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ORIGINAL ARTICLE

Efficacy of hydrogen peroxide wipes for decontamination of AZD1222 adenovirus COVID-19 vaccine strain on pharmaceutical industry materials

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Significance and Impact of Study: The disinfection procedure with hydrogen peroxide® wipes (HPW) resulted in complete *decontamination* of AZD1222 chimpanzee adenovirus strain in formulated recombinant COVID-19 vaccine ($\geq$7·46 and $\geq$7·49 $\log_{10}$ infectious unit [IFU] ml^{-1} for stainless steel [SS] and low-density-polyethylene [LDP] carriers respectively) and active pharmaceutical ingredient ($\geq$8·79 and $\geq$8·78 $\log_{10}$ IFU ml^{-1} for SS and LDP carriers respectively). HPW can be a good option for disinfection processes in pharmaceutical industry facilities during recombinant COVID-19 vaccine production. This procedure is simple and can be also applied on safety unit cabins and sampling bags made of LDP as well.

Keywords
biopharmaceuticals, pharmaceuticals, quality control, vaccines, viruses.

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Abstract

This study aimed to evaluate the performance of accelerated hydrogen peroxide® wipes (HPW) for *decontamination* of the chimpanzee adenovirus AZD1222 vaccine strain used in the production of recombinant COVID-19 vaccine in a pharmaceutical industry. Two matrices were tested on stainless-steel (SS) and low-density-polyethylene (LDP) surfaces: formulated recombinant COVID-19 vaccine (FCV) and active pharmaceutical ingredient (API). The samples were spiked, dried and the initial inoculum, possible residue effect (RE) and titre reduction after disinfection with HPW were determined. No RE was observed. The disinfection procedure with HPW resulted in complete *decontamination* the of AZD1222 adenovirus strain in FCV ($\geq$7·46 and $\geq$7·49 $\log_{10}$ infectious unit [IFU] ml^{-1} for SS and LDP carriers respectively) and API ($\geq$8·79 and $\geq$8·78 $\log_{10}$ IFU ml^{-1} for SS and LDP carriers respectively). In conclusion, virucidal activity of HPW was satisfactory against the AZD1222 adenovirus strain and can be a good option for disinfection processes of SS and LPD surfaces in pharmaceutical industry facilities during recombinant COVID-19 vaccine production. This procedure is simple and can be also applied on safety unit cabins and sampling bags made of LDP as well.

Introduction

The Brazilian National Immunization Program (NIP) coordinates vaccination at national level and offers vaccines established at the National Vaccination Calendar, which is one of the most extensive in the world (Matos *et al*. 2020). The Immunobiological Technology Institute/Oswaldo Cruz Foundation (Bio-Manguinhos/Fiocruz) produces vaccines to supply the NIP. Due to the pandemic, the large-scale production of the recombinant COVID-19 vaccine (VCD) was necessary to achieve the immunization of the population (Matos *et al*. 2020). The ChAdOx1-S/nCoV-19 recombinant vaccine is a replication-deficient adenoviral vector vaccine that expresses the SARS-CoV-2 spike protein gene (WHO 2021).

During sterile biologicals production, cleaning validation plays an important role in reducing the possibility of product contamination from manufacturing equipment. The cleaning process must be validated to guarantee that residues of infectious materials have been removed from the equipment, so they can be used for several products manufacturing (PDA 2012).

Hydrogen peroxide is an environmentally safe chemical residue free disinfectant that has been used for environmental decontamination in various types of industries, including pharmaceutical, generally using vapour equipment (Gradini *et al.* 2019; Vannier and Chewins 2019; Kindermann *et al.* 2020; Ajorio *et al.* 2021, 2022). Other types of application include the use of soaked hydrogen peroxide® wipes (HPW), which seems an attractive choice for different types of surfaces as part of a preventive risk mitigation concept (Rosenberg *et al.* 2011; Kindermann *et al.* 2020).

Previous studies that evaluated the efficacy of HPW to eliminate pathogenic human viruses from clinical interesting surfaces (e.g. tonometer tips) using disc-based carrier assays presented promising results (Threlkeld *et al.* 1993; Gradini *et al.* 2019; Cutts *et al.* 2020, 2021; Malenovská 2020). However, HPW have not been used yet to investigate the virucidal efficacy in surfaces of equipment and facilities in pharmaceutical industries.

