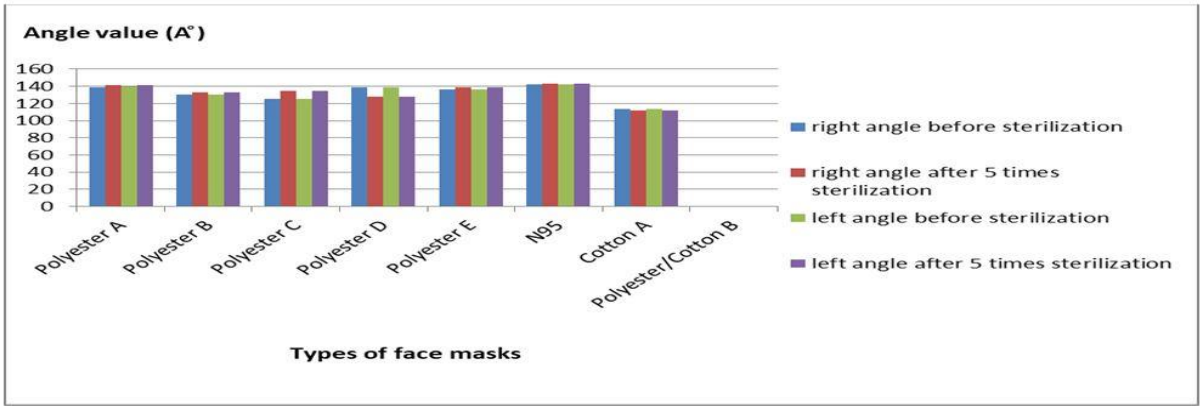


Figure 6 (A and B) Contact angle measurements of different face masks. The values for 6AA) polyester; 6 AB) N95; 6 AC) polyester/cotton mix, and 6 AD) cotton face masks

results were obtained when using a similar methodology for evaluating different types of masks, where surgical masks showed an 89% filtration rate while those for homemade masks ranged from 40% to 83%, depending on the fabric type. The first objective of this study is to validate a method to elute and re-concentrate bacteriophage phiX174 from polyester, polyester/cotton, and cotton face masks. Our results showed that deionized water followed by 3% beef glycine (pH 9.5 or pH 7.2) was the best eluent for bacteriophage phiX174 from all the studied face masks as it showed the highest elution efficiency. This was followed by

the single eluents, 3% beef glycine (at either pH 9.5 or pH 7.2), and finally, deionized water (lowest elution efficiency). This indicates that two consecutive eluents might increase the chance of eluting more viruses that are still adsorbed and/or absorbed on the yarns of the face masks as the viral particles remaining on the yarns of the face masks after elution using deionized water had to be re-eluted using another eluent (3% beef glycine) in our study. Our results also showed that 3% beef glycine (pH 9.5 or 7.2) is better than deionized water as a single eluent from different face masks. The beef glycine solution was previously used to elute enteric and non-enteric viruses from cotton gauze pad fibers used in the concentration of sewage and water samples (Coin 1967; Grabow 1968; Rao and Labzoffsky 1969; Hill et al. 1971; Liu et al. 1971; Ikner et al. 2012), with low viral adsorption efficiency. The higher recovery percentages in our study seen when beef glycine was used as an eluent either directly or after using deionized water compared to the previous studies might be due to the difference in the composition of the materials used in our study. It might be easier to elute viruses from the surface or yarns (threads) of polyester than cotton, as evidenced by the low recovery percentages of cotton masks. This might be attributed to the hydrophobic nature of polyester compared to the hydrophilic nature of cotton. Hence, polyester masks might prevent complete absorption of dispersed droplets (1 to 10 µm) carrying the viral particles, increasing the viral load on the mask itself, unlike that seen in the case of cotton masks. The contact angle of a liquid droplet applied to the substrate surface is affected by both fluid and substrate (Sarah and Ulrich 2018). There is a lack of equilibrium between drop and surface on absorbing substrates. Thus, a dynamic contact angle is measured, indicating the samples' hydrophobicity. The results of the right and left contact angles confirmed the hydrophobic nature of the polyester face masks, as higher contact angles were recorded for these than for polyester/cotton face masks. The contact angles were 0 for the cotton face masks, in which the droplets were completely absorbed. Liquid behavior on porous substrates is determined simultaneously by the spread of the substrate's surface and penetration into the bulk (Holman et al. 2002; Wijshoff 2018). Differences in yarn mass, density, and manufacturing methods within the different face mask types with the same composition might influence the efficiency of the elution process. This might explain the higher elution efficiency of bacteriophage phiX174 from the N95 face mask (100% polyester internally and externally) compared to the other commercial polyester face masks. At different pH values of beef glycine solution (pH 9.5 or pH 7.2), there were no significant differences in the viral loads in the eluate solutions from all the studied face masks. As hydrophobic interactions are considered to be the dominant forces stabilizing viral attachment to the membrane filters at high pH (Farrah et al. 1981), we choose to use beef glycine at pH values ranging from moderate alkaline (pH 9.5) to approximately neutral pH (7.2) as an eluent in this study.

