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Short Report

Virucidal efficacy of an ozone-generating system for automated room disinfection

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SUMMARY

Besides conventional prevention measures, no-touch technologies based on gaseous systems have been introduced in hospital hygiene for room disinfection. The whole-room disinfectant device Sterisafe Pro, which creates ozone as a biocidal agent, was tested for its virucidal efficacy based on Association Française de Normalisation Standard NF T 72−281:2014. All test virus titres were reduced after 150 and 300 min of decontamination, with mean reduction factors ranging from 2.63 (murine norovirus) to 3.94 (simian virus 40). These results will help to establish realistic conditions for virus inactivation, and assessment of the efficacy of ozone technology against non-enveloped and enveloped viruses.

Introduction

Contaminated environments in healthcare settings might be the source for acquisition of microbial pathogens, including viruses. Therefore, effective cleaning and disinfection with conventional methods should interrupt the transmission of viral diseases from these contaminated surfaces in indoor settings. Methods used for disinfection are based on an appropriate product with exact application times and on correct human behaviour, which is sometimes difficult to achieve. Therefore, no-touch automated room disinfection with new agents has been introduced as an alternative to the traditional techniques in hospitals and other medical settings. Consequently, for room decontamination in the medical field, no-touch methods such as hydrogen peroxide vapour, aerosolized hydrogen peroxide and ultraviolet light have been established. There are various commercially available systems on the market, and these differ in terms of application, active agents, effectiveness, practicability and cost [1].

Well-designed clinical studies comparing new no-touch disinfection with traditional disinfecting practice are limited. Weber *et al.* reviewed various comparative trials, and evaluated the effectiveness of ultraviolet devices and hydrogen peroxide systems against pathogens such as meticillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, *Clostridioides difficile* and *Acinetobacter* spp. [2].

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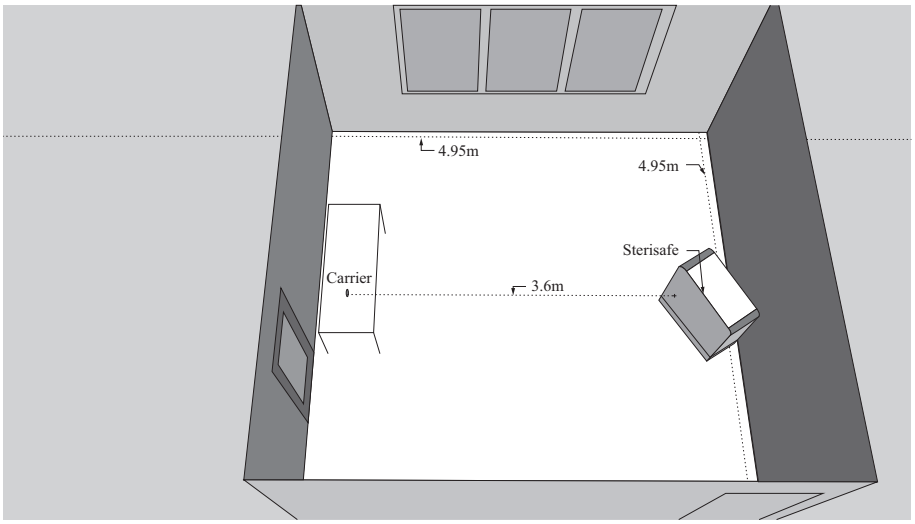

Figure 1. Layout of the room used for testing the air disinfection device. Carriers were placed on a table 3.6 m away from the outlet of the STERISAFE Pro device, as shown.

In contrast to the above-mentioned pathogens, viruses such as human norovirus have not generally been included in these studies, but they can also play an important role in hospital hygiene. Therefore, when starting clinical trials with multidrug-resistant bacteria, important human viruses should also be included in laboratory studies for no-touch room disinfection systems. The present study investigated the inactivation of human adenovirus type 5 (AdV) and murine norovirus (MNV) (mandatory test viruses), modified vaccinia virus Ankara (MVA) and simian virus 40 (SV40). STERISAFE Pro was applied using Association Française de Normalisation (AFNOR) Standard NF T 72—281 under defined conditions [3].

Methods

Automated disinfection system

The ozone-based device STERISAFE Pro Version 1.0 with an integrated nebulizer was tested. This device was supplied by STERISAFE ApS (Copenhagen, Denmark). The STERISAFE Pro device concentrates ambient air with conversion to ozone.

