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NOTE TO THE EDITOR

Hydrogen peroxide vapour (HPV) inactivation of adenovirus

E. Berrie[1], L. Andrews[1], S. Yezli[2] and J.A. Otter[2]

1 Clinical BioManufacturing Facility, University of Oxford, Oxford, UK
2 Bioquell (UK) Ltd, Andover, Hampshire, UK

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Correspondence
Eleanor Berrie, Clinical BioManufacturing Facility, Old Road, Headington, Oxford, OX3 7JT, UK.
E-mail: eleanor.berrie@ndm.ox.ac.uk

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Abstract

Aims: Adenovirus contamination can be problematic in various settings including life science laboratories and during pharmaceutical manufacturing processes. Stringent and effective decontamination procedures are necessary to minimize the risk of personnel exposure or product cross-contamination in these settings. Hydrogen peroxide vapour (HPV) is sporicidal, tuberculocidal and fungicidal with proven efficacy against some viruses. We investigate the efficacy of HPV for the inactivation of a recombinant adenovirus.
Methods and Results: In this study, the survival of a dried recombinant adenovirus (Ad5GFP) was tested before and after HPV exposure to determine the efficacy of HPV at inactivating adenovirus. A > 8-log $TCID_{50}$ reduction resulted from 45-min exposure to HPV in a microbiological safety cabinet.
Conclusions: HPV is effective for the inactivation of a recombinant adenovirus.
Significance and impact of the study: The results suggest that HPV may be useful for adenovirus decontamination in life science laboratories or in manufacturing facilities.

Adenovirus is an important human pathogen causing mild to severe diseases especially in immunocompromised individuals (Kojaoghlanian *et al.* 2003; Goncalves and De Vries 2006). Recombinant adenoviruses are widely used in biomedical and industrial settings because they are an excellent gene transfer tool (Hitt *et al.* 1997; Leen and Rooney 2005). The implementation of effective decontamination procedures in these settings is hence critical to minimize the risk of personnel exposure to the virus and to prevent product cross-contamination (Sofer 1995, 2003). Hydrogen peroxide vapour (HPV) is a vapour-phase method used to decontaminate enclosures ranging from microbiological safety cabinets (MSC) to entire buildings in laboratory, health care, pharmaceutical and other industries (French *et al.* 2004; Johnston *et al.* 2005). HPV is sporicidal, tuberculocidal and fungicidal (Heckert *et al.* 1997; Johnston *et al.* 2005; Hall *et al.* 2007, 2008). However, few studies have investigated its virucidal efficacy (Heckert *et al.* 1997; Pottage *et al.* 2010). We tested the efficacy of HPV for the inactivation of high-titre recombinant adenovirus dried to simulate a laboratory spillage using an inoculated carrier method.

Stainless steel 10-mm-diameter discs (Apex, Sanford, NC, USA) were pretreated to minimize any cellular toxicity problems. They were boiled in 2% Hellmanex II solution (Hellma GmbH + CoKg) for 30 min, irrigated and soaked overnight in purified water. Then discs were drained, transferred to Lancer acid rinse solution (Lancer, UK) overnight at room temperature then drained and washed in a Lancer washing machine (model 910UP) on a cycle with a detergent wash, followed by an acid rinse before rinses in potable water and final rinses in purified water. Discs were dried and autoclaved before use. Purified replication-deficient recombinant human adenovirus Ad5GFP, an E1/E3 gene deleted vector expressing green fluorescent protein (GFP), a reporter gene providing a simple and rapid measure of viral transduction and gene expression in HEK 293 cells (Losert *et al.* 2006) (obtained from Professor Hill's group, University of Oxford), was grown in HEK 293 cells (ECACC) and purified using standard caesium chloride density centrifugation to a titre of approximately 10^{12} $TCID_{50}$ ml^{-1}. The HEK 293 cells allow for adenovirus replication because they stably express the E1 gene. A master cell bank was prepared, and a fresh vial of cells was thawed and established in

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culture for each experiment. Cells were grown in DMEM with high glucose and sodium pyruvate supplemented with 4 mmol l^{-1} L-glutamine, nonessential amino acids and 10% foetal bovine serum. Cells were not allowed to become confluent and were split when between 70 and 80% confluent. Each well of a 96-well microtitre plate was seeded with 150 μl of cell suspension containing 10^5 viable cells ml^{-1} in DMEM, as above but supplemented with 5% foetal bovine serum, nonessential amino acids and 4 mmol l^{-1} L-glutamine and incubated for between 36 and 48 h at 37°C in an atmosphere of 5% CO_2 and air. Plates were used when the cell sheet was subconfluent but there was good cover over the well. Working in a MSC, 10 μl of thawed virus was diluted with 290 μl of serum-free medium ($10^{-1\cdot5}$) and two further decimal dilutions ($10^{-2\cdot5}$ and $10^{-3\cdot5}$) were prepared. For experiment 1, the $10^{-1\cdot5}$ and $10^{-3\cdot5}$ dilutions were used while experiment 2 used the $10^{-1\cdot5}$ and $10^{-2\cdot5}$ dilutions. In experiment 3, a single dilution ($10^{-1\cdot5}$) was tested. Discs were inoculated with 10 μl for each dilution and air-dried for 2 h. Three discs for each dilution were exposed to HPV inside one MSC and three were exposed to laboratory air. In experiment 2, a control wet sample (virus in suspension) was stored on ice in room air in a separate MSC to determine the titre of the starting dilution. A Clarus™ S HPV suite (Bioquell (UK) Ltd, Andover, Hampshire, UK) was used to decontaminate the MSC, as described by Hall *et al.* (2007). Briefly, the Clarus S was placed inside the MSC, which was sealed using adhesive tape, and the UV light was switched off. The HPV equipment exerts a small negative pressure on the chamber to prevent leakage of HPV during the cycle. Liquid hydrogen peroxide (60 ml, 30% w/w) was converted to HPV inside the cabinet and distributed by means of an internal fan. Once the 45-min HPV exposure was complete, HPV was catalytically converted to oxygen and water vapour. The whole decontamination process took approximately 3 h. The Clarus S does not measure the concentration of HPV inside the chamber. *Geobacillus stearothermophilus* biological indicators (BIs) with a population of $>10^6$ spores on stainless steel discs sealed in individual Tyvek pouches were used to validate the HPV decontamination cycles as standard (Apex Laboratories Inc., Sanford, NC, USA). The BIs were positioned in alternating high and low positions at the four corners of the MSC. Control- and HPV-exposed BIs were cultured in 20 ml Trytipcase Soy Broth (TSB) at 60°C and examined for turbidity over 7 days. Three separate HPV fumigation cycles were run.

