Letters in Applied Microbiology

Letters in Applied Microbiology ISSN 0266-8254

ORIGINAL ARTICLE

Evaluation of hydrogen peroxide efficacy against AZD1222 chimpanzee adenovirus strain in the recombinant COVID-19 vaccine for application in cleaning validation in a pharmaceutical manufacturing industry

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Significance and Impact of the Study: Hydrogen peroxide vapour showed efficacy for chimpanzee adenovirus AZD1222 strain elimination in two matrixes: formulated recombinant COVID-19 vaccine (≥5·03 $\log_{10}$) and API (≥6·40 $\log_{10}$) and can be used in pharmaceutical industries facilities, and can be a good choice for pharmaceutical industries facilities disinfection during recombinant COVID-19 vaccine production.

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

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2021/1207: received 17 May 2021, revised 14 December 2021 and accepted 14 December 2021

doi:10.1111/lam.13635

Abstract

This study aimed to evaluate the performance of hydrogen peroxide vapour (HPV) to inactivate the chimpanzee adenovirus AZD1222 vaccine strain used in the production of recombinant COVID-19 vaccine for application in cleaning validation in pharmaceutical industries production areas. Two matrixes were tested: formulated recombinant COVID-19 vaccine (FCV) and active pharmaceutical ingredient (API). The samples were dried on stainless steel and exposed to HPV in an isolator. One biological indicator with population $>10^6$ *Geobacillus stearothermophilus* spores was used to validate the HPV decontamination cycle as standard. HPV exposure resulted in complete virus inactivation in FVC (≥5·03 $\log_{10}$) and API (≥6·40 $\log_{10}$), showing HPV efficacy for reducing chimpanzee adenovirus AZD1222 vaccine strain. However, the optimum concentration and contact time will vary depending on the type of application. Future decontamination studies scaling up the process to the recombinant COVID-19 vaccine manufacturing areas are necessary to evaluate if the HPV will have the same or better virucidal effectivity in each specific production area. In conclusion, HPV showed efficacy for reducing AZD1222 chimpanzee adenovirus strain and can be a good choice for pharmaceutical industries facilities disinfection during recombinant COVID-19 vaccine production.

Introduction

Brazil has a universal, comprehensive and free of charge Unified Health System (SUS) that contributes to reducing inequalities in access to health care and outcomes for over three decades (Castro *et al.* 2019). However, Brazil is one of the countries most severity hit by coronavirus disease 2019 (COVID-19). The fast spread of both cases and deaths of COVID-19 in Brazil had distinct patterns in each state, and the SUS has been attending a large number of hospitalizations and deaths (de Andrade *et al.* 2020). This scenario became even worse with the spreading of the variant of concern P1, that is estimate to be 1·4–2·2 times more transmissible and able to evade immunity comparing to previously non-P1 infection (Faria *et al.* 2021). So, efforts to vaccine the largest

Table 1 Virucidal efficacy of exposure to hydrogen peroxide vapour against AZD1222 chimpanzee adenovirus strain present in formulated COVID-19 vaccine and active pharmaceutical ingredient of COVID-19 vaccine dried in stainless steel surface

	Without exposure to HPV*		After exposure to HPV		Reduction (%)	
Samples	IFU ml^{-1}†	log_{10} IFU ml^{-1}	IFU ml^{-1}	log_{10} IFU ml^{-1}	IFU ml^{-1}	log_{10} IFU ml^{-1}
FVC‡ (T0)	$1{\cdot}81 \times 10^9$	9·26	NR§	NR	NR	NR
API¶ (T0)	$4{\cdot}87 \times 10^{10}$	10·69	NR	NR	NR	NR
FVC	$1{\cdot}32 \times 10^8$	8·12	$\leq 1{\cdot}22 \times 10^3$	≤3·09	$1{\cdot}32 \times 10^8$ (≥99·99)	5·03 (≥61·95)
API	$3{\cdot}08 \times 10^9$	9·49	$\leq 1{\cdot}22 \times 10^3$	≤3·09	$3{\cdot}08 \times 10^9$ (≥99·99)	6·40 (≥67·44)

