Critical evaluation of some microbial contamination removal and inactivation methods for face masks

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Abstract

Reusable face masks are one major protective measure in droplet infection, even the efficiency has been discussed in aerosol distribution and wide use in population. In epidemic and especially in pandemic condition security of supply is compromised due high and sudden consumption of protective devices. When production and storages of masks are not sufficient, the option is that the masks are cleaned and disinfected typically by washing. During the recent COVID crisis has been raised question whether single-use respirators could be decontaminated as well. This is a critical view on some low-tech methods and their efficiency. Because of last resort option ECDC (European Centre for Disease Prevention and Control) encourage national public health authorities and groups studying such methods to share their results as soon as they become available. This paper is a report on studies of some simple decontamination effects and their efficiency.

Introduction

Due to global connectivity, virus infection diseases and related pandemics are among the most pronounced safety risks of modern open society. Typical epidemics repeat themselves in a cyclic but unpredictable fashion, with initial exponential growth. Successfully tackling such crises depends on the availability of safe, acceptable and high-quality protective equipment as well as governmental readiness to influence citizens' uptake of protective behaviours, besides heavy regulations.

Virus infection diseases and related pandemics are one of the most pronounced safety risks of modern open society (Cirillo and Taleb 2020; WHO 2019). The most common human disease is a flu that is an upper respiratory infection caused by viruses. Respiratory viruses spread among humans via droplets, aerosols and contaminated surfaces (Kutter et al. 2018). Obviously, droplets are essential in distribution of viruses (Milton et al. 2013). The aerosol transmission route is emphasized in the research on SARS-CoV-2 virus (Santarpia et al. 2020) and the use of face masks (Li et al. 2020) and social distancing are recommended actions to prevent the spreading of the infection (Ferioli et al. 2020).

In the context of the COVID-19 pandemic, there is a worldwide shortage of respirators such as filtering face pieces (FFP). Therefore, they should be prioritised for the use by front line health care workers who are at increased risk of getting COVID-19 (European Centre for Disease Prevention and Control 2020b; Nguyen et al. 2020). At the same time, the recommendations for the citizens to wear face masks in public has increased the need for alternative, easily available face masks. Wide variety of mask materials and concepts have been tested (Tcharkhtchi et al. 2021), and there are great differences in the protection level they provide. For example, filtration efficiency for 0.03 – 2.5 μm sized particles is 80 – 90% for nonwovens, while with cloth masks the efficiency is 39–65% (Shakya et al. 2017). Professional N95 and FFP2 protective devices seem to be 1.7 (Rengasamy et al. 2009) and even 33 (van der Sande et al. 2008) times more efficient than non-standard masks. Despite some of their weaknesses, in combination with other measures the personal protection devices seem to be effective (Abaluck et al. 2020).

The shortage has been seen as well in increased price of protective masks causing problems to lower income citizens. On June 2020 CEN workshop agreement CWA 17553 Community face coverings - Guide to minimum requirements, methods of testing and use was published, and it was stated that the community face coverings specified as reusable shall withstand the number of cleaning cycles claimed by the producer (at least 5 cleaning cycles) with a minimum washing temperature of 60°C. For the respirators, several different procedures have been tested for decontamination of respirators to mitigate the effects of shortage (Bailar et al. 2006; European Centre for Disease Prevention and Control 2020a; Rubio-Romero et al. 2020). However, there are not published research studies about cleaning the community face masks available yet.

Requirements for any useful method are that they are simple, inexpensive, effective, remaining mask performance, and safe in means that no harmful contaminants nor residuals after decontamination exists.

Steam sterilization is a routinely used sterilisation process in hospitals, but not suitable for respirators. Respirator deformation or failing fit-test after steam sterilization at 134°C was reported in some types of respirators in a study performed in the Netherlands (Rijksinstituut voor Volksgezondheid en Milieu 2020). Research published in 2012(Lore et al. 2012) demonstrated the effectiveness of microwave generated steam (MGS) in inactivating viral particles of influenza virus on two models of N95 respirators. MGS had shown to reduce > 4 logs viable influenza virus on N95 respirators, with only one of the six models tested showing a slight changes of the foam at the nose cushion (Heimbuch et al. 2011). Additionally, physical deformation for certain N95 models related to inner foam nose cushion was reported, yet maintained adequate aerosol penetration and filter airflow resistance after three disinfection cycles (Bergman et al. 2010). Disinfection using steam bags inactivated 99.99% bacteriophages from N95 respirators (Fisher et al. 2011). The steam had little effect on the filtration efficiency, which still remained above 95%. In a recent pre-print (Liao et al. 2020), it was shown that steam treatment on N95 compatible melt-blown fabric did not considerably impact the efficiency and pressure drop in three steam treatment cycles. In a study (Bergman et al. 2011), the authors reported how three applications of MGS did not cause significant changes (pass rate ≥ 90%) in respirator fit in the three types of N95 respirators tested.

Heat treatment did not cause considerable degradation of filtration properties on melt-blown fabrics (the material out of which respirators are constructed). Heat treatment was performed with static-air oven at 75°C for 30 min per cycle and tests were performed up to 20 cycles (Liao et al. 2020). Minor or no change in filtration efficiency and pressure drop was detected with heat treatment up to 100°C. In this publication the authors highlight that steam may decrease filtration efficiency and that humidity should be kept low when approaching 100°C (Liao et al. 2020). Similar results were obtained when dry heat was used at 70°C for up to 60 minutes on fabric from N95 respirators (Fischer et al. 2020). They found that filtration performance was not reduced after a single decontamination cycle, but after subsequent rounds of decontamination filtration efficacy decreased. Dry heat decontamination also inactivated SARS-CoV-2 more rapidly on N95 fabric than steel. The authors highlighted that dry heat and exposure time long enough ensure the reduction of viruses (Fischer et al. 2020). Viscusi et al. (2009) found that different models of respirators melted and changed filtering properties in different temperatures. They reported melting in some models when temperature above 100°C was applied.

The disinfection methods presented above for FFP decontamination and reuse are only considered as extraordinary last-resort methods due to shortage of FFP supplies. They should be applied after a careful evaluation of the situation and after exploring the possibility of resource-conscious, rational use of FFPs, for example by extending the FFP lifespan, and having in mind the product use instructions provided by FFP manufacturers. After all not much has been published on decontaminating of surgical single use face masks or community face coverings corresponding to the use in public defined by national authorities.

One useful method for microbe deactivation is ultraviolet germicidal irradiation (Fisher and Shaffer 2011). UV-radiation is used widely in different industrial processes and medical applications to decontaminate different substrates. Traditional mercury lamps with wavelength of 254 nm (UVC radiation) have been employed widely. Main inactivation mechanism is pyrimidine-dimer formation between thymine bases which inhibits microbe DNA or virus RNA reproducibility (Kowalski 2009). In recent years more UV sources have been developed and they are becoming less expensive and less risky to use.

CDC (Centers for Disease Control and Prevention) recommends for mask decontamination UV dosage 1 J/cm^2 applied preferably in intervals. This is considerably high dosage and e.g. corona virus in water inactivates with markedly lower dosages (Zhao et al. 2020). It is also recommendable to treat both sides of the mask (Derraik et al. 2020). Previous studies have shown 99.9 % decontamination of MS2 and influena virus when applying the recommended dose of 1 J/cm^2 (Mills et al. 2018).

Various technologies have been applied to decontaminate respirators from different microbes including viruses. Several of these, like autoclaves and use of hydrogen peroxide vapour, are possible for professional use, but not for common people. In the following we fact test some of the possible low technology last-resort methods without an intention to recommend them in use under normal conditions where respirators are available. Due to the rapidly emerging demand for face masks, more understanding on their re-use is needed. In this study the used methods were chosen from the technologies available at home. A single-use surgical face mask and a home-made cloth face mask were selected for the study, as they are available for citizens and the use of them do not increase the possible shortage of respirators for health care professionals. The following microbe inactivation and removal methods were studied: boiling, machine wash, steam and heat treatments, ironing, long storage at airy room temperature, and intensive UV irradiation. We are discussing the effect of these methods on efficiency of removing microbes and filtration performance.

