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Evaluating the virucidal efficacy of hydrogen peroxide vapour

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SUMMARY

Background: Surface contamination has been implicated in the transmission of certain viruses, and surface disinfection can be an effective measure to interrupt the spread of these agents.

Aim: To evaluate the in-vitro efficacy of hydrogen peroxide vapour (HPV), a vapour-phase disinfection method, for the inactivation of a number of structurally distinct viruses of importance in the healthcare, veterinary and public sectors. The viruses studied were: feline calicivirus (FCV, a norovirus surrogate); human adenovirus type 1; transmissible gastroenteritis coronavirus of pigs (TGEV, a severe acute respiratory syndrome coronavirus [SARS-CoV] surrogate); avian influenza virus (AIV); and swine influenza virus (SwIV).

Methods: The viruses were dried on stainless steel discs in 20- or 40-μL aliquots and exposed to HPV produced by a Clarus L generator (Bioquell, Horsham, PA, USA) in a 0.2-m^3 environmental chamber. Three vaporized volumes of hydrogen peroxide were tested in triplicate for each virus: 25, 27 and 33 mL.

Findings: No viable viruses were identified after HPV exposure at any of the vaporized volumes tested. HPV was virucidal (>4-log reduction) against FCV, adenovirus, TGEV and AIV at the lowest vaporized volume tested (25 mL). For SwIV, due to low virus titre on the control discs, >3.8-log reduction was shown for the 25-mL vaporized volume and >4-log reduction was shown for the 27-mL and 33-mL vaporized volumes.

Conclusion: HPV was virucidal for structurally distinct viruses dried on surfaces, suggesting that HPV can be considered for the disinfection of virus-contaminated surfaces.

Introduction

Viruses are important causes of acute and chronic diseases in humans and animals, and frequently cause community-acquired and nosocomial infections. Surface contamination with viruses is common in households, industrial settings and healthcare facilities, and the role of these contaminated surfaces in the transmission of certain viruses, such as norovirus, is recognized increasingly.[1] Many viruses have a low infectious dose and are shed at high titres from infected individuals, even when the infection is asymptomatic.[2] They can contaminate dry surfaces, survive for extended periods and be transmitted to susceptible hosts from surfaces.[2,3] Once dried on inanimate surfaces, viruses are less susceptible to disinfection than when

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hydrated in suspension.[4] This susceptibility is further reduced by the presence of organic soil and viral clumping.[4]

Disinfection of surfaces is an effective method for reducing the risk of exposure to viruses and interrupting their spread.[5] However, some viruses, such as norovirus, are resistant to some commonly used hospital disinfectants.[6,7] In addition, conventional manual disinfection of surfaces is labour intensive, and it appears that a two-stage disinfection procedure, including surface rehydration followed by disinfection, is required for effective inactivation of viruses dried on surfaces.[4,6] Reliance on an operator to ensure appropriate selection, formulation, distribution and contact time of the agent further limits the repeatability and efficacy of the manual disinfection process. Hence, viral contamination can persist after standard or even enhanced manual environmental cleaning and disinfection.[6,8–10]

Hydrogen peroxide vapour (HPV) is a novel 'no-touch' automated decontamination technology that removes the reliance on the operator to ensure distribution, contact time and process repeatability, and has therefore been used for environmental decontamination in various settings to improve the efficacy of disinfection.[11] Two types of HPV are available: non-condensing vaporized hydrogen peroxide (VHP) technology (Steris) and condensing HPV technology (Bioquell). Condensing systems inject hydrogen peroxide until the air in the enclosure becomes saturated and hydrogen peroxide begins to condense on surfaces. Non-condensing systems dry the vapour stream as it is returned to the generator.[12] Both condensing and non-condensing systems are registered with the Environmental Protection Agency, and have well-established efficacy against bacterial spores and other microbes.[12–14] A study published in the 1990s evaluated the efficacy of a non-condensing VHP system against a range of viruses.[14] However, limited evidence is available for the virucidal activity of condensing HPV systems. Recently, several studies have demonstrated the in-vitro activity of condensing HPV systems against individual viruses, including feline calicivirus (FCV),[11] adenovirus,[15] lactococcal bacteriophages[16] and MS2 coliphage.[17] However, to the authors' knowledge, no studies published to date have evaluated the efficacy of condensing HPV systems against a range of viruses exposed under the same conditions.