*Hydrogen peroxide vapour.
†Infectious units.
‡Formulated COVID-19 vaccine.
§Not realized.
¶Active pharmaceutical ingredient of COVID-19 vaccine.

number of people in the shortest possible time is crucial to reduce the propagation of P1. The failure to avoid this propagation can also facilitate the emerging of new variants of concern, isolating Brazil as a threat to global health security leading to a complete humanitarian crisis (Castro *et al.* 2021).

The Immunobiological Technology Institute/Oswaldo Cruz Foundation (Bio-Manguinhos/Fiocruz) from Brazil is producing the recombinant COVID-19 vaccine due to a technology transfer with AstraZeneca and Oxford University (FIOCRUZ 2021). The ChAdOx1-S/nCoV-19 [recombinant] vaccine is a replication-deficient adenoviral vector vaccine against COVID-19. The vaccine expresses the SARS-CoV-2 spike protein gene, which instructs the host cells to produce the protein of the S-antigen unique to SARS-CoV-2, allowing the body to generate an immune response and to retain that information in memory immune cells. Efficacy shown in clinical trials in participants who received the full series of vaccine (two doses) irrespective of the interval between the doses was 63·1%, based on a median follow-up of 80 days, but tended to be higher when this interval was longer (WHO 2021).

Bio-Manguinhos/Fiocruz produce vaccines to supply the Immunization National Program of Brazil (INP) from the Ministry of Health (Domingues *et al.* 2020), and due to the severity of the pandemic in Brazil, the large production of COVID-19 vaccine is extremely necessary to achieve the immunization of all population (Castro *et al.* 2021). The production of sterile biological products, as COVID-19 (recombinant) five doses vaccine, is a complex process that requires the implementation of good manufacturing practices (GMP). GMP is the aspect of quality assurance that ensures that the products are consistently produced and controlled based on appropriate quality standards for intended use and as required by the product specification (WHO 2016; Brasil 2019). Cleaning validation plays an important role in reducing the possibility of product contamination from pharmaceutical manufacturing equipment/system. It demonstrates that the cleaning process adequately and consistently removes product residues, process residues and environmental contaminants from the equipment/system, and can be safely used for the manufacture of defined subsequent products (PDA 2012).

Due to the COVID-19 pandemic, Bio-Manguinhos/Fiocruz had to reorganize its production areas to meet the large production scale of COVID-19 for the INP. In this scenario, the validation of cleaning process to eliminate residues of AZD1222 chimpanzee adenovirus strain from the equipments/systems from production areas are necessary in order to guarantee the GMP. To achieve this goal, we evaluated the performance of hydrogen peroxide vapour (HPV) to inactivate the chimpanzee adenovirus AZD1222 vaccine strain used in the production of COVID-19 vaccine in two different matrixes for application in cleaning validation in pharmaceutical industries production areas.

Results and discussion

The results of samples titration are present in Table 1. No IFU were observed in the negative controls and the ACs presented satisfactory results according to established criteria's. The biological indicator (*G. stearothermophilus*) exposed to HPV was inactivated, while the non-exposed used as control had visible growth following the 7 days of incubation.

The FVC and API used for spiking after 7 h in room temperature contained 9·26 and 10·69 log_{10} IFU ml^{-1} respectively (Table 1). After the inoculation and drying in the stainless steel surface the titre loss observed was of 1·14 and 1·20 log_{10} IFU ml^{-1}. However, it was not possible to perform the assay with drying controls for the process (controls that are inoculated and exposed to the

conditions during the HPV cycle, but not HPV itself) so, to calculate the titre loss only associated with the period of HPV cycle could not be realized.