Materials And Methods

Face masks

The tested medical face mask was a commercially available Type I model according to EN 14683 (2019). Homemade cloth mask was sewn from a commercial cotton fabric (plain weave, 150 g/m^2, yarn densities: weft 21 yarns/cm and warp 25 yarns/cm). To mimic the cloth mask in the testing of inactivation and removal methods for microbes, twofold cotton fabric pieces was oversewn together. For the filtration tests the size of samples was 20x20 cm^2 and for the microbiological testing 10x10 cm^2. For UV decontamination commercially available cloth mask, surgical face mask, copper face mask and FFP2 filtering half mask were used. Figure 1

Decontamination methods

The methods for the decontamination i.e. inactivation and removal of microbes were selected so that they could be done at home. Five different methods were studied:

1) Washing: samples were washed in a washing machine in a laundry bag with a commercial laundry detergent at 60°C and dried in a drying rack at ambient temperature for at least 2 hours.
2) Boiling: samples were let to stay in boiling water with laundry detergent for 5 minutes, rinsed with cold tap water and dried.
3) Steam treatment: a commercial home model steam cleaner (Kärcher SC 3.000) was used to treat the samples from both sides with hot steam generated from tap water.
4) Heat treatment: samples were placed in a heating cabinet (Memmert UFE 400) to mimic home oven at 70°C for 30 minutes.
5) Ironing: samples were ironed from both sides with a clothing iron. The temperature setting for cotton fabric was three points (i.e. about 200°C) with steam. The medical face mask did not withstand the same temperature, it started to melt, thus the medical face mask was ironed with one point temperature setting (i.e. about 100°C) without steam.
6) In separate set UVC radiation with Biocid lamp enabling 1.8 mW/cm2 (containing two Philips UVC-lamps TUV 36T5 HO 4P SE, wavelength 250 nm), from distance 10 cm with 2, 5 and 10 minute exposure times. Corresponding dosages were 0.216 J/cm^2, 0.54 J/cm^2 and 1.08 J/cm^2.

Microbiological methods

The mask samples were contaminated with mixture of microbes containing:

• *Aspergillus niger* VTT D-81078; 1.5 x 10^6 cfu/ml

• *Bacillus atrophaeus* spores VTT E-052737; 1.4 x 10^7 cfu/ml

• *Saccharomyces cerevisiae* VTT C-96203; 6.5 x 10^7 cfu/ml

• *Pseudomonas fragi* (gram negative) VTT E-98200T; 2.1 x 10^3 cfu/ml

• *Micrococcus luteus* (gram positive), VTT E-91474; 1.2 x 10^7 cfu/ml

• MS2 bacteriophage DSM 13767; 5.0 x 10^6 pfu/ml.

In the middle of mask sample 0.1 ml of microbe suspension was added and mask samples were packed in plastic bag and taken to the decontamination treatments. After each treatment, the mask sample was packed in sterile stomacher bag and kept in refrigerator before culturing.

In the microbiological studies, untreated samples were contaminated and thereafter they were treated with the decontamination methods. Selected samples were let to stay at room temperature for 72 hours without any decontamination treatment. Additionally, samples were decontaminated with ten treatment cycles and thereafter they were contaminated again and the decontamination treatment was repeated once more.

After the decontamination procedures the number of survived microbes was determined using culturing methods. The efficacy of inactivation and removal treatments was defined by comparing results of treated samples to untreated reference sample. Microbes were detached from masks by adding 50 ml of extraction liquid (1 g/l Peptone, 5 g/l NaCl and 2 g/l Tween 20) on stomacher bag containing mask sample (Finnish Standards Association, 2019). The samples were homogenized with stomacher for 1 min. Dilution series was performed. Bacteria were cultured on Trypticase Soy Agar (TSA) using 10 ml of sample for pour plate technique and 0.1 ml of dilutions for spread plate technique. Yeasts and moulds were cultured on YM-agar using 0.1 ml of dilutions for spread plate technique. MS2 bacteriophage was cultured on Nutrient agar (NA) together with host, *Eschericia coli* and incubated in 37°C for one day.

Filter performance

To determine the effect of the decontamination procedures on the performance of the face masks, their key characteristics were measured from untreated samples and from samples after one and ten treatment cycles. The particle removal efficiency was measured by placing the face mask in a holder with an open area of 78 cm^2 and challenging it with polydisperse diethylhexyl sebacate (DEHS) particles. The particle concentrations upstream and downstream of the mask were measured with an optical particle counter (PMS Las X II) in the size range of 0.1–5 µm. The air flow rate through the mask was 28 lpm, giving a face velocity of 6.5 cm/s. The corresponding breathing resistance was measured across the mask with a pressure meter (Mikor TT470S).

Microscopy

Samples were studied with thermo emission JEOL JSM 6360 LV SEM-imaging equipment. Imaging parameters were: voltage 10 kV, distance 19 mm and spot size 40. Samples were gold sputtered prior the imaging for 120 s with Balzers sputter coater (SCD 050).

Results

The visual evaluation of the cloth mask after the decontamination treatments showed that the treatments with water, washing and boiling, caused slight wrinkling of the samples, as expected. Surprisingly, the surgical face mask remained usable after these treatments, also. The other treatments, ironing, steam and heat treatments, did not have an effect on the visual look of the samples. In UV radiated samples visual changes were not observed. Figure 2.

The effect of inactivation treatments on the structure of samples was studied with SEM. For both surgical and cloth masks, the washing treatments caused most prominent changes in the structural level compared to the other treatments. In the surgical face mask, washing made the both layers of the mask slightly bulkier and the fibres were loosened from the structure compared to the untreated sample, Fig. 3. In the cloth mask, the yarns were more compactly packed together so that the spaces between the yarns were smaller after the washing, Fig. 4.

Inactivation and removal efficiency of microbes

The inactivation and removal of different microbes on the masks are shown in Figs. 5–8. The results for the single-use surgical face mask treated once is presented in Fig. 5 and in Fig. 6 the treatments has been repeated 10 times. Similarly, Fig. 7 and Fig. 8 show the results for the cloth mask. The limit of detection was 1000 CFU per mask.

As can be seen, for the single-use surgical face mask, boiling and washing with detergent was the most efficient cleaning method giving over 3-Log reduction even for the sturdy *B. atrophaeus* spores while heat treatment and ironing were effective against the other test microbes. After the heat treatments with oven and ironing, only *B. atrophaeus* bacteria were still present in the samples. The repeated treatment cycles did not have effect on the disinfection efficiency. In addition to the decontamination treatments, one sample was only stored in an airy room temperature for 72 hours. During the 72 hours storage, the levels of *B. atrophaeaus, M. luteus* and MS2 did not change, but the levels of *P. fragi, A. niger* and *S. cerevisiae* were decreased.

Similarly, boiling was the most efficient cleaning method for the cloth mask. Washing with detergent was able to disinfect other microbes, but it is interesting to note that washing resulted in only 1-Log reduction of *B. atrophaeus* on the cloth mask. In addition, the heat treatment in oven was not as effective for the cloth mask as for the single-use surgical mask. This may be due to the hydrophilic nature of cotton which enhanced the penetration of liquid contaminated with bacteria compared to the surgical face mask which is more hydrophobic due to its thermoplastic materials. Ironing was more effective for cloth mask, because the material withstood higher temperature compared to surgical mask. 72 hours storage was not effective method to decontaminate cloth mask.

Comparison with UV decontamination

Results showing 2, 5 and 10 minutes of UV treatment effects on cloth mask, surgical mask and FFP2 contaminated with *S. aureus* and MS2 microbes are in Fig. 9. In cloth mask significant decontamination was not observed, but in surgical mask both microbes were decontaminated already after 2 minutes. In FFP2 microbes decline as a function of UV dosage.

In Fig. 10 is expressed UV radiation decontamination efficiency for different materials. In cloth masks was not reached even one logarithmic unit decay, while in surgical mask that was up 6-log, which means 99.9999% disinfection. In FFP2 2 minutes radiation provides 99%, 5 minutes 99.9% and 10 minutes results up to *S. aureus* 99.9999% and MS2 99.99%.

In Fig. 11 is expressed how copper affects on UV decontamination. In 30 second UV radiation decreased 99.9999% *S. aureus and* 99.9% MS2 from copper mask, while in surgical mask *S. aureus* decreased 99.9% and MS2 90%. After two minutes UV radiation no growth was observed from masks.

In a separate test was studied effect of added sun lotion and make-up product. Some 50% reduction was observed in UV radiation efficiency.

Transmittance of UV-radiation in mask was measured with UVP UVX Radiometer: UVX-25 Sensor (Serial no. E32998), when lamp was 0.1 m from the mask surface on opposite of it. Surgical mask transmitted 0.16 mW/cm², which is 8.9% of total radiation on the mask. Neither cloth mask nor FFP2 transmitted the UV radiation. As separated layers cloth mask transmitted only 0.01 mW/cm², while FFP2 outer 0.5 mW/cm², middle 0.1 mW/cm² and inner layer 0.1 mW/cm².

