UNIVERSITY of VERONA
Department of DIAGNOSTICS AND PUBLIC HEALTH

Assessment of the ozonation procedure for the sanitization of FFP2 masks and protective suits.

The Association of Voluntary Public Assistance Croce Verde (Verona branch) uses the ozone generated by the Sany-car tool as sanitization method for ambulances after their use, following the 27-minutes protocol for sanitizing environments up to 25 m^3 as per the tool data sheet.

According to the technical data sheet, this tool is reported to be effective in sanitizing bacteria, fungi, moulds, and inactivating viruses on the surfaces of the medium that is subjected to treatment.

On the inactivation scale of microorganisms and microscopic life forms, the most resistant microorganisms are the prions, followed by spores, mycobacteria, gram-negative bacteria, naked viruses, gram-positive bacteria and then viruses with envelope.

SARS-CoV-2 is also included among the viruses with envelope.

The purpose of the assessment was to evaluate whether this procedure used as such for sanitizing ambulances could have an effect not only on the surfaces of the vehicle but also on the FFP2-type masks and protective suits used by the staff, in order to allow their reuse.

Since it was not possible to work with the SARS-CoV2 virus, as the virus must first be isolated and put into cell culture in order to be replicated in the laboratory, we proceeded to contaminate the masks subjected to the treatment with gram-negative bacteria, which in the scale are placed at a higher level of resistance than viruses.

Materials and methods:

The bacteria used for contamination were a multi-resistant *Klebsiella pneumoniae* strain and a multi-resistant *Pseudomonas aeruginosa* strain.

Cut-outs of FFP2 mask and protective suit of about 1cm^2 provided by Croce Verde were used.

The contamination of masks occurred in two ways: the first by immersion in a bacterial suspension at different concentrations; in the second contamination mode, the bacterial suspension - always at different concentrations - was sprayed from a distance of about 30-40cm towards the external part of the mask using a spray bottle, in order to mimic the mode of contamination by droplets originating from sneezes.

For each contamination mode and for each different bacterial concentration, two cut-outs of mask and two of protective suit were used. For each test, one mask and one protective suit cut-out was used as reference sample, while the other two cut-outs were treated – as a test – with ozone, that is the treatment for sanitizing the ambulances.

The initial bacterial suspension, both for *K. pneumoniae* and *P. aeruginosa*, was 0.5 McFarland corresponding to 10^9 microorganisms per ml. The initial suspension was then diluted by factor 10 to 10^4. The tested concentrations were 10^8, 10^7, 10^6, 10^5 and 10^4

After contamination and ozone treatment, the cut-outs were left to dry for a few hours.

Before positioning them in the infused agar plate, the cut-outs were immersed in sterile distilled water for two minutes. In case of contamination by spraying, the positioning took place with the contaminated surface on the surface of the plate.

Plates were incubated at 37 °C for 24 hours, after which colony growth was measured.

Results

The following are the results of the trial. In total, the ambulance was subject to 4 sanitization operations, two on masks and protective suits contaminated by immersion and two on masks and protective suits contaminated by spray.

Table 1: analysis of masks and protective suits sanitization after contamination by immersion in suspensions at various *K. pneumoniae* concentrations.

CFU/ml	*K. pneumoniae* contamination by immersion			
	reference		Ozone treatment	
	Protective suit	mask	Protective suit	mask
10^4	+	+	-	-
10^5	++	+++	-	-
10^6	+++	++++	+	++
10^7	+++	+++++	++	+++
10^8	++++	+++++	+++	++++

Picture 1: masks and protective suits contaminated by immersion in a bacterial suspension of 10^4 CFU/ml without ozone treatment (left) and after ozone treatment (right)

Figure 2: masks and protective suits treated by immersion in a suspension of 10^8 CFU/ml of *K. pneumoniae* without ozone treatment 8 (left) and after ozone treatment (right)

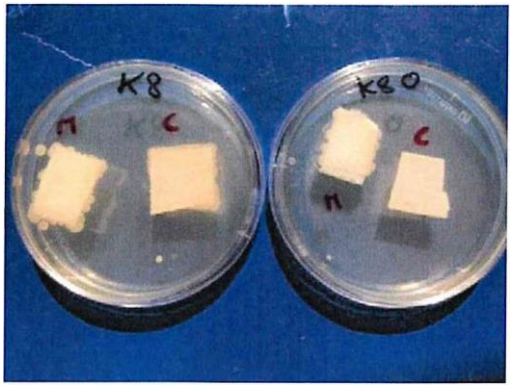


Table 2: analysis of masks and protective suits sanitization after contamination by immersion in suspensions at various concentrations of *P. aeruginosa*