The quantification process (plaque assay) for bacteriophage phiX174 depends on the quantification of the infectious viral particles. There was no significant difference between the results of the viral loads in the beef-glycine eluate or after using organic flocculation as a secondary concentration method. Previous reports indicated that beef extract might inhibit the molecular techniques and/or plaque assay of some enteric viruses when concentrated in water and wastewater. Plaque assays and molecular techniques are sensitive to organic inhibitors, such as humic and fulvic acids, which are naturally present in waste-, surface-, and tap water (Farrah et al. 1976; Sobsey and Glass 1984; Sobsey and Hickey 1985; Ikner et al. 2012). Richards and Weiheimer (1985) reported that 3% beef extract significantly reduced plaque counts and sizes. The heterogeneous organic composition of beef extract further contributes to this inhibition. This might be explained by the nature of some viruses, such as bacteriophage phiX174 which might not be affected by the inhibitory effect of beef extract during the plaque assay or by the lack of water or wastewater matrices containing organic inhibitors. Another possibility is the lower efficiency of organic flocculation compared to other methods, such as PEG precipitation as a secondary concentration process (Le Guyader et al. 2009; Pérez-Sautu et al. 2012; El-Senousy et al. 2013). This is consistent with our results showing significantly higher efficiency of the PEG precipitation method than the organic flocculation method as a re-concentration method.

Our results also showed an inversely proportional relationship between the viral loads in the aerosols in the experimental cabin with that eluted from the different face mask types. This might result in a higher presence of viruses in the aerosols, especially those un-adsorbed and/or unabsorbed in the face masks. Therefore, the higher elution rates, which might indicate higher adsorption and/or absorption of viruses on the face masks, might decrease the viral load in the aerosols. Herein, we used glass impingers with a flow rate of 12 L per min with 25 mL of 0.1% tryptone nutrient broth according to Harstad (1965) to concentrate viruses from the aerosols. This has been frequently used previously to concentrate viruses from aerosols (Tseng and Li 2005; Zhao et al. 2014; Bekking et al. 2019; Chen et al. 2021). However, till now, there is no standard method to concentrate viruses from aerosols. Recently, Raynor et al. (2021) reported that high-flow rate samplers recovered higher quantities of the virus than low-flow samplers. However, lower flow rate samplers were able to better measure the air concentrations of infectious viruses and viral RNA. To detect and accurately assess airborne viruses in animal agriculture and other settings, a two-sampler approach may be warranted. A high-flow sampler is likely to provide low limits of detection to determine if the virus is present in the air. If a virus is detected, a lower flow sampler might then be used to accurately measure airborne virus concentrations. In addition to viruses in aerosols, other viral numbers might still be adsorbed and/or absorbed into

the face masks, depending on the efficiency of the elution process. Another important factor is the efficiency of the concentration method for viruses from aerosols. These factors might explain the differences between the inoculated and quantified viruses either in the eluate solutions or in the aerosols.