Filtration performance

Some of the treatments had a strong effect on the performance of the surgical face mask as shown in Fig. 12. Washing and boiling deteriorated the particle removal efficiency significantly. On the contrary, heat treatment improved the filtration efficiency somewhat but increased at the same time the breathing resistance. Steam cleaning and ironing did not affect significantly the pressure drop nor the filtration efficiency. The biggest changes occurred already after the first disinfection time; ten times treated face masks behaved in quite same way than masks treated once.

Unlike the surgical face mask, there were no big changes in the filtration efficiency of the cotton mask (Fig. 13). The removal efficiency remained poor for submicron particles and increased with size for particles larger than 1 µm. The biggest changes were observed in the flow resistance: it almost doubled from the initial 24 Pa to 45 Pa after washing at 60°C. Steam cleaning increased the pressure drop somewhat (to 33 Pa) but other cleaning methods did not have a significant effect on the breathing resistance. The particle removal efficiencies and pressure drops were similar with the ten times treated face covers to the ones treated once.

Discussion

According to ECDC (2020) among the various methods for decontamination of FFPs, several options show a favourable profile when considering effectiveness while not causing significant deterioration in filtration and breathability at least for some decontamination cycles. Such options include ultraviolet germicidal irradiation (UVGI), ethylene oxide, hydrogen peroxide vapour and to some extent dry and moist heat. However, ethylene oxide is not favourable because it is carcinogenic. In the development project of the Finnish Defence Forces Research Institute, we have showed that cleaning disposable respirators is industrially possible with gaseous hydrogen peroxide treatment without deteriorating the properties of the respirators. These experiments showed that hydrogen peroxide vapour at sufficiently high concentrations and long treatment times resulted in over 6 Log reductions of the hardy *Bacillus atrophaeus* spores. However, hydrogen peroxide treatment is not safe at home. In addition, few studies have investigated the possible use of dry microwave for respirator decontamination. Microwave oven irradiation was used on nine models of respirators (N95 and P100) and it melted samples from two respirators models (Viscusi et al. 2009). In addition, the metallic nose pin in some respirator models makes the method impractical.

In the context of COVID-19 pandemic, the use of reusable cloth face masks has increased (Cotrin et al. 2020) and the need for further studies for the decontamination has been identified (Konda 2020). In this study, the decontamination methods were selected so that they are safe to do at home. They were evaluated based on their effect on removal of microbes and filtration efficiency for surgical and reusable cloth face masks.

The washing and boiling treatments were the most effective decontamination methods for the surgical face mask, but at the same time the filtration efficiency of submicron particles was drastically decreased. The degradation of the protection capability of the surgical face mask after washing and boiling may be due to the loss of the electric charge of the filtration material. The slight increase of the filtration efficiency after the heat treatment was observed also by (Yan et al. 2020) and they suggested that this was due to the decreased moisture level in heat treated masks. We suggest that the slight increase in filtration efficiency after the heat treatment is not because of melting of the polymer (not detected with SEM) but because of the relaxation of orientation in the structure. Whereby the mask shrunk so that the fibres shrunk slightly in the longitudinal direction and swell in the transvers direction. In addition, the ironing and steam treatments did not have an effect on the filtration efficiency, other studied microbes were removed but sturdy *B. atrophaeus* was not removed by these treatments. *B. atrophaeus* are known to be highly resistant against disinfectants for example H_2O_2 sterilization, plasma processed air (Raballand et al. 2008; Schnabel et al. 2014)

Boiling was found to decontaminate the cloth mask effectively, and in addition, this treatment did not change the flow resistance. The almost doubled flow resistance of the cloth mask after the washing treatment was likely because of the structural changes seen in SEM images where the yarns were packed more tightly together. In any case, the particle removal efficiency of surgical face mask after the cleaning efforts was higher than that of cotton masks, which did not filtrate submicron particles practically at all. The poor filtration performance is not a surprise because the improvised materials are not designed for respiratory protection.

UV radiation is not expected to have any effect on filtering capacity. Radiation has no material damaging effects observed and UV radiation has neither effect on triboelectric charging on the material. Some reports mention N95 mask damaged with UV radiation 950 J/cm^2 dosage (Zhao et al. 2020), but that is fully impractical value.

Conclusions

The structure of the surgical face mask used in this study was mechanically resistant to up to ten washes. However, washing and boiling were the most detrimental for the single-use surgical face mask. Both methods caused a significant loss in filtration efficiency. Half an hour at temperature of 70°C was not enough to destroy sturdy *B. atrophaeus*, but destroyed the other studied microbes effectively. Additionally, the method may be uncertain, because the temperature control of household ovens is not accurate in this temperature range. Ironing removed microbes quite effectively. However, for the surgical face mask ironing reduced the air permeability and thus increased breathing resistance.

Of the inactivation and removal methods for the microbes tested, community face mask made of cotton cloth was best cleaned by boiling for at least 5 minutes. Machine washing at 60°C was the second best way to reduce the microbial contamination level. However, the washing increased the breathing resistance remarkably. For the cotton cloth mask, the decontamination procedures did not affect the particle removal efficiency which was already poor, especially for submicron particles.

After any of the microbe inactivation methods tested, the single-use surgical face masks had a better filtering performance than the cloth mask. However, the tested treatments of the single-use surgical face mask impaired significantly its protective performance or were ineffective in microbe inactivation, or are difficult to control so that they cannot be recommended for professional use. Finally, these tests were carried out with a specific types of masks, and therefore it is necessary to be cautious when other types of masks are considered.

Further can be concluded, that 1 J/cm^2 UV-radiation is sufficient for microbe decontamination but collected dirt may decline the effect. Thick, dense or dark material also reduce the UV radiation efficiency, and for effective microbe deactivation dosage must be increased and UV decontamination repeated on both sides. Copper seems to have no marked effect on mask UV decontamination when time is more than one minute.