The aim of this study was to evaluate the performance of HPW to *decontaminate* the AZD1222 chimpanzee adenovirus vaccine strain, used in the production of VCD in different matrices and surfaces for application in facilities cleaning validation.

Results and discussion

The results of the samples and reference material (RM) titrations are presented in Table 1. The carrier's disinfection procedure was satisfactory and no visible growth of bacteria was observed in brain–heart infusion broth (BHI) after incubation. No infectious unit (IFU) was observed in the negative controls and the RM presented satisfactory results according to the established criteria. The formulated recombinant COVID-19 vaccine (FCV) and active pharmaceutical ingredient (API) used for spiking, after 1 h in room temperature (20–25°C), contained 7·88 and 9·18 $\log_{10}$ IFU ml^{-1} respectively (Table 1).

After the inoculation and drying, no titre loss was observed for both matrices and surfaces (Table 2). According to standard NF-T-72-281:2014 (L'Association Francaise de Normalisation 2014) difference between inoculum control and residue effect (RE) must be $<0{\cdot}5$ $\log_{10}$ IFU ml^{-1}. In the present study, the differences observed were $\leq 0{\cdot}04$ $\log_{10}$, indicating absence of RE in the test. In disinfected carriers, no IFU was observed in any inoculated dilution, and the calculated titre was $\leq 0{\cdot}42$ $\log_{10}$ IFU ml^{-1}. Therefore, the disinfection procedure resulted in complete *decontamination* of AZD1222 chimpanzee adenovirus strain present in FCV ($\geq 7{\cdot}46$ and $\geq 7{\cdot}49$ $\log_{10}$ IFU ml^{-1} for stainless-steel [SS] and low-density-polyethylene [LDP] carriers respectively) and API ($\geq 8{\cdot}79$ and $\geq 8{\cdot}78$ $\log_{10}$ IFU ml^{-1} for SS and LDP carriers respectively).

Table 1 Titre of AZD1222 chimpanzee adenovirus vaccine strain present in the FCV, API and RM

Sample	Series	Titre (IFU ml^{-1})	Average titre (IFU ml^{-1})	CV (%)	Log_{10} average titre (IFU ml^{-1})
FCV	1	$1{\cdot}35 \times 10^{9}$	$1{\cdot}51 \times 10^{9}$	14·3	7·88
	2	$1{\cdot}66 \times 10^{9}$			
API	1	$2{\cdot}88 \times 10^{10}$	$3{\cdot}00 \times 10^{10}$	5·7	9·18
	2	$3{\cdot}12 \times 10^{10}$			
RM	1	$1{\cdot}68 \times 10^{9}$	$1{\cdot}68 \times 10^{9}$	0	9·23
	2	$1{\cdot}68 \times 10^{9}$			

API: active pharmaceutical ingredient of COVID-19 vaccine; CV, coefficient of variation; FCV, formulated COVID-19 vaccine; IFU: infectious units; RM, reference material.

Table 2 Virucidal efficacy of peroxide wipes against AZD1222 chimpanzee adenovirus vaccine strain present in formulated COVID-19 (recombinant) vaccine and active pharmaceutical ingredient of COVID-19 (recombinant) vaccine dried in stainless-steel (SS) and low-density-polyethylene (LDP) surfaces

Sample	Surface	Carrier	Average titre (IFU ml^{-1})/ carrier	Log_{10} average titre (IFU ml^{-1})/ carrier
FCV	SS	IC	$7{\cdot}60 \times 10^{7}$	7·88
		RE	$6{\cdot}97 \times 10^{7}$	7·84
		DC	$\leq 2{\cdot}63 \times 10^{0}$	≤0·42
	LDP	IC	$8{\cdot}15 \times 10^{7}$	7·91
		RE	$8{\cdot}02 \times 10^{7}$	7·90
		DC	$\leq 2{\cdot}63 \times 10^{0}$	≤0·42
API	SS	IC	$1{\cdot}62 \times 10^{9}$	9·21
		RE	$1{\cdot}65 \times 10^{9}$	9·22
		DC	$\leq 2{\cdot}63 \times 10^{0}$	≤0·42
	LDP	IC	$1{\cdot}60 \times 10^{9}$	9·20
		RE	$1{\cdot}60 \times 10^{9}$	9·20
		DC	$\leq 2{\cdot}63 \times 10^{0}$	≤0·42