STERISAFE Pro progresses through three phases: the building phase, with increasing ozone levels; the decontamination phase, with a constant ozone concentration; and the cleaning phase, when ozone is removed. Two different decontamination cycle lengths were evaluated for their virus-inactivating properties: 150 min and 300 min. The 150-min decontamination period was tested once, and the 300-min decontamination period was tested twice.

Cell culture and viruses

A549 cells and CV-1 cells were passaged in Eagle's Minimum Essential Medium with Earle's BSS (Biozym Scientific GmbH, Catalogue No. 880120). BHK-21 cells were passaged in Eagle's Minimum Essential Medium with Hank's BSS (Biozym Scientific GmbH, Catalogue No. 880144). RAW 264.7 cells were passaged in Dulbecco's modified Eagle medium (Biozym Scientific GmbH, Catalogue No. 880005). Before the inactivation assays, viruses had been passaged three times in their respective cell lines (AdV in A549 cells, MNV in RAW 264.7 cells, MVA in BHK-21 cells and SV40 in CV-1 cells). Virus test suspensions were produced by inoculating respective cells at a multiplicity of infection of 0.1. On showing cytopathic effects, cells were subjected to three freeze—thaw cycles followed by low-speed centrifugation to separate the virus from cell debris. All virus stocks were stored at -80 °C. The choice of test viruses for this study was adopted from the AFNOR standard [3] and the quantitative suspension test published by Deutsche Vereinigung zur Bekämpfung der Viruskrankheiten e.V [4].

Carrier preparation

Stainless steel carriers (Article No. 4174—3000, GK Formblech GmbH, Berlin, Germany) were placed in a container with 5% (v/v) Decon 90 for 60 min and rinsed twice with distilled water at room temperature. Subsequently, the carriers were submerged in 70% (v/v) propan-2-ol for 15 min, air-dried under laminar air flow and sterilized (steam sterilization). For the preparation of virus inoculum, 19 parts of virus suspension were mixed with 1 part of 10% skimmed milk solution (Fluka Analytical, Article No. 70166—500) to a final concentration of 0.5%. Fifty microlitres of virus inoculum was applied to the carriers and dried.

No-touch disinfection and virus recovery

The virus-inactivating properties of ozone generated by STERISAFE Pro for automated room disinfection were evaluated based on AFNOR Standard NF T 72—281:2014, with AdV, MNV, MVA and SV40 as test viruses. This standard has been established for more than 25 years, and was updated in 2014 [3]. It allows aerial surface disinfection systems to make a virucidal claim.

The floor of the test room measured 4.95 m x 4.95 m, with a height of 2.55 m and a calculated volume of 62.48 m^2. The target ozone concentration was 80 ppm, with relative humidity (RH) of 90%, in accordance with the manufacturer's instructions. The distance between the ozone application unit and the contaminated carriers was 3.60 m (Figure 1). The carriers