Declarations

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Figures

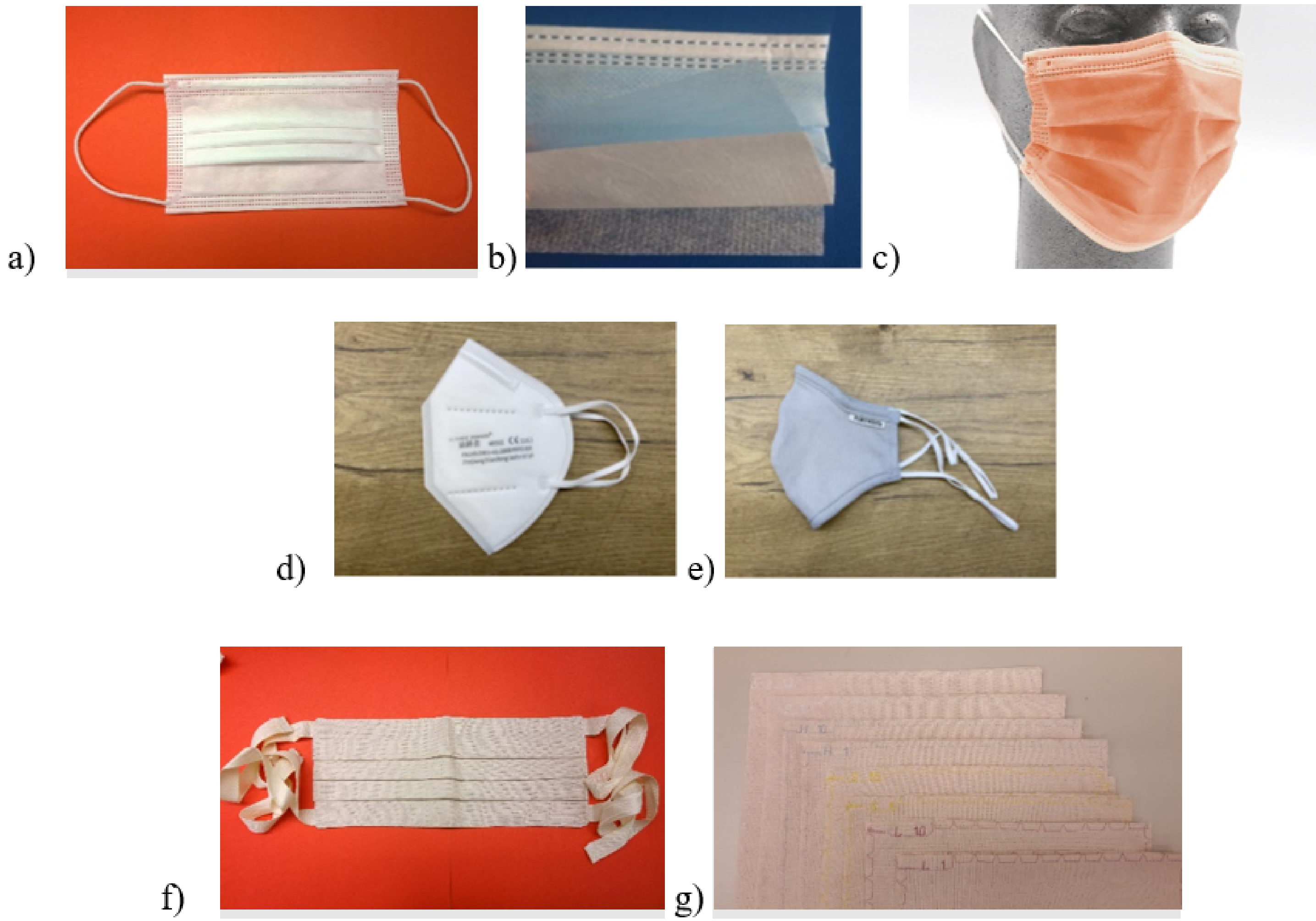

Figure 1

Samples a) surgical face mask, b) Shengquan Disposable Medical Face Mask (EN 14683:2019 Type IIR REF SMDP20603) triple layered structure of surgical face mask: polyester nonwovens in between of which is meltspun polyprylene layer, c) Copper-Inside Medical Mask (EN 14683:2019 Type IIR), d) NM FFP2 Filtering half mask M9502 (EN 149:2001 + A1:2009) material: polypropylene nonwoven and spunlace-PP, e) antimicrobial doupple layer cloth mask Portwest CV34 –(cover: 100 % PESi 160 g/m^2, inliner: 100 % CO with Texpel MicroTM treatment 130 g/m^2), f) homemade cotton mask and g) oversewn cotton samples for the testing of inactivation and removal efficiency of microbes.

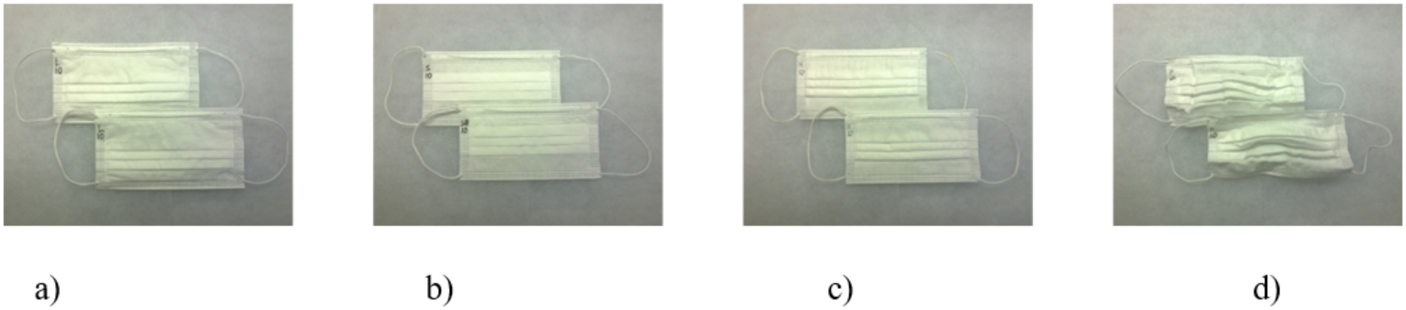

Figure 2

Photos of the surgical face masks after the different decontamination treatments repeated 10 times. a) oven, b) ironing, c) steam and d) washing.

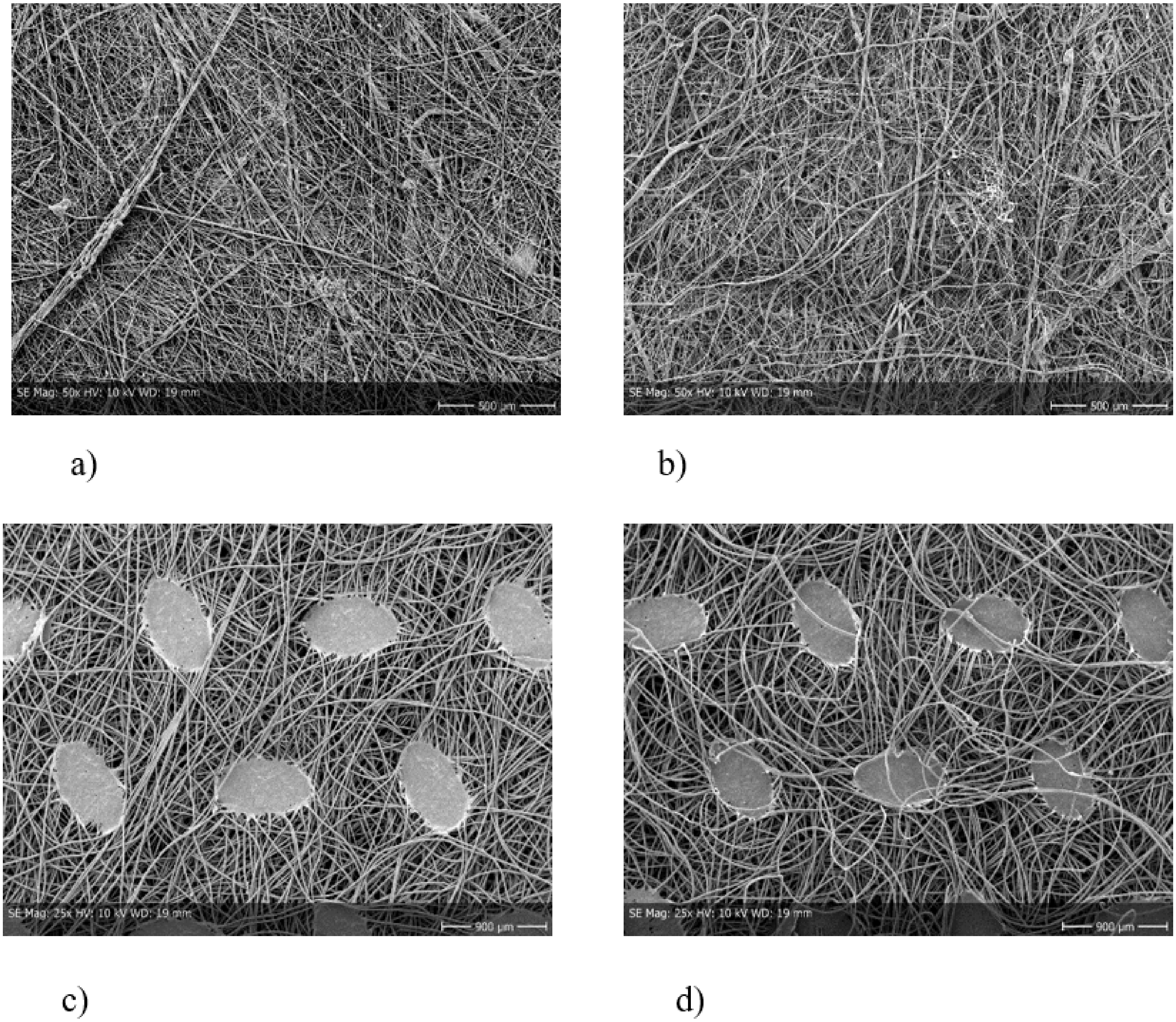

Figure 3

SEM images of surgical face mask a) untreated middle layer and b) washed middle layer; c) untreated top layer and d) washed top layer

a) b)