For FVC and API samples exposed to HPV, even kept at 5 ± 3°C for 1 h, it was not possible to use the dilution 10^0 to calculate the titre due to the death of the monolayer, probably associated with the presence of hydrogen peroxide residues in the samples. Excluding this dilution, HPV exposure resulted in complete inactivation of AZD1222 chimpanzee adenovirus strain present in FVC and API, presenting a reduction ≥99·99% of IFU per ml and ≥61·95% of $\log_{10}$ IFU ml^{-1}.

The hydrogen peroxide is a 'no-touch' automated residue-free decontamination technology that removes the reliance on the operator to ensure distribution, contact time and process repeatability and can be scaled up to decontaminate rooms, laboratories and entire buildings (Tuladhar *et al.* 2012; Vannier and Chewins 2019; U.S. FDA 2020). HPV has been used for environmental decontamination in pharmaceuticals industries (Gradini *et al.* 2019; Vannier and Chewins 2019; Kindermann *et al.* 2020), but few studies were realized concerning its application for viruses disinfection in cleaning validation programmes (Kindermann *et al.* 2020) and none using the AZD1222 chimpanzee adenovirus strain. In this study, a validated cycle using HPV was used to disinfect AZD1222 chimpanzee adenovirus strain present in FVC and API of recombinant COVID-19 vaccine in the isolator, that is commonly used for evaluation of HPV efficacy on viruses elimination in small-scale experiments before application in the areas in fact (Tuladhar *et al.* 2012; Otter *et al.* 2013).

Unfortunately, it was not possible to evaluate the IFU in the dilution 10^0 in the samples exposed to HPV due to the death of the monolayer. Even using the strategy of including 1 h aeration step at 5 ± 3°C to wait the conversion of the hydrogen peroxide into water and oxygen (Tuladhar *et al.* 2012) the residual could not be eliminated. It is known that the temperature of 5 ± 3°C was not ideal for the conversion of hydrogen peroxide, and higher temperatures would be better, but this could also lead to viral titre loss. Probably the addition of catalase in the culture medium (Abd-Elghaffar *et al.* 2016) can be a better strategy to be tested to remove the residue of hydrogen peroxide and increase the limit of detection of the methodology applied (Ajorio *et al.* 2021).

Inferring whether the death of the monolayer had not occurred in 10^0 dilution of samples exposed to HPV, probably no IFU would have been found and the viral titre reduction would be ≥6·03 and ≥7·40 $\log_{10}$ IFU ml^{-1} for FVC and API, respectively, representing an increase of the reduction to at least ≥ 71·95%, in $\log_{10}$ IFU ml^{-1}. These results obtained showed that the HPV decontamination process performed was effective to inactivate AZD1222 chimpanzee adenovirus strain. These results were similar to those reported by Tuladhar *et al.* (2012), that described that HPV was able to reduce viability >5 $\log_{10}$ of adenovirus type 5 (reference strain, Hu/adenovirus/type 5/6270/1988/Ethiopia) in the stainless steel surface. Other studies that used different viruses and/or different matrices also reported satisfactory performance of HPV. Disinfection on stainless steel resulted in complete inactivation of poliovirus, rotavirus, murine norovirus, and influenza A virus (Tuladhar *et al.* 2012). HPV effectivity was evaluated against viruses of veterinary interesting, and HPV exposure resulted in complete inactivation of human adenovirus type 1 (hADV-1), representing a reduction of >4·83 $\log_{10}$ (Goyal *et al.* 2014). Vannier and Chewins (2019) reported that HPV reduced 7·5 $\log_{10}$ of foot and mouth disease virus strain obtained from a vaccine manufacturing facility. Kindermann *et al.* (2020) reported complete inactivation of bovine viral diarrhoea virus (>5·2 $\log_{10}$), minute virus of mice (>5·0 $\log_{10}$) and Hepatitis A virus (>4·0 $\log_{10}$) after at least 55 min of HPV exposure. Ajorio *et al.* (2021) reported complete inactivation of yellow fever virus 17DD vaccine strain in formulated yellow fever vaccine (≥4·42 log10 PFU ml^{-1} reduction) and for yellow fever viral suspension—active pharmaceutical ingredient (API) samples 3·17 log10 PFU ml^{-1} (64·3%) reduction after HPV exposure in isolator.