API: active pharmaceutical ingredient of COVID-19 vaccine; DC: disinfected carrier; FCV, formulated COVID-19 vaccine; IC: inoculum control; IFU, infectious units; RE: residue effect; SS, stainless steel surface; LDP, low-density-polyethylene surface.

Sattar and Maillard (2013) used the term decontamination to encompass both the physical removal and the inactivation functions of such wipes. In the present study, the ready-to-use commercial HPW showed satisfactory results for decontamination (removal and inactivation) of AZD1222 chimpanzee adenovirus strain in both matrices and surfaces evaluated. However, as wipes without hydrogen peroxide were not included as controls, the contribution of the hydrogen peroxide and the mechanical intervention cannot be measured individually.

Hydrogen peroxide acts against viruses by forming HO free radicals which react with thiol groups in proteins and nucleic acids, thus inhibiting the infection replication process (Finnegan *et al.* 2010). Previous studies reported problems with hydrogen peroxide residue (death of cell monolayer in titrating assay) during the evaluation of liquid (1–30%) and HPV virucidal activity (Ajorio *et al.* 2021, 2022). According to the manufacturer, the concentration of accelerated hydrogen peroxide in Oxivir TB Wipes (OTW) is 0·50–0·55%, which is lower than the used in others studies (Ajorio *et al.* 2021, 2022) and can explain why no RE was observed in the present study.

According to NF T72-281:2014 (L'Association Francaise de Normalisation 2014), a $>4\ \log_{10}$ reduction is recommended for virus decontamination and HPW was sufficient to reach this target ($\geq 7{\cdot}46\ \log_{10}$) for all matrices and surfaces evaluated. The OTW leaflet describes that the product has virucidal activity against Influenza virus H1N1. These results were similar to those of other authors that evaluated the virucidal efficacy of HPW against adenovirus strains and reported complete *decontamination* ($>4{\cdot}83$ to $>8\ \log_{10}$) (Berrie *et al.* 2011; Cutts *et al.* 2021). Cutts *et al.* (2020) observed a reduction of $\sim 5{\cdot}5\ \log_{10}$ after 5 s and $\sim 6{\cdot}4\ \log_{10}$ after 60 s of Ebola virus Makona variant, and complete *decontamination* ($\sim 6{\cdot}2\ \log_{10}$) after 5 s of vesicular stomatitis virus in impregnated 4 × 4 cm wipes with 1:40 solution of accelerated hydrogen peroxide. When applied in SS surfaces, *decontamination* of $\sim 6\ \log_{10}$ was observed for both viruses. In plastic surfaces, such as Goldmann tonometers and pneumotonometer tips, similar results were reported (Threlkeld *et al.* 1993; Malenovská 2020). Threlkeld *et al.* (1993) evaluated the efficacy of 3% HPW over adenovirus 8 and observed no virus recovery with an initial inoculum varying from 1·9 to $3{\cdot}5\ \log_{10}$. Malenovská (2020) used Alphacoronavirus 1 as a surrogate virus to investigate the persistence of coronavirus dried on a plastic carrier. Using wipes saturated with a combination of disinfectant agents, that included 0·5% hydrogen peroxide and 5 min of contact, complete *decontamination* was achieved ($\geq 3{\cdot}5\ \log_{10}$).

In conclusion, HPW showed efficacy for AZD1222 chimpanzee adenovirus vaccine strain *decontamination* in FCV and API matrices, being a promising disinfectant method for surfaces of SS and LPD in production areas and equipment. This procedure is simple and can be applied on safety unit cabins and sampling bags made of LDP as well.