Figure 4

SEM images of a) untreated cotton mask and b) after washing treatment

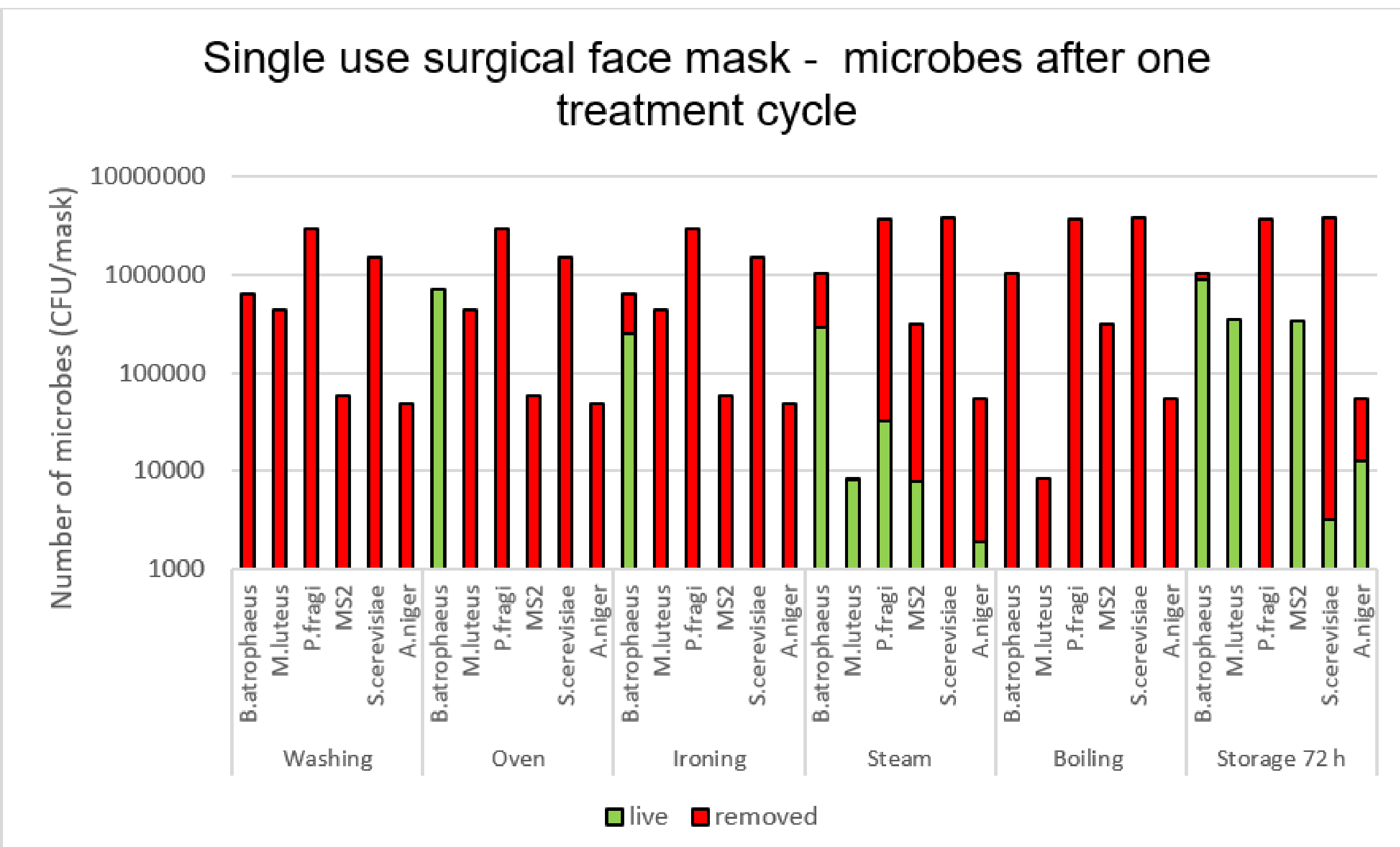

Figure 5

Disinfection efficiency of the various methods with the single use surgical mask after one treatment.

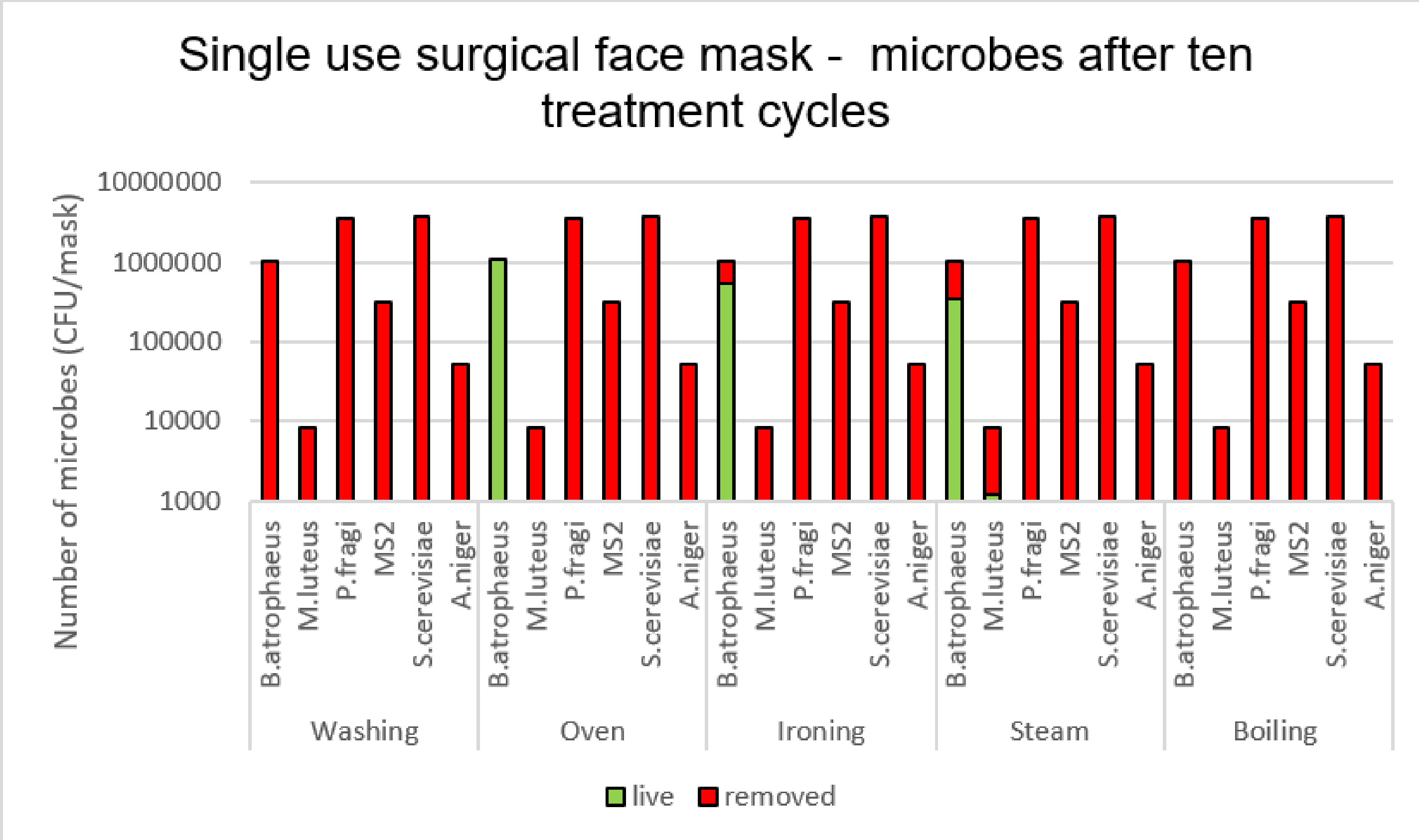

Figure 6

Disinfection efficiency of the various methods with the single use surgical mask after ten treatment cycles.

