MYCOSCIENCE DECONTAMINATION TESTING OF 3M N-95 RESPIRATORS BY ETHYLENE OXIDE STERILIZATION METHOD

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In recent weeks, Mycoscience Inc. has received multiple inquiries from healthcare facilities and academic institutions regarding ways to decontaminate or sterilize medical surgical masks and N-95 respirators. Technically these devices are recommended by the CDC, OSHA, FDA, as well as by the manufacturers' for single-use only. However, due to the Covid-19 crises and the dire shortages in the supply chain, it may be necessary to reuse these respirators after decontamination inactivates the SARS-CoV-2 virus and other potential pathogenic agents.

BACKGROUND INFORMATION:

In an eerie prediction of our current crises, Viscusi *et al.* (November 2009) , published an article in the *Annals of Occupational Hygiene* (available on the NIH website and PubMed) raising concern over the potential shortage of respirators in an influenza pandemic. The article states:
"During an influenza pandemic, a shortage of filtering facepiece respirators (FFRs) may occur if manufacturing production is unable to meet the demand or if FFR stockpiles become depleted. According to a 2006 report from the National Academies' Institute of Medicine, over 90 million N95 FFRs will be needed to protect workers in the healthcare sector during a 42-day influenza pandemic outbreak (Bailar *et al.*, 2006). Guidance provided by the Centers for Disease Control and Prevention (CDC) states that once an FFR is worn in the presence of an infected patient, it should be considered potentially contaminated and not be reused by the same person or a coworker (CDC, 2007). A contaminated FFR could potentially serve as a fomite and lead to self-inoculation or spread of the organism to patients and other healthcare workers. Guidance from the Occupational Safety and Health Administration (OSHA) considers FFRs to be one-time-use devices when used in the presence of infected patients and advises employers and employees to only reuse FFRs during a pandemic if FFRs are in short supply and the device has not been obviously soiled or damaged (e.g. creased or torn), and it retains its ability to function properly (OSHA, 2007)."

Viscusi *et al.* , went on to test N95 and P100 respirators after different decontamination methods using the facilities and equipment at the NIOSH Personal Protective Technology Laboratory in Pittsburgh, PA. They found that filtration performance after one-time decontamination treatments using ethylene oxide (EtO), and hydrogen peroxide (vaporized and liquid forms) was observed to have filter aerosol penetration values that remained within acceptable limits of the National Institute for Occupational Safety and Health (NIOSH) certification criteria. While VHP may have some distinct advantages over EO, VHP sterilizers are not common at healthcare facilities and have relatively small chambers. Recently, FDA granted an Emergency Authorization Use for the Battelle CCDS Critical Care Decontamination System™ (a large mobile VHP decontamination system from Battelle) that can sterilize as many as 5,000 N-95 FFR's per cycle, but there are few of these systems available for use. However, it does serve as a statement regarding the decontamination efficacy of VHP methodology.

STUDY OBJECTIVE:

The goal of this study was to further evaluate a limited number of N95 FFRs to determine the effects of EO sterilization in order to reuse the FFRs. The biological decontamination methods used in this study included only EO sterilization. Following treatment by EO the N-95 respirators were evaluated for changes in physical appearance/odor (observational analysis), laboratory performance (filter aerosol penetration and filter airflow resistance), sterilization of the BI's located inside the FFR, and EO residuals in the form of Ethylene Oxide and Ethylene Chlorohydrin.

METHODOLOGY:

A total of (5) N-95 FFR's were used in this study. **All the FFR samples were 3M N-95 Respirators Model #8110-S. The sample quantity was limited to the remaining quantity of N-95 masks at Mycoscience Inc**. We were not able to obtain any other masks due to the critical shortage. Four of the FFR's were individually packaged in Crosstex Duo-Check™ self-sealing sterilization pouches and included a Crosstex BG-106 Biological Indicator (BI) containing $1x10^6$ CFU of *Bacillus atrophaeus* spores placed underside of the FFR. *Bacillus atrophaeus* is the recommended BI for the EO sterilization process. The pouched samples were then shipped to Northeast Scientific, a reprocessing facility in Waterbury, CT that has eight EO sterilizers available. The single remaining FFR was not EO sterilized and kept as a non-processed control sample to compare against the sterilized test samples. After completion of the EO cycle, one of the four sterilized samples was kept at -20^oC and sent to Ethide Labs for testing of EO residuals. The three remaining EO processed FFRs, along with the non-processed control were subjected to the evaluation testing listed below.