According to NF T72-281:2014 standard for surface disinfection processes by air, a >4-$\log_{10}$ reduction is recommend for viruses decontamination (L'Association Francaise de Normalisation, 2014). In the present study, the exposure to HPV was sufficient to reach this target (≥5·03 $\log_{10}$) for both matrixes evaluated. Chewins (2018) reported that standard test methods based on the application of a liquid disinfectant to a surface or to a suspension are not suitable for determining the efficacy of automated airborne disinfection systems. When evaluating hydrogen peroxide decontamination systems, organisms of local concern and relevant matrixes challenges should be used to show in-use efficacy. With the increasing use of automated whole-room disinfection devices, the novel testing standard needs to be designed with relevant reduction targets to facilitate the comparison between studies (Ali *et al.* 2018; Ajorio *et al.* 2021).

In conclusion, hydrogen peroxide showed efficacy for reducing AZD1222 chimpanzee adenovirus strain. Our study is limited by its small scale, and the fact that was not possible to evaluate the IFU in the dilution 10^0, which decreased the limit of detection of the methodology. Future decontamination studies scaling up the process to the recombinant COVID-19 vaccine manufacturing areas are necessary to evaluate if the HPV

will have the same or better virucidal effectivity and the improvement of the methodology to eliminate hydrogen peroxide residue in the samples. As in production areas of biological industries the HPV decontamination is realized after a cleaning step with water and neutral detergents (PDA 2012; WHO 2016), the HPV effective could be even higher due to the reduction of organic matter after this cleaning step.

Materials and methods

Viruses samples and reference material

The chimpanzee adenovirus AZD1222 vaccine strain was used for spiking experiments. The vaccine strain was tested in two presentations: (i) formulated COVID-19 vaccine (FCV) titre $1{\cdot}81 \times 10^{9}$ IFU ml^{-1} lot: 213VCD003Z; (ii) API of COVID-19 vaccine titre $4{\cdot}87 \times 10^{10}$ IFU ml^{-1} lot: 0000023181 (cryovault 1/12). One assay control (AC) lot: C00443-00003, developed and supplied by AstraZeneca, was used as reference material (RM) to validate the assays. This AC possess inferior and superior confidentiality limits previously established. Water for injection (WFI, Bio-Manguinhos/Fiocruz, Rio de Janeiro, Brazil) was used as the negative control.

Chimpanzee adenovirus AZD1222 virus titration

The titres of FCV, API, RM and samples were determined using the infectious unit (IFU) methodology described by Oxford University and AstraZeneca and implemented and validated in Bio-Manguinhos/Fiocruz.

Two independent aliquots of undiluted and serial 10-fold dilutions (10^{0} to 10^{-6}) of the samples were prepared in DMEM medium (Life Technologies, New York, NY) supplemented with 1% of penicillin-streptomycin (Life Technologies), and 10·0% of heat-inactivated fetal bovine serum (Life Technologies) followed by inoculation of 0·1 ml into poly-D-lysine coated 24-well plates (using two wells/dilution) containing 0·9 ml of HEK-293 (ATCC® CRL-1573TM) ($2{\cdot}8 \times 10^{5}$ cells ml^{-1}) and incubated at 37 ± 2°C with 5 ± 1% CO_2 and ≥85% relative humidity for 47 ± 1 h. Two non-inoculated wells were used as controls in each microplate. Complementary negative controls included the inoculation of 0·1 ml of WFI and supplemented DMEM medium used in the assays into two wells.