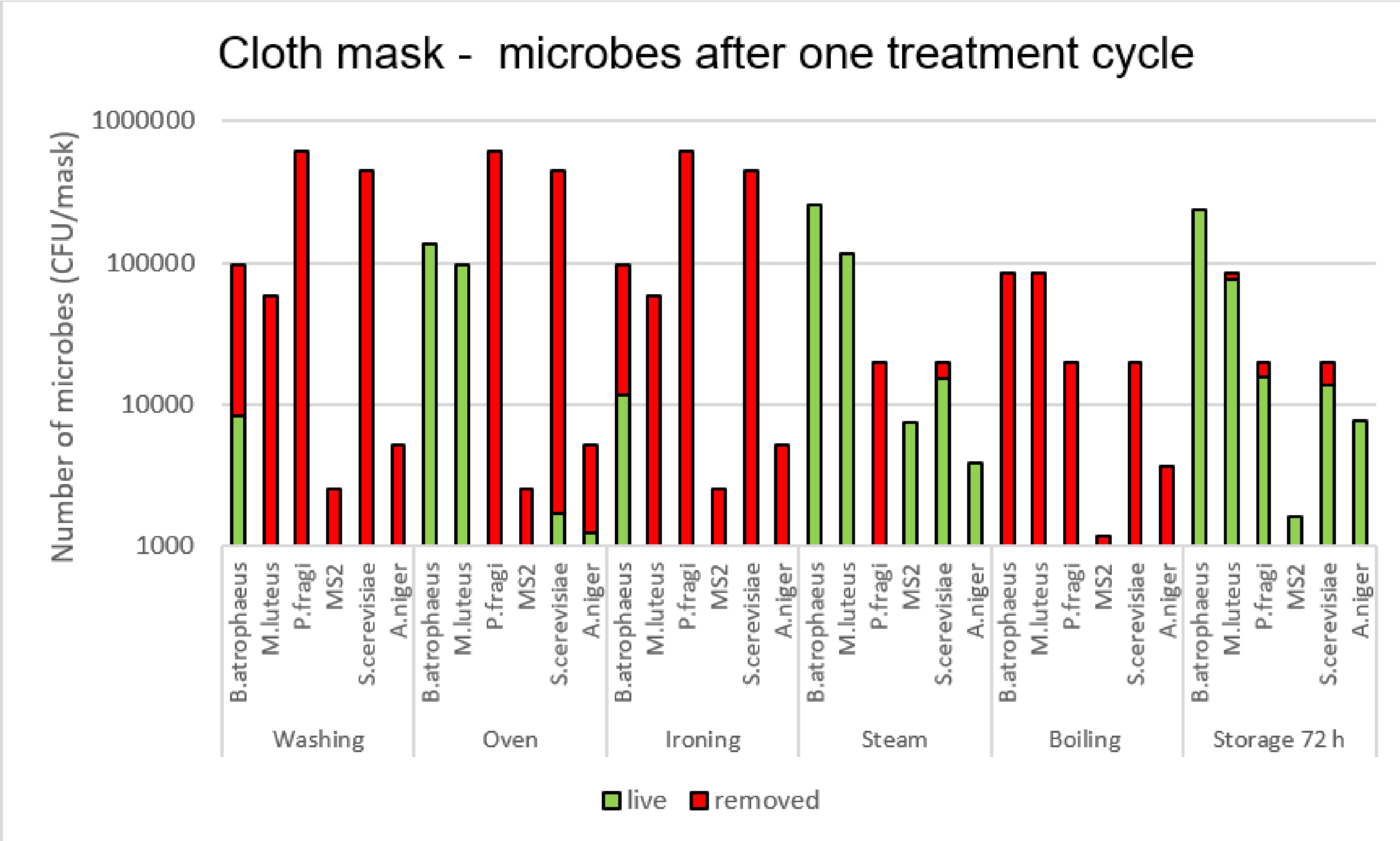

Figure 7

Disinfection efficiency of the various methods with the cloth mask after one treatment.

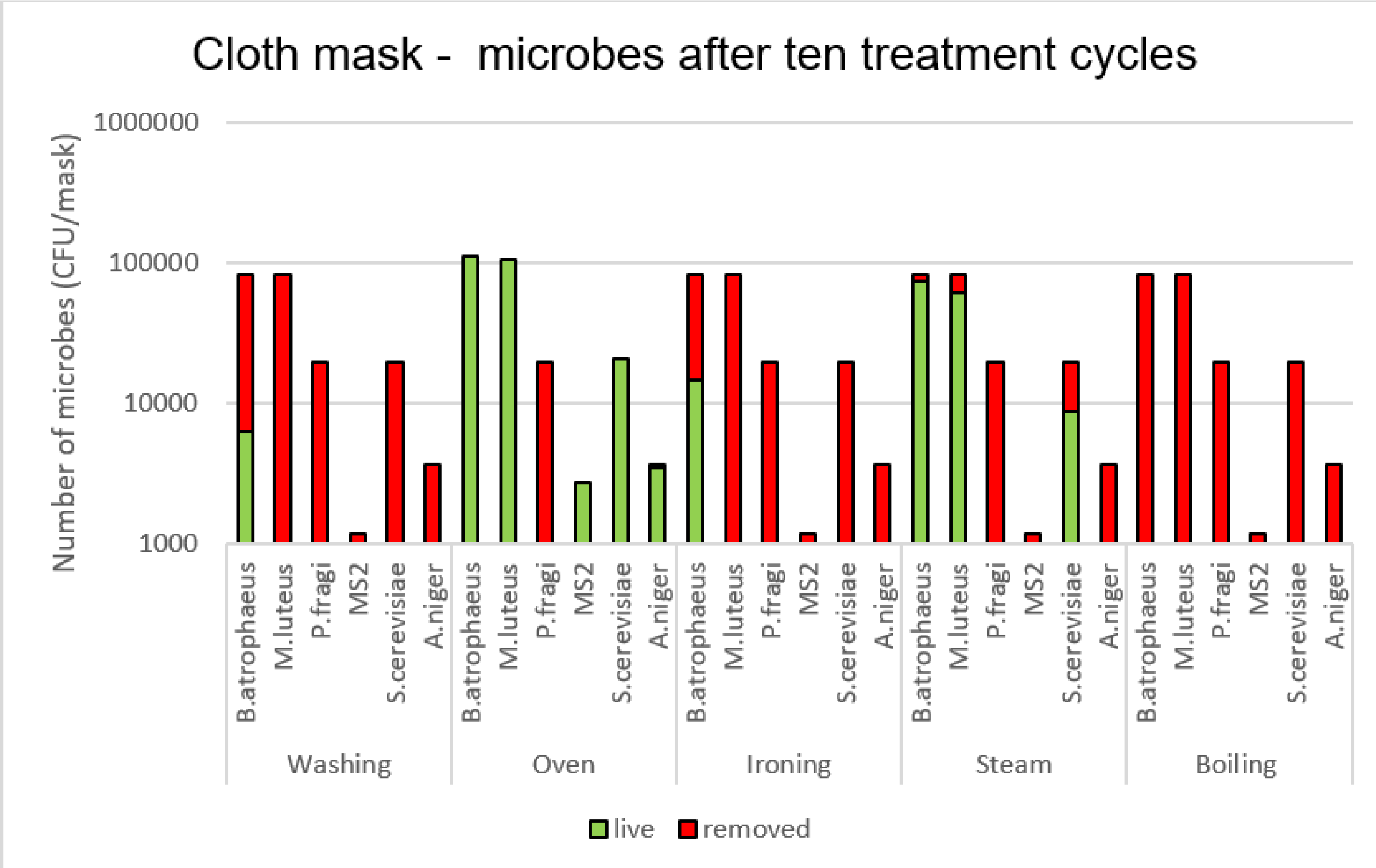

Figure 8

Disinfection efficiency of the various methods with the cloth mask after ten treatment cycles.