Materials and methods

Virus samples, RM and chimpanzee adenovirus AZD1222 titration

The chimpanzee adenovirus AZD1222 vaccine strain was used for spiking experiments. The vaccine strain was tested in two presentations: (1) FCV and (2) API of VCD. One batch of VCD (lot: MRVCD01/21) was used as RM to validate the assays. Lower and higher confidentiality limits were previously established in a control chart: confidence limits = 9·23–9·32 $\log_{10}$ IFU ml^{-1}. Phosphate-buffered saline (PBS) pH 7·2 (Sigma-Aldrich, Saint Louis, MO) was used as negative control.

The titres of FCV, API, RM and samples were determined using the IFU methodology described by Ajorio *et al.* (2022). Two independent aliquots of undiluted and serial 10-fold dilutions of the samples were prepared in Dulbecco's Modified Eagle Medium (DMEM) (Life Technologies, New York, NY) supplemented with 1% of penicillin–streptomycin (Life Technologies) and 10% of heat-inactivated foetal bovine serum (Life Technologies). Afterwards, inoculation of 0·1 ml into poly-D-lysine-coated 24-well plates containing 0·9 ml of HEK-293 (ATCC® CRL-1573TM) ($2{\cdot}8 \times 10^5$ cells/ml) and incubated at 37°C with 5% CO_2 and ≥85% relative humidity for 47 h was realized. Two non-inoculated wells were used as controls in each microplate. After incubation, the cells were fixed with methanol (J.T. Baker, Chaganas, Republic of Trinidad and Tobago) and then washed with PBS pH 7·2 (Sigma-Aldrich). Afterwards, mouse anti-adenovirus (Abcam, Waltham, MA) was added in each well. After 60 min, the liquid was removed and the plates were washed with PBS. Then, rabbit anti-mouse IgG-HRP antibodies (Abcam) were added in each well. After 60 min, the liquid was removed and the plates were washed with PBS. Then, 1× DAB substrate kit (Thermo Fisher, Rockford, IL) was added in each well. After 10 min, the liquid was removed and the plates were washed with UltraPure DNase/RNase free water (Thermo Fisher). After, 1·0 ml of UltraPure water was added in each well and cell counting was realized using an inverted light microscope (Zeiss, Göttingen, Germany) with a 10× ocular lens with 20 mm of field diameter and a 10× objective lens. The infectious titre in $\log_{10}$ IFU ml^{-1} was calculated using the following equation:

$$\text{Titre}\left(\log_{10}\ \text{IFU ml}^{-1}\right) = (\text{Average stained cell counts} \times \text{number of fields} \times \text{dilution factor})/\text{sample volume}$$

where the number of fields into an ocular lens with 20 mm of field diameter and 10× objective lens = 61, and the sample volume = 0·1 ml.

When no IFU was found in any dilution, the assay detection limit was calculated considering 1 IFU in the total volume of the lowest dilution inoculated.

Carriers' preparation

Two different surface carriers were tested: SS and LDP. They were disinfected according to standard NF-T-72-281:2014 (L'Association Francaise de Normalisation 2014).

To verify the efficacy of disinfection and sterilization procedure, two carries of each type were added to tubes containing 20 ml of BHI (Merck, Darmstadt, Germany) and incubated at 32·5°C for 14 days.

Spiking experiments: RE and HPW virucidal activity evaluation

Two matrices were tested: FCV and API in SS and LDP surface carriers, based on the methodology described on standard NF-T-72-281:2014 (L'Association Francaise de Normalisation 2014). Five carriers of each type were placed in one Petri dish and spiked with 50 µl of PBS. After 1 h, the spiking inoculums were completely dried and the carriers were disinfected with OTW (Diversey, Ontario, Canada) with circling friction movements with pressure for 5 min, as recommended by the manufacturing instructions. Then, each carrier was added to tubes containing 10 ml of supplemented DMEM medium. The tubes were vortexed for 30 s and the contents were transferred to one flask (pool). This pool (~50 ml) containing the OTW residue was aliquoted into four new tubes (10 ml/tube) and further used for the RE evaluation (item 2.4.2). A schematic diagram of residue preparation is shown in Fig. 1.