After incubation, the medium was removed and 1·0 ml of methanol (J.T. Baker, Trinidad and Tobago) were added and the plates were maintained at ambient temperature for 30 ± 5 min. The methanol was removed and the wells were washed three times with 1·0 ml of phosphate buffered saline pH 7·2 (PBS, Sigma-Aldrich, Saint Louis, MO). Afterward, 250 μl of mouse anti-adenovirus (Abcam, Boston, MA), diluted 1:10 000 in 1% bovine serum albumin (BSA, Sigma, New York, NY)/PBS solution (Sigma), were added in each well and the plates were maintained at ambient temperature for 60 ± 10 min on an orbital rocker set at 50–100 rev min^{-1}. The liquid was then removed and the plates were washed three times with 1·0 ml of PBS. Then, 250 μl of rabbit anti-mouse IgG-HRP antibody (Abcam), diluted 1 : 20 000 in 1% BSA/PBS solution, were added in each well and the plates were maintained at room temperature for 60 ± 10 min on an orbital rocker set at 50–100 rev min^{-1}. Th liquid was removed and the plateswere washed three times with 1·0 ml of PBS. Then, 250 μl of 1× DAB substrate kit (Thermo Fisher, Rockford, IL) were added in each well and plates were maintained at ambient temperature for 10 ± 2 min on an orbital rocker set at 50–100 rev min^{-1}. Afterward, the liquid was removed and the plates were washed three times with 1·0 ml of UltraPure DNase/RNase free water (Thermo Fisher, New York, NY). After the final water wash step, 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 fields that presented 8–124 brown stained cells/field were selected and stained cells were counted into five fields (two fields from area A, two from area B and one from area C) as illustrated in Fig. 1.

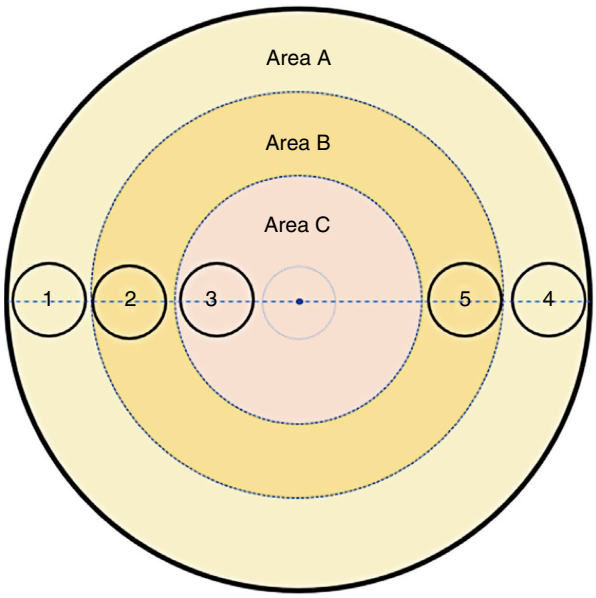

Figure 1 Schematic of field counting areas. Area A—Width of approximately one field around edge of well; Area B—Area of approximately one field away from edge of well; Area C—Middle of well.

Table 2 System and sample suitability criteria's applied to valid the determination of the titre of AZD1222 virus

	Parameter	Criteria
System suitability	Assay control cell count is within the range at one or more effective dilution level	8–124 brown stained cells/field
	Calculate the infectious titre of assay control	50% ≤Expected titre ≤200%
	Each negative control field	≤5 stained cells
Sample suitability	Average stained cell count of samples is within the range at one or more effective dilution level	8–124 brown stained cells/field
	% coefficient of variance titre among sample duplicates at each effective dilution level	≤30%

The infectious titre in IFU/mL was calculated using the following equation:

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

NOTE: the number of fields into an ocular lens with 20 mm of field diameter = 61; and the sample volume = 0·1 ml.

The system and sample suitability criteria's to valid the assay are presented in Table 2.