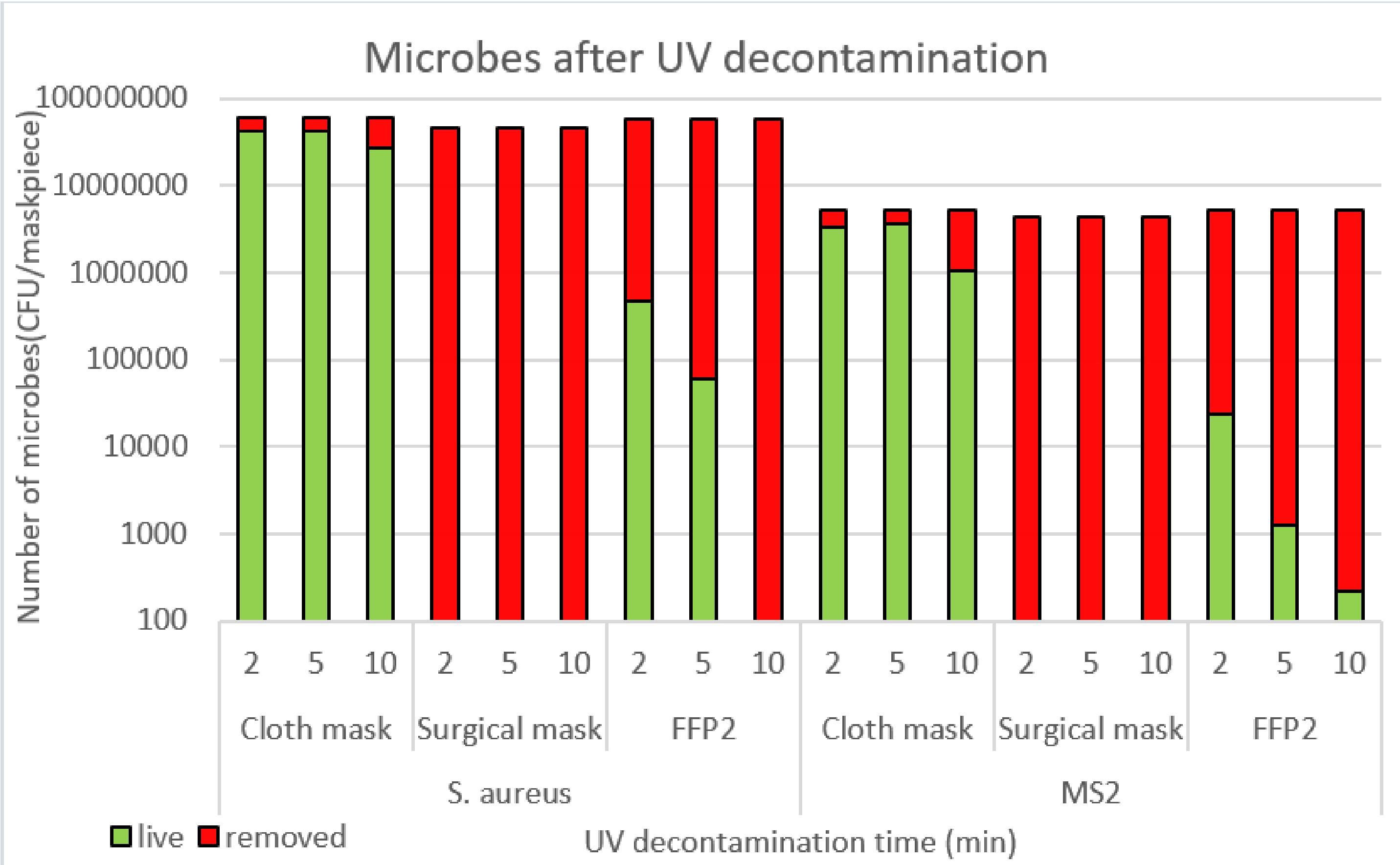

Figure 9

Microbe contents after UV-radiation, green reflects to living and red decontaminated microbes.

