# Evaluation of the Decontamination Effectiveness of Three Processes in Respiratory Protective Devices


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## Abstract & Goal


In order to combat the dissemination of COVID-19, the government guaranteed and encouraged the use of respiratory protection devices, giving priority to health professionals. However, the mandatory use of facial masks leads to their scarcity and excessive production of waste, mostly they are considered disposable[1–4]. Countries that favored the use of masks by the public at the beginning of the Covid-19 outbreak experienced lower mortality related to the virus[2,4,6].

There are different Respiratory Protective Devices (RPD) types: surgical face mask, KN95 face mask, and social face mask. Surgical face mask constitutes a physical barrier for respiratory protection that was conceived for not be shared and not be used more than once[7,8]. The KN95 face mask is designed to provide a seal around the nose and mouth. It is classified as a RPD with a very efficient filtration of airborne particles[7]. Social face masks are expected to be reused; therefore, they must be washed and capable of withstanding high temperatures[2].

Studies have demonstrated the importance of adopting cleaning, disinfection and monitoring strategies for environmental surfaces and medical devices to prevent the transmission of pathogens[9,10]. Hydrogen Peroxide Vapor (HPV) was used in the decontamination of a wide range of microorganisms and the material decontaminated has no toxicity to human health[2]. The effect of decontamination by HPV has been examined in surgical face masks and KN95 face masks and their respective elastic strips contaminated with pig coronavirus (PRCV). The results showed a reduction of five orders of magnitude of indicators in contaminated masks[12]. Microwave vapor bags were also applied in the decontamination of N95 respirators. Although the results for N95 respirators show a reduction of 3 logs of the indicator, they did not comply with FDA guidelines and requirements[13]. Health professionals also tested the disinfection of elastomeric respirators with sodium hypochlorite (NaOCl) at a concentration of 1000 rpm. The effectiveness of the process was achieved after professionals followed a Standard Operating Procedure (SOP)[14].

**Goal:** The objective of this work was to evaluate the efficiency of the decontamination processes, HPV, immersion in commercial bleach, and steam in microwave bags in N95 face masks, surgical and cloth face masks. During this study we determined which of these processes lead to the sterilization. Additionally, the number of treatments was also evaluated to conclude if it interferes with the efficiency of the decontamination process.

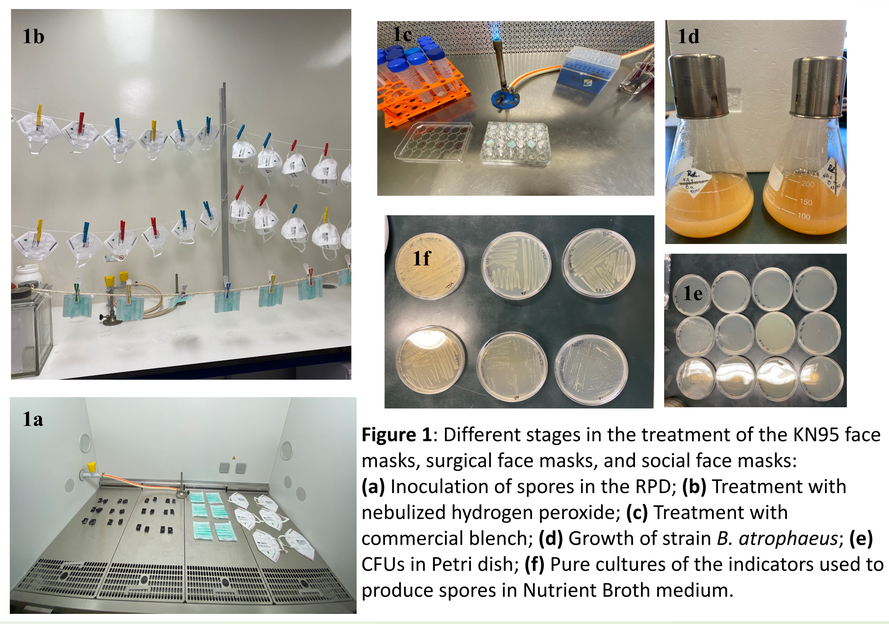
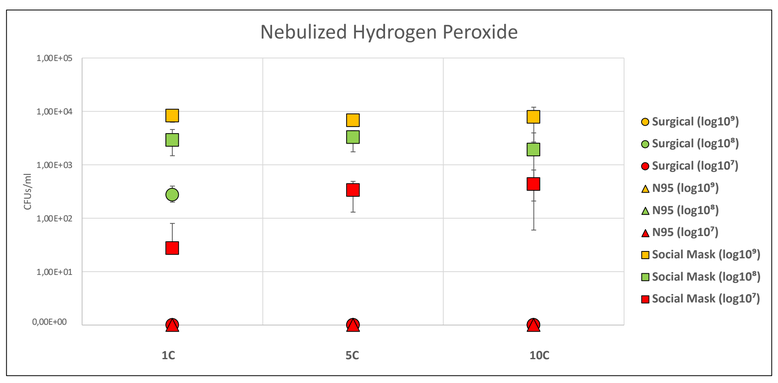
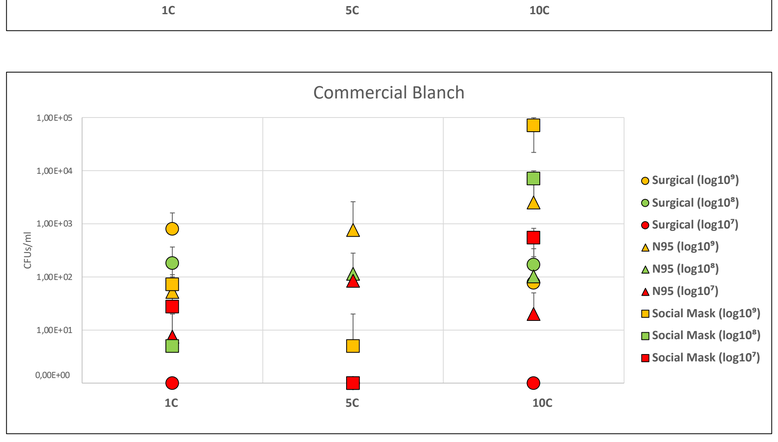