In a biological safety cabinet, six Petri dishes were opened, and four carriers (two SS and two PBD) were placed into each dish. The carriers of three plates were spiked with 50 µl of FCV and the inoculum were spread with a bacteriological loop (Cralplast, São Paulo, Brazil). The same was made in API samples. After 1 h, the spiking inoculum were completely dried and the carriers of one plate of each matrix were added to tubes containing 10 ml of supplemented DMEM, used for the initial inoculum determination. The carriers of a second plate of each matrix were added to tubes containing 10 ml SS or LDP OTW residue (Fig. 1) for the RE evaluation. The carriers of the remaining plates were submitted to disinfection with OTW with circling friction movements with pressure for 5 min, as recommended by the manufacturing instructions (disinfected carrier). Then, they were added to tubes containing 10 ml of supplemented DMEM. All tubes were vortexed for 30 s and submitted to titration as previously described. A schematic diagram of sample spiking is shown in Fig. 2.

The FCV and API flasks used for spiking were kept inside the biological safety cabinet under the same conditions and two aliquots of each were taken and titrated. One vial of RM was analysed to validate the assay.

The $\log_{10}$ IFU ml^{-1} percent of reduction was calculated by comparing the titre of the undisinfected with the disinfected samples, using the following equation:

$$\%\text{titre reduction}\left(\text{IFU ml}^{-1}\right) = 100-[(\text{average of } \textit{disinfected samples} \times 100)/(\text{average of } \textit{undisinfected samples})].$$

Author contributions

Vinicius Pessanha Rhodes: the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, final approval of the version to be submitted. Ana Carolina Ferreira Ballestê Ajorio: the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, final approval of the version to be submitted.

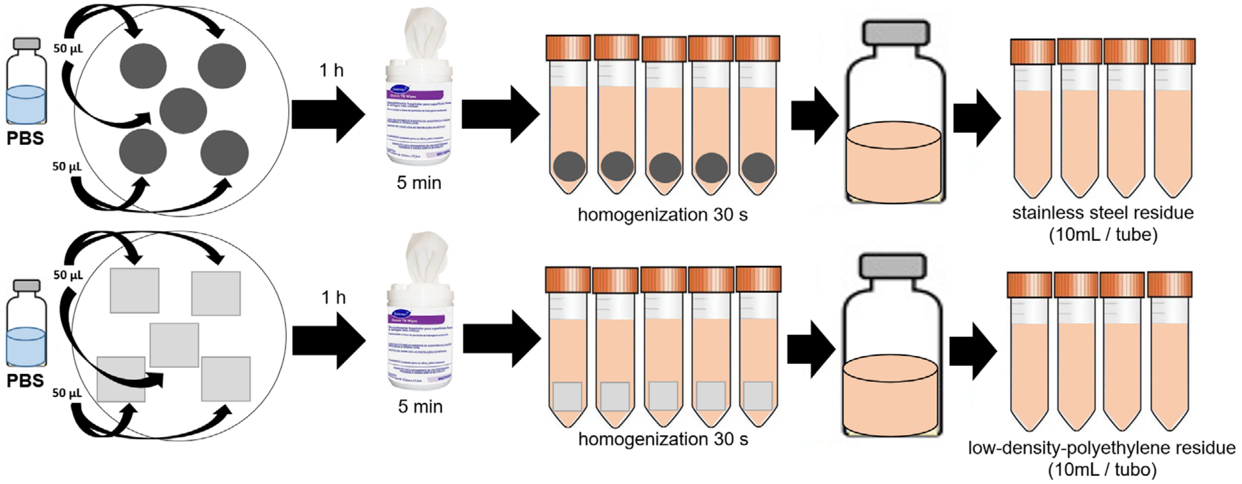

Figure 1 Schematic diagram for preparation of stainless-steel and low-density-polyethylene residue for evaluation of residue effect. PBS: phosphate-buffered saline pH 7·2.