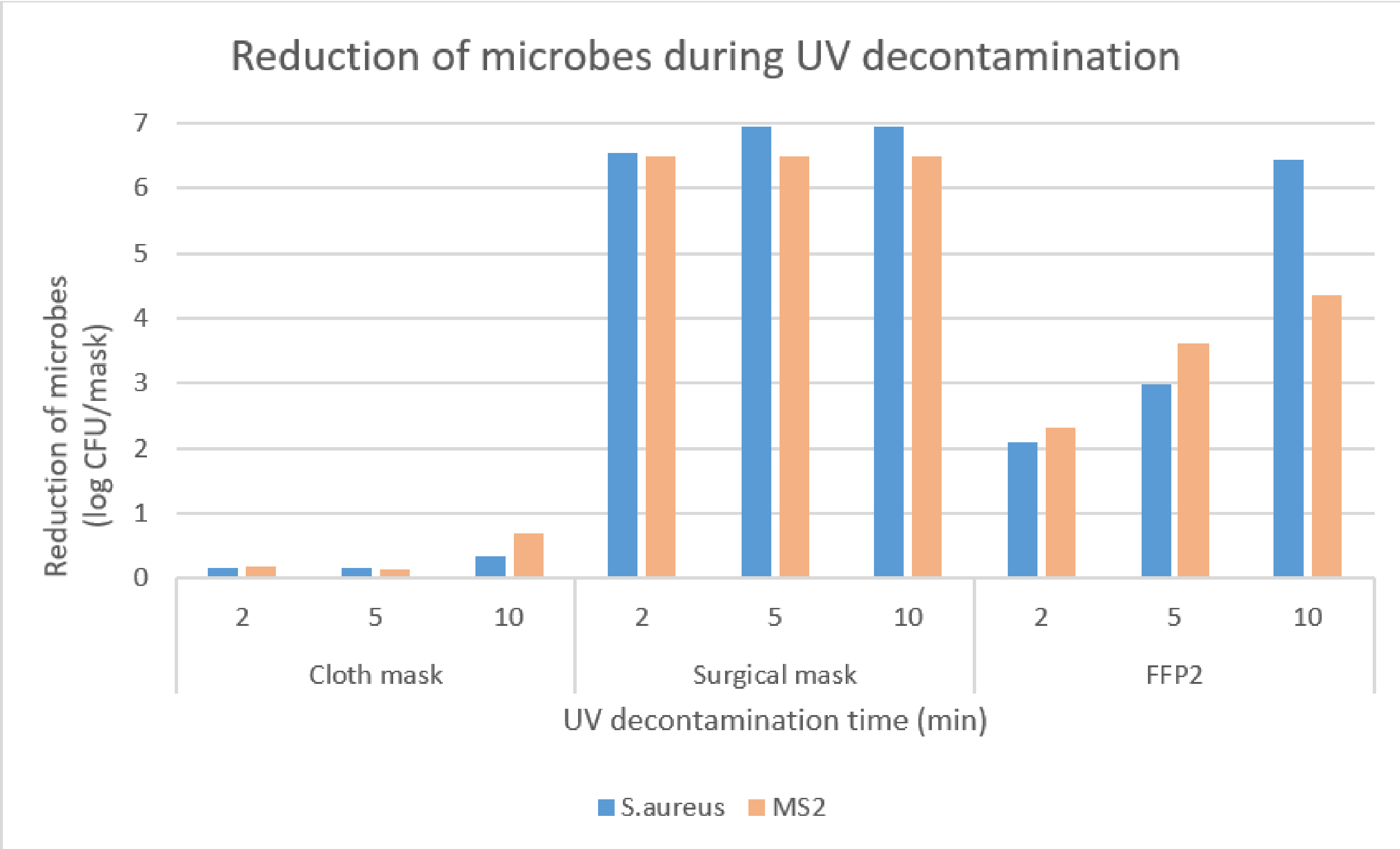

Figure 10

Microbe reduction in UV-treatments

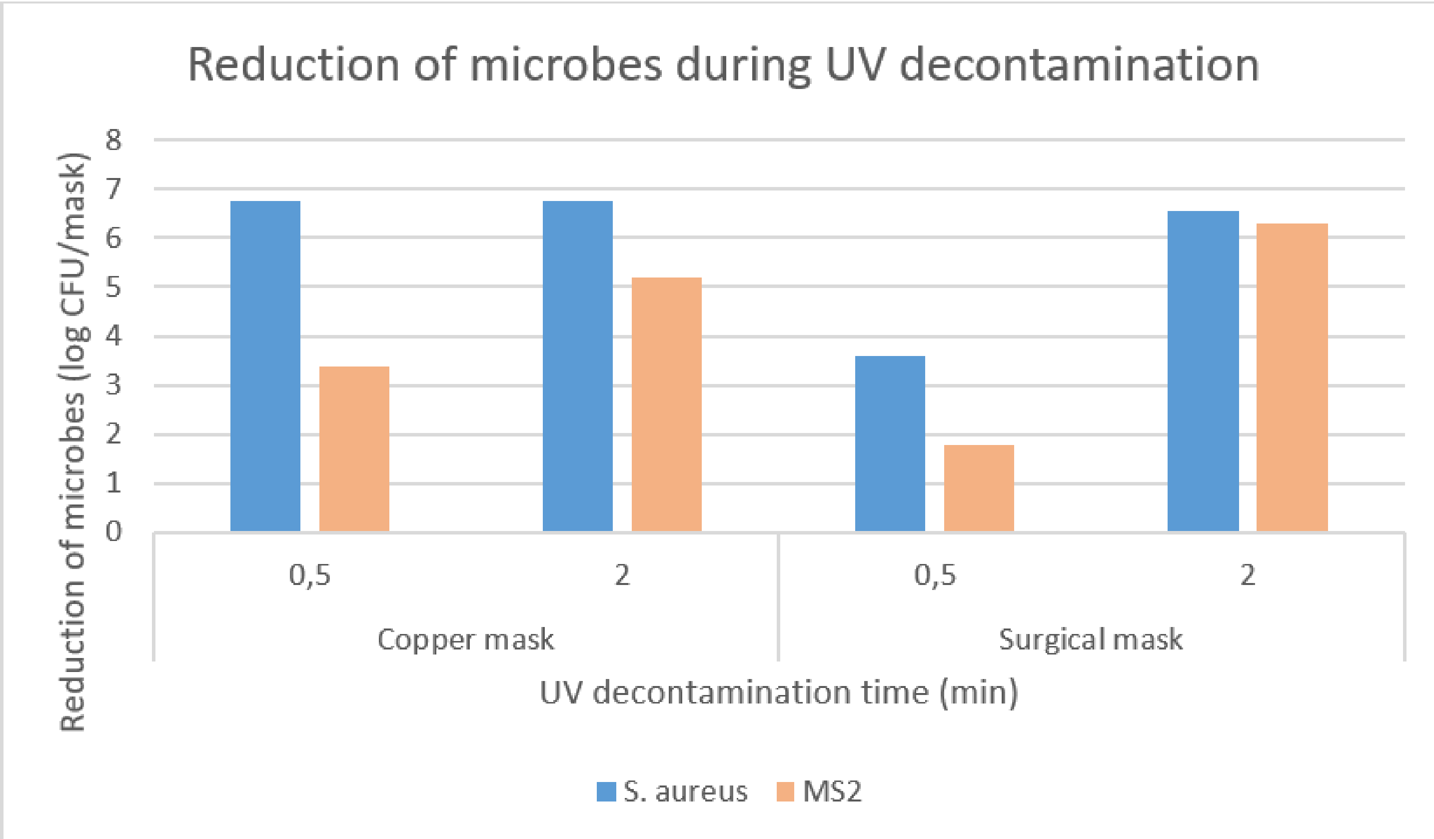

Figure 11

Comparison between copper mask and surgical mask

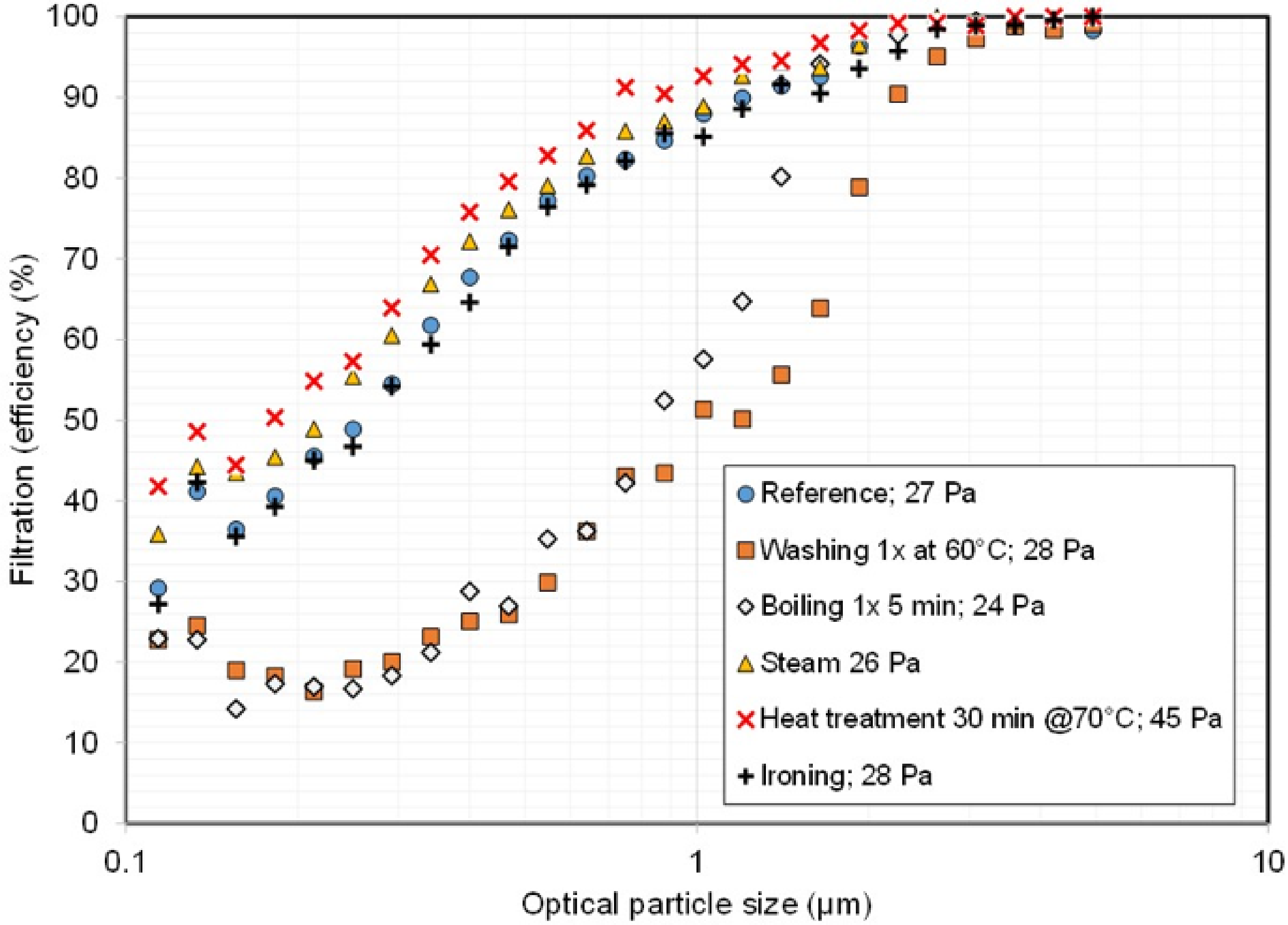

Figure 12

Measured filtration efficiency as a function of particle size of differently disinfected surgical face masks after first treatment cycle.

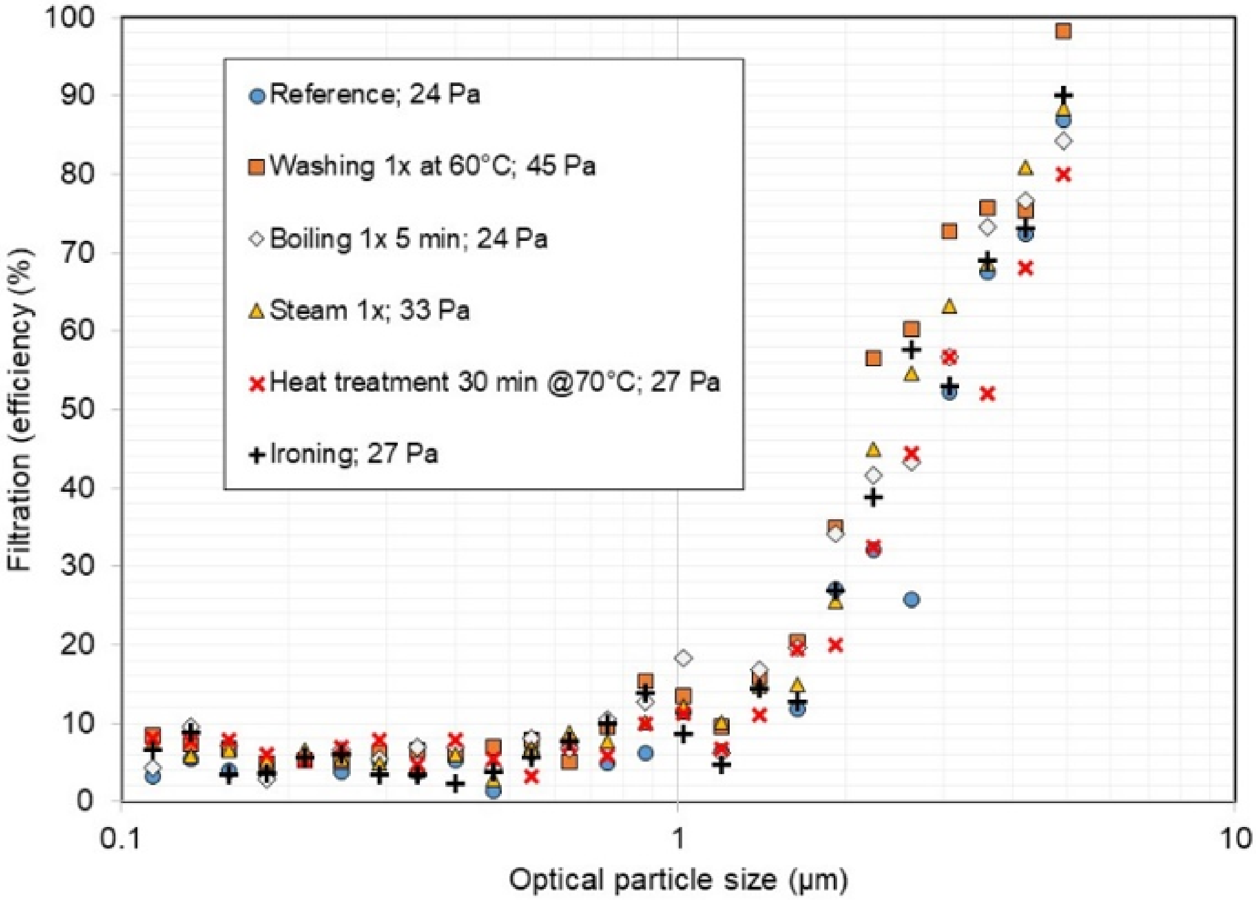

Figure 13

Measured filtration efficiency as a function of particle size of differently disinfected cloth masks.