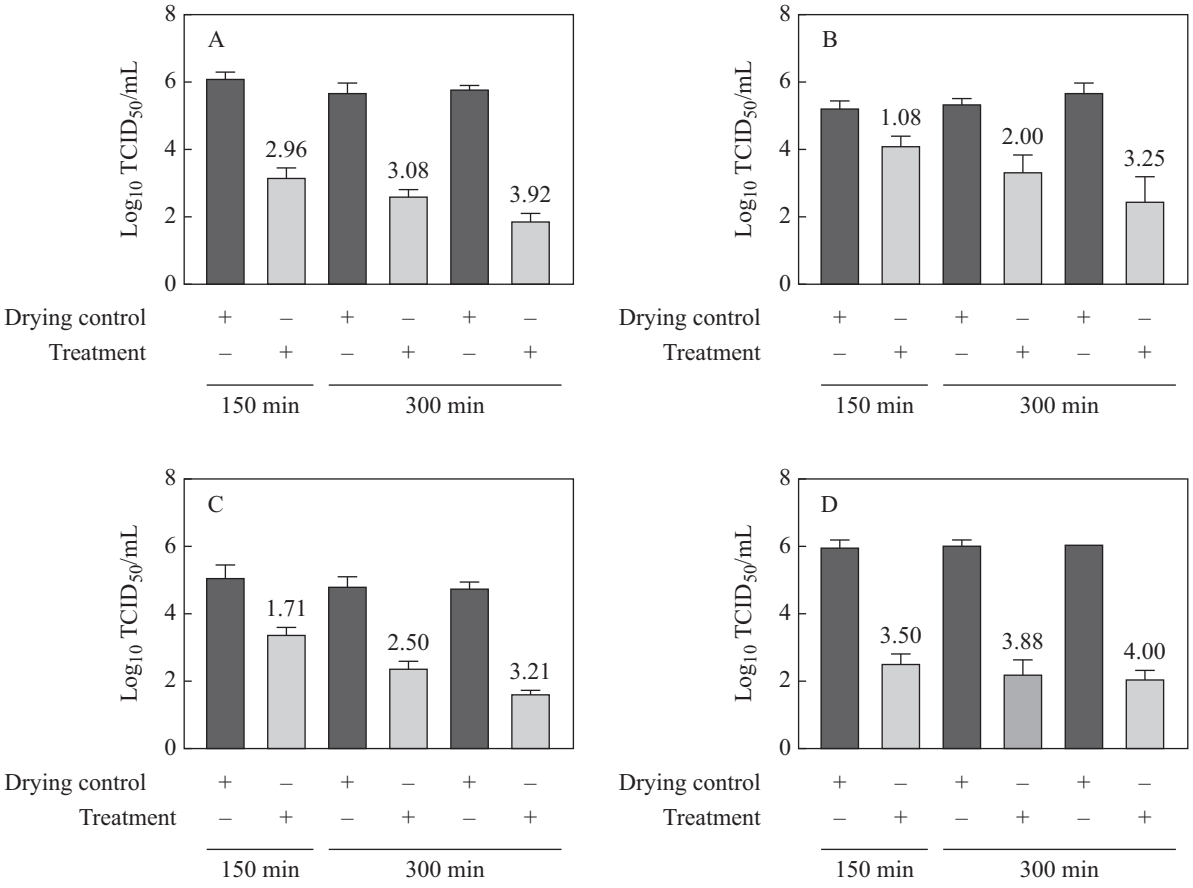

Figure 2. Titre reduction of four test viruses after exposure to ozone (two exposure times). (A) Human adenovirus type 5. (B) Murine norovirus. (C) Modified vaccinia virus Ankara. (D) Simian virus 40. Respective virus titres with and without treatment are shown. Calculated reduction factors are displayed on top of the grey bars. $TCID_{50}$, tissue culture infectious dose 50%.

(always three in parallel) with the dried inoculum were deposited in a slat with the contaminated side facing away from the ozone application unit (Figure 1). After the final cleaning phase in the test room, the carriers were transferred for elution in a 25-mL vial with 10 mL of medium without fetal calf serum (FCS), and vortexed for 60 s. Directly after elution, a series of 10-fold dilutions of the eluate in ice-cold maintenance medium were prepared and inoculated on cell culture. Controls to ozone treatment were inoculated and dried in the same way, but stored in another room without ozone treatment prior to elution and titration.

Preparation of controls

A virus control before drying is needed as the control of the initial virus titre in the test assay, determination of stability after drying and for evaluation of neutralization of the disinfectant. For this control, 50 μL of virus inoculum was added to 9.950 mL of medium without FCS (elution).

In addition, two virus controls directly after drying (VCt0) and three carriers for each exposure time tested were incorporated. For VCt0, the elution took place immediately after drying the virus inoculum in 10 mL of medium without FCS. The elution for VC at the respective time points was run in parallel with room disinfection, as described. Additionally, a cytotoxicity control, a cell control and a reference control (formaldehyde) for internal validation were incorporated.

Determination of infectivity

After addition of 0.1 mL of freshly trypsinized cells (10–15 x 10^3 cells per well), infectivity was determined using endpoint titration, transferring 0.1 mL of each dilution into eight wells of a microtitre plate, beginning with the highest dilution. The cell suspension was adjusted. Microtitre plates were incubated at 37 °C in a 5% CO_2 atmosphere. The cytopathic effect was read using an inverted microscope. Calculation of the tissue culture infectious dose 50% ($TCID_{50}$)/mL was calculated using Spearman and Kärber's method.

Calculation of virus-inactivating properties

The virus-inactivating properties of treatment with the STERISAFE Pro were measured by subtracting the mean virus titres (after treatment) from the virus titres that resulted in parallel without ozone treatment, and indicated as reduction factors (RFs).

Results

Virus titres measured on the contaminated carriers were reduced depending on the length of the decontamination period (Figure 2). The ozone concentration tested was able to reduce the different virus titres after 150 min from 1.08 (MNV) to 3.50 (SV40).