**Figure 1**: Different stages in the treatment of the KN95 face masks, surgical face masks, and social face masks: **(a)** Inoculation of spores in the RPD; **(b)** Treatment with nebulized hydrogen peroxide; **(c)** Treatment with commercial blench; **(d)** Growth of strain *B. atrophaeus*; **(e)** CFUs in Petri dish; **(f)** Pure cultures of the indicators used to produce spores in Nutrient Broth medium.

## Effect of three decontaminating treatments



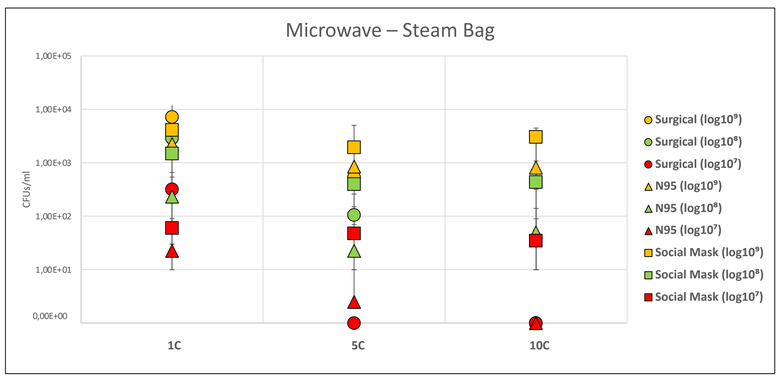




**Figure 2:** The effect of three decontaminating treatments. Inoculated surgical face mask, KN95 face mask, and cloth face mask for decontaminated via exposure to contact with Nebulized Hydrogen Peroxide, Immersion in Commercial Blanch, and contact with Steam in Microwave (steam bag). There was a reduction of six orders of magnitude of the bacterial indicator in the surgical face masks in contact with nebulized hydrogen peroxide and immersion commercial blanch, and the KN95 face masks in contact with nebulized hydrogen peroxide, and contact with steam in the microwave (steam bags). Small reductions of the indicator were observed in the treatment of cloth face with nebulized hydrogen peroxide; in 44% of these masks, there was a reduction of five orders of magnitude.

## Conclusions

- The nebulized hydrogen peroxide sterilized the KN95 and surgical masks since it reduced by six orders the magnitude of the bacterial indicator, as established by the ISO[15] Standard.
- A reduction of between five and four orders of magnitude of the microbial indicator was obtained in social masks nebulized with hydrogen peroxide, which corresponds to disinfection.
- The disinfection by immersion in commercial bleach sterilized the surgical masks, as established by the ISO[15] Standard. In KN95 face masks and cloth face masks reduced five orders of magnitude, which corresponds to disinfection.
- The disinfection with steam microwave sterilized KN95 face masks as established by ISO15. In surgical and cloth face masks since it reduced five and four orders of magnitude, respectively, corresponded to disinfection.
- The number of treatments did not affect the efficiency of the decontamination process.

### Methods

The masks were analysed progressively at the end of each treatment cycle. The treatment cycles were divided into three groups: 1 cycle (1C), 5 cycles (5C) and 10 cycles (10C). In order to proceed with the microbiological analysis, the masks corresponding to 5C and 10C groups were contaminated with different concentrations of the spores[15,16] at the end of the 4th and 9th treatment cycle, respectively and dried overnight.