Our second objective was to study the effect of sterilization of face masks at 126°C for 30 mins and at 0.15 Mpa on the viral elution efficiency from these masks as an application of the validated method. Our results indicated no significant differences in the viral elution efficiency from all studied face masks before, after one cycle, or five autoclaving cycles. This was evident when the elution efficiency of bacteriophage phiX174 from autoclaved and non-autoclaved face masks (after one cycle or five cycles of autoclaving) or the number of viruses in the aerosols were compared in parallel to the dispersion/elution process using non-autoclaved and autoclaved face masks (after one cycle or five cycles). This might indicate that autoclaving does not affect the efficiency of the face mask yarns to adsorb and/or absorb virus and that probably no changes occur in the yarns, such as cutting, grooves, breaks, and any other defects. This was confirmed in our study when the face masks were examined using SEM before and after five autoclaving cycles. This can also be validated by the results of textile composition before autoclaving and after one and five autoclaving cycles, in which no significant differences were observed almost in all face masks (polyester, polyester/cotton, and cotton). Also, no significant differences were observed in the tensile strength and elongation of all studied face masks before and after one and five autoclaving cycles. We also confirmed the lack of significant differences between the measured right and left contact angles for all the studied face masks before and after five autoclaving cycles. Moreover, there were no significant differences between the air permeability of the five studied polyester face masks before and after five autoclaving cycles. de Man et al. (2020) reported that multiple heat sterilization procedures did not change the permeability of face masks for small particles. Also, face masks can be reprocessed with minimal reduction of particle filtration efficiency by exposing them to 121°C steam for 15 mins or H_2O_2 plasma sterilization (van Straten et al. 2021), which enables the reuse of face masks. Moreover, families can easily do this at home using a standard pressure cooker. It might be difficult for some families, especially in poor countries, to follow the WHO guidelines for wearing and appropriately changing face masks because of the high cost of daily face masks for all family members.

Therefore, autoclaving the face masks at home using a pressure cooker might solve this economic problem. However, the efficiency and the filter breathability might be compromised by sterilization in an autoclave and ethanol treatment, especially for the filtered N95 face mask. Physical damages were observed in N95 respirators after autoclaving. The effect depends on several factors, such as particle size, breathing flow rate, protective device, and type of treatment (Grinshpun et al. 2020). This result contradicts ours, indicating no changes in air permeability for the five studied polyester face masks. However, we could not successfully test the air permeability test for N95 face masks. Riepe et al. (1999) showed that re-sterilization of polyester vascular grafts at 134°C with 2.4 bar steam pressure for 6 mins did not change the textile strength, single-filament strength, weight, infra-red spectroscopy, and electron microscopy of the surface. Therefore, it was concluded that it is safe to use once-autoclave-re-sterilized surplus un-coated polyester grafts, provided sterility is guaranteed. Moreover, Yen et al. (2022) also showed that 25 cycles of vaporized hydrogen peroxide reprocessing of 3M 1860/1860S N95 respirators did not compromise filtration efficiency, seal check, or qualitative and quantitative fit.

Conclusions

Our conclusions may be summarized as first, deionized water followed by beef-glycine 3% (at either pH 9.5 or pH 7.2) is a better eluent of bacteriophage phiX174 from polyester, polyester/cotton, and cotton face masks than beef-glycine 3% (at either pH 9.5 or pH 7.2) as a single eluent. Also, deionized water has a lower efficiency as a single eluent for the bacteriophage phiX174 virus for all the types of studied face masks. Second, PEG precipitation has higher efficiency than organic flocculation as a secondary concentration process for bacteriophage phiX174 eluted from different types of face masks (polyester, polyester/cotton, and cotton) using beef-glycine 3% (at either pH 9.5 or pH 7.2). So, elution of bacteriophage phiX174 from different types of face masks using beef-glycine 3% (at either pH 9.5 or pH 7.2) followed by PEG as a secondary concentration method achieved the highest percentage of the viral recovery. Finally, autoclaving face masks for up to five cycles (at 126°C for 30 minutes and 0.15 Mpa for each cycle) does not significantly affect their characteristics such as composition, tensile strength, elongation, air permeability, contact angles, and no changes between viruses in the eluate solutions when using autoclaved and non-autoclaved face masks even after five cycles of autoclaving.

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Author Contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Waled Morsy El-Senousy, Faten Hassan Hassan Abdellatif,

Hend Mohamed Ahmed, and Sherif Abd-Elmaksoud. The first draft of the manuscript was written by Waled Morsy El-Senousy and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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