The resulting RFs were consistently higher for the longer decontamination period. The RFs after a 300-min decontamination cycle were 2.63 (MNV), 2.86 (MVA), 3.50 (AdV) and 3.94 (SV40). For AdV and SV40, the calculated RFs reached $\geq$3.00 ($\geq$99.9%) for both MNV and MVA values resulted below.

Discussion

There are various automated room disinfectant systems with different active agents on the market, which may reduce the reliance of operators performing surface disinfection with germicides. Many studies have focused on resistant bacteria and fungi, but data for important human pathogenic, non-enveloped viruses are missing for surface disinfection. Therefore, this laboratory study included examinations following the AFNOR standard with AdV and MNV as non-enveloped viruses. Both viruses are also named, together with the porcine parvovirus, in the recently published European Norm EN 17272:2020, which describes a test method for automated airborne room disinfection [5]. Here, an additional validation control was implemented, and further validation of the room to be treated was performed. In the present study, both MVA (enveloped) and SV40 (non-enveloped) were incorporated to gain better insight into virus inactivation by ozone. Both of these viruses are used as test viruses in German guidelines describing a quantitative suspension test [4].

With an exposure time of 300 min, AdV and SV40 were inactivated by 3 log steps based on a calculation with data for untreated carriers. For MNV and MVA, RFs of 2.00/3.25 (mean 2.63 MNV) and 2.50/3.21 (mean 2.86 MVA), respectively, were measured. Due to a wide variety of test parameters in an examination room, variation will be greater compared with laboratory experiments performed in a laminar flow box. By incorporating corresponding controls in a non-treated room, the decline in virus titres is based solely on the effect of the ozone-producing device, and not on any further decline in virus titres after drying.

Interestingly, these results show that the enveloped virus (MVA) was more resistant to ozone than the non-enveloped viruses. In quantitative suspension tests in Germany and Europe [4,6], MVA is used as a test virus for all enveloped viruses, as it is generally more fragile than non-enveloped viruses from different families when testing hand, surface or instrument disinfectants with different active ingredients. There is an urgent need to clarify whether other enveloped viruses causing respiratory tract infections might show similar behaviour. Recently, a study showed that the bacteriophage Φ6 (Phi 6) and the enveloped bovine coronavirus L9, as surrogates of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), were reduced by $\geq$4 $\log_{10}$ steps when testing the same ozone-based device with a decontamination time of 60 min. The ozone concentration and RH were identical, but other important test parameters, such as room parameters, differed [7]. Therefore, a direct comparison of virus stability towards ozone between the enveloped viruses MVA and bovine coronavirus is not possible. In addition, a small ozone generator with disinfecting and deodorizing air purifier functions was able to reduce SARS-CoV-2 after 60 min of exposure to 6.0 ppm ozone at 55 °C by 3 $\log_{10}$ steps [8].

The risk of toxicity from aerosol exposure to ozone is well known, and a carcinogenic effect in animal models has been discussed. However, the new disinfection technology can be used in the hospital setting, as the indoor air concentration of ozone is lower and rooms are safe to enter after a completed cycle.

As a strong oxidizing agent, ozone has been widely used as a disinfectant in drinking water treatment plants. Additionally, the ability of ozone gas to inactivate norovirus placed on dried samples has been reported. These examinations were performed at various locations within an office room, in a cabin of a cruise liner, and in a hotel room. It was found that feline calicivirus (a former surrogate of human norovirus) and norovirus itself were inactivated by 3 $\log_{10}$ steps when ozone gas was produced from a portable commercial generator. Therefore, it was suggested that this technique might also be important for healthcare facilities [9].

Furthermore, a study from Quebec performed in a rotative environmental aerosol chamber showed that 0.23 ppm ozone for 40 min at 85% RH achieved inactivation of MNV by 2 $\log_{10}$ steps. Furthermore, it was demonstrated that the length of time and RH were highly significant for virus inactivation [10].

In conclusion, these results clearly demonstrate the virus-inactivating potential of ozone in a laboratory experiment with enveloped and non-enveloped viruses. There is a need for clinically relevant studies to compare no-touch disinfection systems with traditional liquid disinfectants against important non-enveloped stable viruses, such as norovirus or rotavirus, for reduction of nosocomial virus infections.

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Conflict of interest statement
None declared.

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