CFU/ml	*P. aeruginosa* contamination by immersion			
	reference		Ozone treatment	
	Protective suit	mask	Protective suit	mask
10^4	+	+	-	-
10^5	++	+++	-	-
10^6	+++	++++	+	++
10^7	+++	+++++	++	+++
108	++++	+++++	+++	++++

Table 3: analysis of masks and protective suits sanitization after contamination by aerosol in suspensions at various concentrations of *K. pneumoniae*:

CFU/ml	*K. pneumoniae* contamination by aerosol			
	reference		Ozone treatment	
	Protective suit	mask	Protective suit	mask
10^4	+	+	-	-
10^5	+	++	-	-
10^6	++	+++	-	-
10^7	+++	++++	+	++
10^8	+++	++++	+	++

Figure 3: Masks and protective suit contaminated by aerosol in a suspension with 10^4 CFU/ml of *Klebsiella pneumoniae* and treated with ozone on the right and untreated on the left

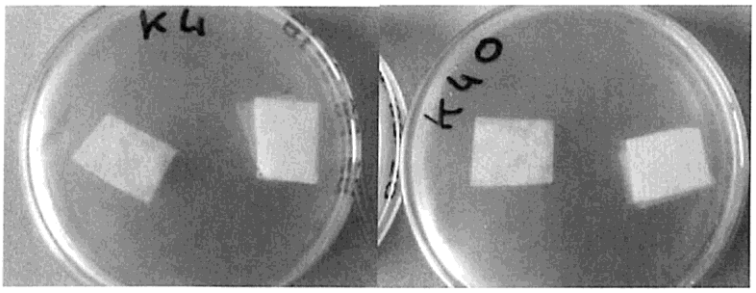
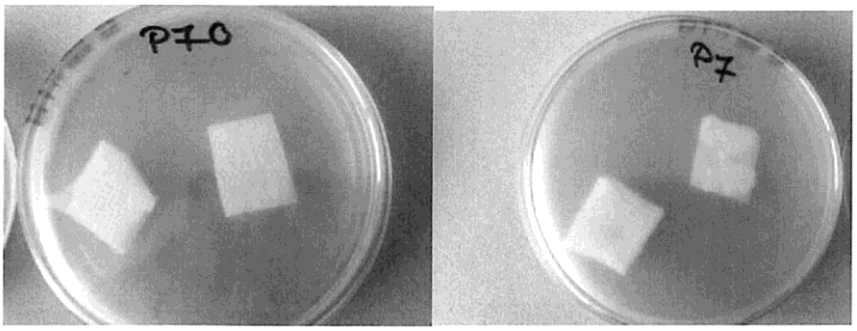


Table 4: analysis of masks and protective suits sanitization after contamination by aerosol in suspensions at various concentrations of *P. aeruginosa*

CFU/ml	*P. aeruginosa* contamination by aerosol			
	reference		Ozone treatment	
	Protective suit	mask	Protective suit	mask
10^4	+	+	-	-
10^5	+	++	-	-
10^6	++	+++	-	-
10^7	+++	++++	-	+
10^8	+++	++++	+	++

Figure 4: Masks and protective suit contaminated by aerosol suspension with 10^7 CFU/ml of *Pseudomonas aeruginosa* and treated with ozone on the left and untreated on the right



Conclusions:

FFP2 masks and protective suits are made of different materials and the contamination is easier for the masks than the white coats, which are water-repellent.

Contamination by immersion in a bacterial suspension always results in a higher charge compared to aerosol contamination, obtained by spraying the assessed bacterial suspension at a distance of 30-40cm, in order to mimic the contamination by sneezing.

With the immersion method, the ozone treatment carried out in an ambulance managed to eliminate the microorganisms used at the lowest concentrations, i.e. 10^4 and 10^5 CFU/ml, with 100% effectiveness.

With the aerosol method, the ozone treatment carried out in an ambulance was more effective, eliminating even higher initial charges, which in any case at the beginning were

lower in the reference sample as well. In this case, especially for protective suits, decontamination levels of up to 10^7 CFU/ml were reached, with a reduction of the charge at the highest concentration of 10^8 CFU/ml of about 80%.

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