As such, this study evaluated the in-vitro virucidal efficacy of a condensing HPV system against a number of structurally distinct viruses of importance in the healthcare, veterinary and public sectors. These included a small non-enveloped RNA virus (FCV, a norovirus surrogate), a larger non-enveloped DNA virus (human adenovirus) and three enveloped RNA viruses: transmissible gastroenteritis coronavirus (TGEV, a severe acute respiratory syndrome coronavirus [SARS-CoV] surrogate), avian influenza virus (AIV) and swine influenza virus (SwIV).

Materials and methods

Viruses

The following five viruses were used: FCV (strain 255) as a surrogate of human norovirus, TGEV (Purdue strain) as a surrogate for SARS (severe acute respiratory syndrome) virus, human adenovirus type 1 (hADV-1), AIV (A/chicken/Maryland/2007[H9N9]) and SwIV (A/swine/Minnesota/2010 [H3N2]). FCV, TGEV and hADV-1 were grown in CRFK, ST and A-549 cells, respectively, while AIV and SwIV were grown in MDCK cells. The cells were grown in Eagle's MEM (Mediatech, Herndon, VA, USA) supplemented with 150 IU/mL penicillin, 150 μg/mL streptomycin, 50 μg/mL neomycin, 1 μg/mL fungizone and 8% foetal bovine serum. Viruses were harvested from infected cells by three freeze–thaw cycles followed by centrifugation at 2000 ***g*** for 20 min. The supernatant was aliquoted and stored at −80 °C until use.

HPV exposure

Sterile 10-mm-diameter 18/8 stainless steel (grade 304) discs (Mesa Labs, Lakewood, CO, USA) were inoculated with 20 μL of virus suspension of FCV, hADV-1 and TGEV. For AIV and SwIV, 40 μL of virus suspension was used because their initial titres were lower than those of the other three viruses. No additional soiling was added, apart from the 8% foetal bovine serum in the culture medium. After virus application, the discs were placed inside a biosafety cabinet to dry for 30 min. After drying, the discs were placed in 24-well tissue culture plates (one disc per well) without lids. For each experiment, three inoculated discs were exposed to HPV in an environmental chamber and one disc was kept in a separate control plate, which was kept outside the environmental chamber at room temperature for the duration of the test. Three independent tests were performed for each vaporized volume of hydrogen peroxide.

HPV was produced using a Clarus L generator (Bioquell, Horsham, PA, USA). The Clarus L generator, which is situated outside the enclosure, converts 35% w/w liquid hydrogen peroxide into HPV using a vaporizer heated to 120 °C, and circulates the HPV through the environmental chamber via a supply and return hose. Hydrogen peroxide was injected at 2 mL/min for 1, 2 or 5 min followed by 1.5 mL/min for 15 min equating to three different volumes: 25, 27 and 33 mL. The concentration of HPV and temperature in the environmental chamber during the cycle was not measured. Following HPV injection, the air in the environmental chamber was routed through an activated carbon filter to break down the hydrogen peroxide to oxygen and water vapour. When the concentration of HPV in the environmental chamber reached <1 ppm, as determined by a hydrogen peroxide hand-held sensor through a sampling port, all test discs were removed. The total exposure time, including injection and aeration (the breakdown of hydrogen peroxide), was approximately 2–3 h, varying with the amount of hydrogen peroxide being vaporized. After completion of each run, discs were removed from the environmental chamber and titrated to determine the amount of surviving virus along with the control disc.

In addition to the virus test discs, four Tyvek-packaged *Geobacillus stearothermophilus* biological indicators (BIs) (Mesa Labs) with a certified population of >6-$\log_{10}$ spores/disc were placed in the corners of the environmental chamber in alternating high and low locations and used as a standard indicator for the HPV decontamination cycles.[18] BIs were removed from the environmental chamber following HPV exposure, transferred into test tubes containing trypticase soy broth, incubated at 65 °C, and examined for bacterial growth daily for seven days. An unexposed BI was transferred into trypticase soy broth and incubated with each batch as a positive control.