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

Spiking experiments

The spiking experiments was performed based on the previously described by Ajorio *et al.* (2021). HPV disinfection was performed using duplicate samples of FVC and API. The stainless steel surface AISI316 of a 3-gloves isolator (La Calhène, Vélizy Cedex, France) was divided into 12 areas (A–L) of 25 cm² and were spiking with 0·5 ml of: A, D, G and J with WFI (negative control); B, C, E and F with FVC; and H, I, K and L with API (Fig. 2). The isolator was kept in circulation mode and the FVC and API flasks used for spiking were kept inside the isolator under the same conditions. After 6 h, the spiking inoculums were completely dry and the residue of areas A, B, C, G, H and I were collected with sterilized swabs (TX715 Alpha Sampling Swab Texwipe, Kernersville, NC) and added to tubes containing 5·0 ml of supplemented DMEM medium. The solutions were sterilized using 0·22 μm filter (Merck Millipore, Carrigtwohill, Ireland), transferred to new tubes, vortexed for 10 s and titrated. The FVC and API vials used for spiking were withdraw of the isolator and one aliquot of each

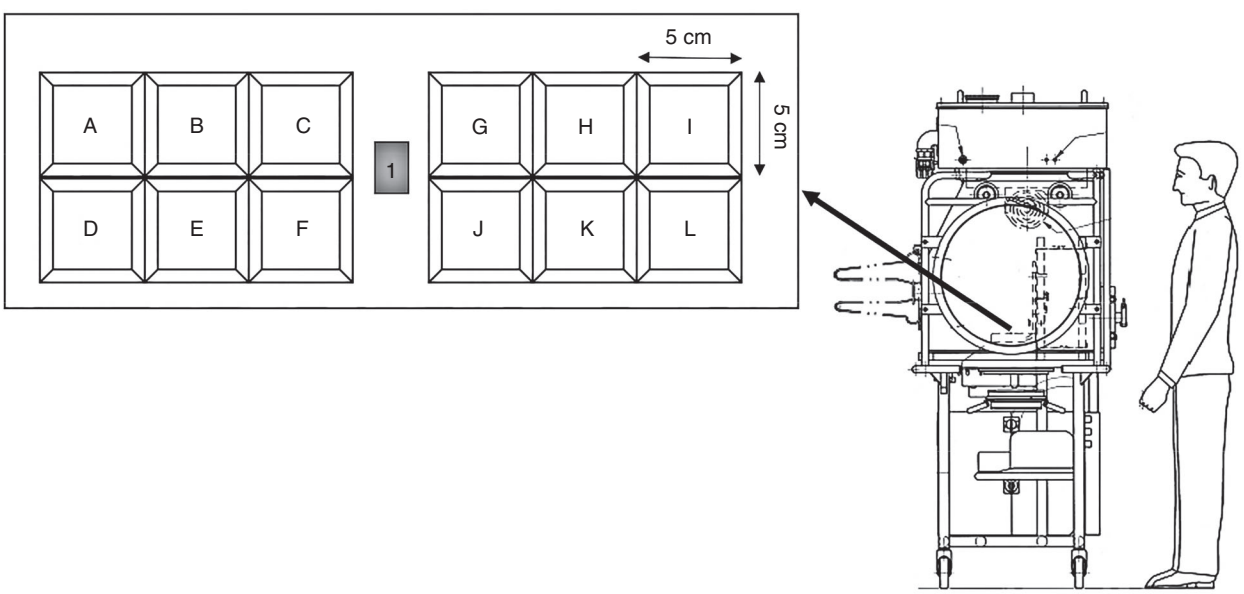

Figure 2 Schematic diagram of sample spiking in the stainless steel AISI316 surface of 3-gloves isolator (La Calhène, Vélizy Cedex, France). Samples locations are indicated by letters: A, D, G and J with water for injection (negative control); B, C, E and F with formulated recombinant COVID-19 vaccine; and H, I, K and L with active pharmaceutical ingredient of recombinant COVID-19 vaccine. Grey square indicate with number 1 was the position of *Geobacillus stearothermophilus* biological indicator.