Following the HPV cycle, the exposed and unexposed adenovirus samples were transferred to another MSC in which 50 μl of DMEM supplemented with 2% foetal bovine serum, nonessential amino acids and 4 mmol l^{-1} L-glutamine was added to the discs and the virus was triturated 15 times. The virus was then transferred to 450 μl DMEM medium supplemented with 5% foetal bovine serum, nonessential amino acids and 4 mmol l^{-1} L-glutamine and decimal dilutions prepared. The culture medium was removed from the microtitre plate cell monolayers, and eight replicate wells were inoculated with 50 μl of the appropriate dilution of virus. Plates were incubated for 2 h at 37°C in an atmosphere of 5% CO_2. After the virus absorption period, 100 μl DMEM medium supplemented with 5% foetal bovine serum, nonessential amino acids and 4 mmol l^{-1} L-glutamine was added and incubated for 3 days. The plates were sealed, examined using a fluorescence microscope and scored positive for virus replication if they contained cells expressing GFP. It was possible to clearly see a single cell expressing GFP. This detection technique has been shown to be a quick and effective way of detecting viable adenovirus when assessing decontamination procedures (Losert *et al.* 2006). The $TCID_{50}$ of each resuspended virus was estimated using the Karber method (Karber 1931):

$$\begin{aligned}&\text{Negative log of } TCID_{50} \text{ endpoint} =\\&(\text{negative log of the highest virus concentration used})\\&-[(\text{sum of \% infected wells at each dilution}/100)\\&-0\cdot50\times\text{log dilution}]\end{aligned}.$$

All treated *G. stearothermophilus* BIs were inactivated by the process while all the untreated controls grew. No virus was detected in any of the HPV-exposed samples but virus was recovered from all of the untreated samples (Table 1). The untreated virus suspension at the end of the experimental period contained in excess of 10 logs of

Table 1 Negative log of $TCID_{50}$ endpoint for the control untreated 50 μl samples

Sample	Replicate 1 ($TCID_{50}$)	Replicate 2 ($TCID_{50}$)	Replicate 3 ($TCID_{50}$)	Mean ($TCID_{50}$)	Standard deviation ($TCID_{50}$)
Experiment 1, neat	−9·7	−9·6	−8·9	−9·4	0·4
Experiment 1, 10^{-2}	−8·3	−7·9	−8·6	−8·2	0·3
Experiment 2, neat	−7·9	−8·4	−8·4	−8·3	0·3
Experiment 2, 10^{-1}	−6·9	−8·7	−7·2	−7·6	0·9
Experiment 3, neat	−8·2	−8·6	−7·7	−8·1	0·4

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$TCID_{50}/50$ μl volume. One to two logs of virus were lost because of drying, or the recovery method was incomplete when compared with the wet reference sample tested in experiment 2, which had a titre of 10·9 log of $TCID_{50}$. The level of adenovirus inactivation ranged between 7·7 and 9·7 log of $TCID_{50}$ for dried samples (mean 8·6, standard deviation, 0·7).

Adenoviruses are capable of survival when dried onto surfaces for more than 8 weeks (Mahl and Sadler 1975). Various liquid chemical disinfectants are capable of inactivating adenovirus, including halogens, aldehydes, quaternary ammonium compounds, phenolics and alcohols (Sattar *et al.* 1989; Rutala *et al.* 2006). However, chemical disinfectants may not be suitable for large-scale decontamination of biomedical and manufacturing facilities because of practical constraints including reliance on the operator for correct formulation, adequate distribution and contact time, toxic residues and incompatibility with equipment and sensitive electronics (Dettenkofer and Block 2005). One carrier test found that 3% liquid hydrogen peroxide was not effective for the killing of dried adenovirus (Rutala *et al.* 2006). In contrast, we found that HPV generated from 30% hydrogen peroxide was effective for inactivating high-titre recombinant adenovirus.

HPV is delivered as a vapour phase so does not rely on the operator to ensure adequate distribution and contact time. HPV is compatible with sensitive electronics and does not leave toxic residues because it is broken down into water and oxygen (Rutala *et al.* 2008). Therefore, we believe that HPV could be a useful decontamination method in settings where contamination with adenoviruses could result in harmful personnel exposure or product cross-contamination (Sofer 1995, 2003).

Our study is limited by its small scale and the use of only one recombinant adenovirus tested under unsoiled conditions. Future studies should address the virucidal efficacy of HPV under different testing conditions and with different viruses. In summary, HPV was able to inactivate a high titre of dried adenovirus and could be a useful method for the decontamination of enclosures contaminated with adenovirus and other viruses. In response to these findings, we have implemented HPV as a routine decontamination method between manufacturing campaigns in our facility.

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Conflict of Interest Statement

J.A.O. and S.Y. are employed by Bioquell (UK) Ltd. E.B. and L.A. have no conflict of interest.

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