**- Contact with Nebulized Hydrogen Peroxide**

Microbial indicator: endospores of *Geobacillus stearothermophilus*. The masks were subjected to the hydrogen peroxide nebulized by the NOCOLYSE® equipment. The type of treatment and volume of the product to be dispersed was indicated by the manufacturer. The curative treatment consisted of the emission of hydrogen peroxide in two distinct moments with an interval of 30 minutes between the 1st and 2nd application, and 2h30 minutes between the 1st and 2nd of a new treatment, according to the manufacturer's recommendation.

**- Immersion in Commercial Blench**

Microbial indicator: endospores of *Bacillus atrophaeus.* The masks were immersed in a solution of deionized water with 0.01% sodium hypochlorite for one minute and air dried[17].

**- Contact with Steam in Microwave (steam bag)**

Microbial indicator: endospores of *Geobacillus stearothermophilus*. The metal rods were removed from the masks and micro-steam bags (Medela®) were used. The process followed the manufacturer's instructions[18].

**Evaluation of the disinfection method:**

After undergoing 1C, 5C and 10C, the masks were subject to microbiological analysis. They were suspended in 0.85% saline solution for 1 h. Then, 100 µl of this solution was plated in Nutrient Agar (NA), and Tryptic Soy Agar (TSA) medium, and incubated for 24 h, at 30ºC or 50ºC, according to the microorganism analysed. Following the ISO 149371[15] standard (International Organization for Standardization) and to assess the efficiency of the disinfection method, the CFUs (Colony Forming Units) were counted.

## Inactivation of three decontaminating treatments

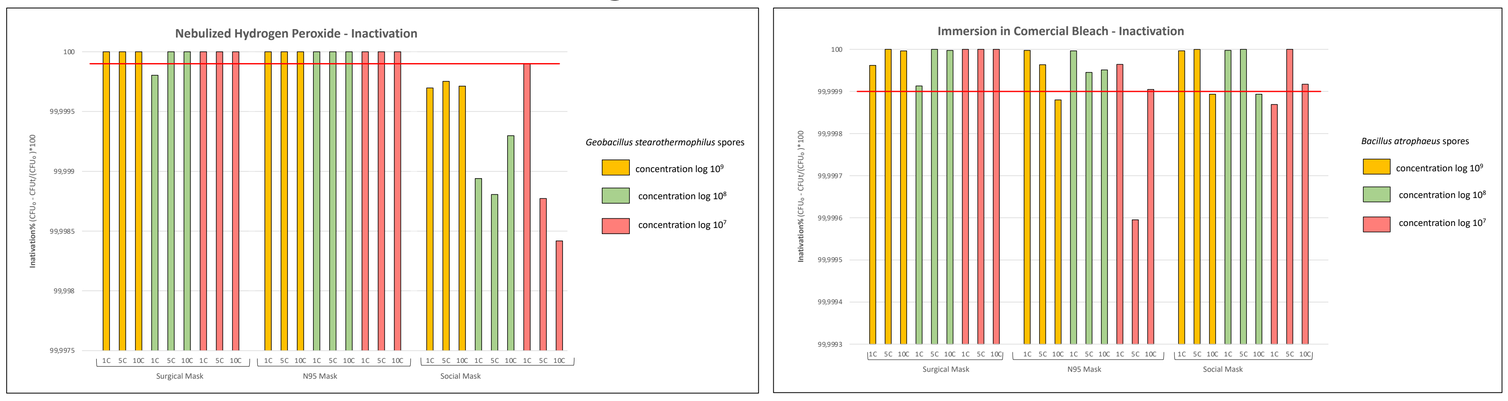



**Figure 3:** Effectiveness of the endospores inactivation by three decontamination processes of surgical, KN95 and cloth face masks. The red line indicates the Standard Effectiveness established by ISO 14937[15]. The inactivation values were obtained through the ratio between the difference of the inoculated CFUs and the CFUs recovered after treatment and the total inoculated CFUs. The inactivation process with nebulized hydrogen peroxide was completely efficient in KN95 masks with a reduction of 6 orders of magnitude, but showed low efficiency in social masks decontamination. Inactivation with commercial bleach resulted in a reduction of more than 6 orders of magnitude for all surgical masks, but was only efficient in 78% and 67% of the KN59 and social masks analyzed, respectively. Microwave steam inactivation was a very efficient process for all KN95 masks disinfection. However, in 66% of the surgical masks and in 55% of the social masks, the endospores inactivation was not complete with a reduction of just 5 orders of magnitude.

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