ETHYLENE OXIDE STERILIZATION:

EO is used in a wide range of work settings as a sterilant or fumigant, including healthcare, facilities and for medical device sterilization of products that cannot tolerate high temperatures or gamma irradiation. The EO sterilizer used in this study was a 3M Ster-Vac™ sterilizer with an EO process that has a 16-hour total processing cycle (3-hour preconditioning with a 1-hour EO exposure followed by a 12-hour heated aeration) and has a $8.0ft^3$ chamber volume. EO residuals remaining on FFRs following decontamination were not believed to be a concern because the sterilization process includes a final aeration cycle to remove residual EO gas. This hypothesis was confirmed by EO residual testing. **The residual levels for ethylene oxide (EO) and ethylene chlorohydrin (ECH) were tested and verified to be well below the limits established in ISO 10993-07. Furthermore, all the BI's tested in this run were negative for growth after the prescribed 7-day incubation in Trypticase Soy Broth media. Successful kill of the BI's indicates a Sterility Assurance Level (SAL) of $1x10^6$.**

VISUAL EVALUATION

After EO sterilization, the remaining (3) sterilized samples and the non-sterilized control sample were inspected and scrutinized carefully for any visible sign of dimensional changes, degradation or changes in materials that could be noted in texture or of the respirator (softness, pliability, coarseness, roughness, etc.) and any detectable odors. **None of the sterilized samples had any discernable changes from the non-sterilized control sample.**

FILTER PENETRATION TESTING - VIABLE AEROSOL CHALLENGE

The FFR's were individually tested for filter aerosol penetration using a modification of ASTM F2101-19 using a biological aerosol of *Staphylococcus aureus* ATCC 6536. Penetration levels were determined in a specially constructed glove box manufactured by Terra Universal. This aerosol chamber contains two DeVilbiss nebulizers used to generate the microbial aerosol under pressure. The FFR samples, (both the unprocessed control and the three EO sterilized test samples) were individually placed into filter holder attached to a SAS impactor-type air sampler containing a Trypticase Soy Agar plate. The air sampler was set to pull a 100-Liter air sample and placed inside the glove box where the aerosol was delivered. This process allowed quantification of any *S. aureus* organisms passing through the filter material of the FFR by counting the colonies on the agar plates. **There was no significant difference in filter aerosol penetration between the (3) sterilized samples and the non-sterilized control** and all the samples blocked >95% of the S. aureus organisms from passing through the mask compared to the positive control without a filter mask in place.

AIRFLOW TESTING - FILTER AIRFLOW RESISTANCE

For the control and post-decontamination test samples, a calibrated Sper Scientific Model 840085 manometer was also used to measure filter airflow resistance in millimeters of water column height pressure (mmH_2O). The setup incorporates a vacuum source and the manometer to measures the pressure differential across the N-95 mask. **There was no significant difference in airflow resistance between the (3) sterilized samples and the non-sterilized control sample**.

CONCLUSIONS:

The effects of the EO decontamination methods on filter aerosol penetration and filter airflow resistance and physical appearance of the tested N95 respirators were negligible in our limited sample size. There were no significant differences found between the fresh, non-sterilized control respirator and those that underwent the EO sterilization prior to viable filter aerosol penetration and filter airflow resistance. EO residuals do not appear to be a concern in validated cycles as heated aeration effectively removed the residuals. Even after extended 24-hour extraction techniques, the EO and ECH residuals were well below the limits established in the ISO 10993-7 standard for EO residuals levels. This particular EO cycle also verified also provided a high level of sterility assurance as verified by the kill of the BI's located inside the FFRs.

In light of these results, and the results published in the November 2009 article by Viscusi et al, both EO and VHP sterilization appear to be the most promising FFR decontamination methods. Where available, VHP has shorter turnaround times than EO, but outside of the aforementioned large capacity Battelle system, concerns remain about the throughput capabilities of the small VHP chambers. EO sterilizers are relatively more common in healthcare facilities and typically have larger chamber capacity, although the turnaround time is longer for EO sterilization cycles than for VHP cycles.

CAUTIONS AND PRECAUTIONS:

Care must be taken when handling used masks with potential pathogen exposure. Always wear gloves when handling a used FFR, especially on the exterior portion. A 3% hydrogen peroxide (H2O2) wipe can be used to hold and/or wipe the exterior portion while the interior of the FFR is examined for any damage, tears, or significant organic debris that can alter the filter or airflow efficacy. Such compromised FFR's should be disposed of by standard biohazardous waste methods. FFR's that are only slightly soiled on the interior may be wiped with a 3% H2O2 wipe as well, but do NOT use the same wipe used to wipe the exterior. The FFR should then be placed in a suitable EO compatible sterilization pouch that has an imbedded EO sterilization indicator. It is recommended that each pouch be labeled with the identity of the healthcare facility, the date pouched, and the ID of the original user.

Note: It is the responsibility of the user facility to determine which FFR's are suitable for reuse and determine the method of decontamination. The information presented here is intended for research purposes only.

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Finally, thanks to all the heroic nurses, doctors, lab techs, and all the other healthcare workers out there on the front lines tending to the testing and needs of the victims of this pandemic. God bless them all.