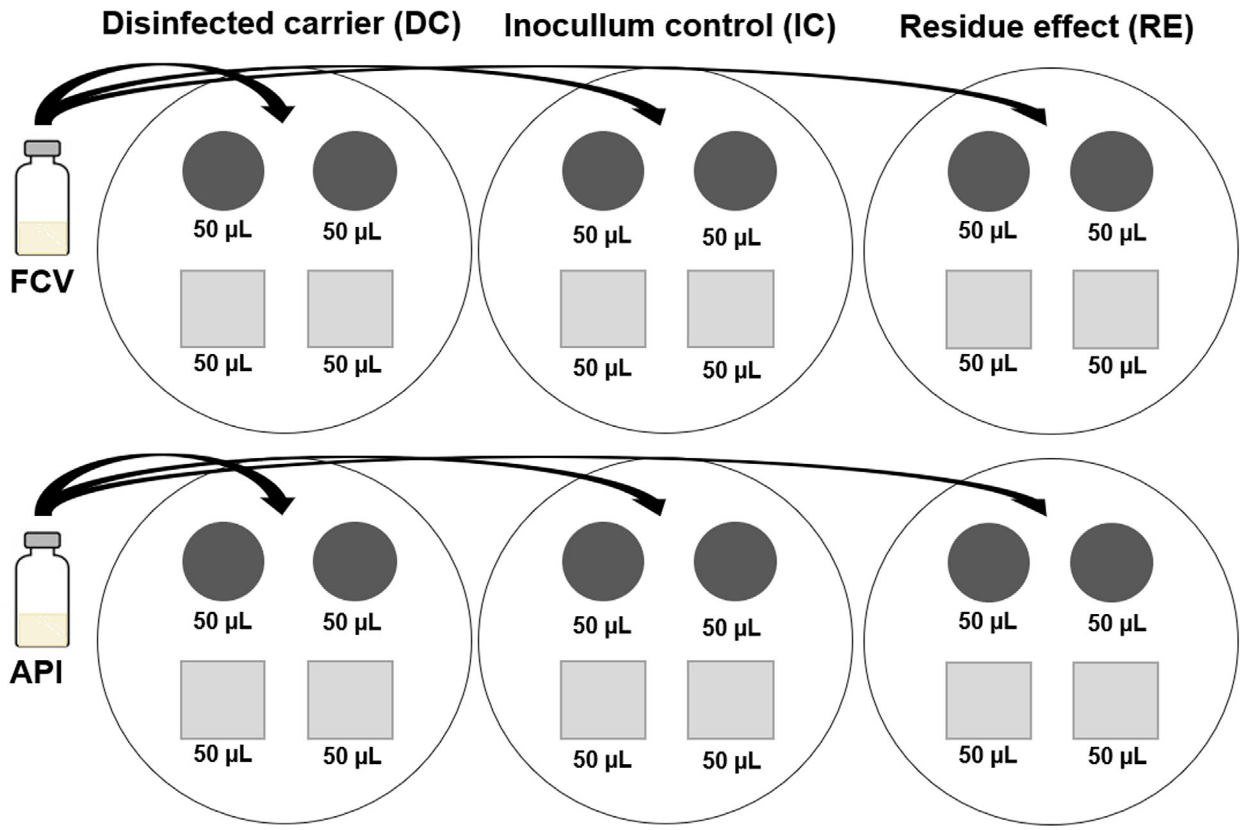

Figure 2 Schematic diagram of sample spiking in the stainless-steel discs (2·0 cm diameter) and low-density-polyethylene (square pieces of 3·0 cm^2) for virucidal evaluation of Oxivir TB Wipes containing hydrogen peroxide. API, active pharmaceutical ingredient; FCV, formulated recombinant COVID-19 vaccine.

Luciana Veloso da Costa: the conception and design of the study, acquisition of data, analysis and interpretation of data, revising it critically for important intellectual content, final approval of the version to be submitted. Anderson Peclat Rodrigues: the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, final approval of the version to be submitted. Vanessa Alvaro Diniz: the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, final approval of the version to be submitted. Rebeca Vitória da Silva Lage: the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, final approval of the version to be submitted. Igor Barbosa da Silva: the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, final approval of the version to be submitted. Marcelo Luiz Lima Brandão: acquisition of data, analysis and interpretation of data, revising it critically for important intellectual content, final approval of the version to be submitted.

Conflict of Interest

No conflict of interest declared.

Data availability statement

The data that supports the findings of this study are available in this article.

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