was taken and titrated. One vial of AC was analysed in this moment to validate the assay. One biological indicator with population $>10^6$ *Geobacillus stearothermophilus* spores (Bioquell, Andover, UK) was positioned in the center of the isolator (Fig. 2) and used to validate the HPV decontamination cycle as standard. A Clarus C HPV generator (Bioquell, Andover, UK) was used to produce HPV from $30\%_{(w/w)}$ HPL. A validated cycle previously established was used: gassing injection of 2·5 g min^{-1} for 20 min, followed by gassing dwell of 0·5 g min^{-1} for 30 min, achieving 482·4 ppm; aeration time of 5 h. Disinfection was performed with relative humidity of 63·2–63·8% and room temperature was ranged from 18·2 to 20·3°C inside the laboratory.

After the decontamination cycle (~5 h), the residue of the areas D, E, F, J, K and L were collected, as previously described, and titrated. In order to eliminate possible residual hydrogen peroxide, which causes total monolayer death, the tubes containing the collected samples were kept at 5 ± 3°C for 1 h. After, the samples were titrated. One vial of AC was analysed in this moment to validate the assay.

Control and HPV-exposed biological indicator were grown in 20 ml Trytipcase Soy Broth (Merck, Darmstadt, Germany) at 60°C and examined for turbidity over 7 days.

The percentage of reduction in IFU/mL was calculated by comparing the titre of the unexposed with the HPV exposed samples, using the following equation:

$$\%\ \text{of titre reduction (IFU ml}^{-1}) = 100 - [(\text{average of HPV exposed samples} \times 100)/(\text{average of unexposed samples})]$$

Acknowledgements

The authors thank Bio-Manguinhos/Fiocruz.

Conflict of Interest

None declared.

Author contributions

Ana Carolina Ferreira Ballestê Ajorio contributed to conceptualization; data curation; formal analysis; investigation; methodology; validation; visualization; roles/writing—original draft. Vinicius Pessanha Rhodes contributed to conceptualization; data curation; formal analysis; investigation; methodology; validation; visualization; roles/writing—original draft. Anderson Peclat Rodrigues contributed to data curation; investigation; validation; writing—review & editing. Vanessa Alvaro Diniz contributed to conceptualization; supervision; writing—review & editing. Josiane Machado Vieira Mattoso contributed to conceptualization; supervision; writing—review & editing. Igor Barbosa da Silva contributed to conceptualization; supervision; writing—review & editing. Débora Michele Morone D'Aiuto contributed to conceptualization; supervision; writing—review and editing. Marcelo Luiz Lima Brandão contributed to conceptualization; data curation; formal analysis; supervision; validation; visualization; roles/writing—original draft.

References

Abd-Elghaffar, A.A., Ali, A.E., Boseila, A.A. and Amin, M.A. (2016) Inactivation of rabies virus by hydrogen peroxide. *Vaccine* **34**, 798–802.

Ajorio, A.C.F.B., Rhodes, V.P., Rodrigues, A.P., Moreira, F.M., Diniz, V.A., Mattoso, J.M.V., Silva, I.B., Guedes Junior, D.S. *et al.* (2021) Evaluation of hydrogen peroxide virucidal efficacy against yellow fever virus 17DD vaccine strain for application in a vaccine manufacturing industry. *J Pharm Biomed Anal* **204**, 114264.

Ali, S., Yui, S., Muzslay, M. and Wilson, A.P.R. (2018) Test parameters for efficacy evaluations of aerial hydrogen peroxide decontamination systems. *J Hosp Infect* **98**, 438–439.

de Andrade, C.L.T., Pereira, C.C.A., Martins, M., Lima, S.M.L. and Portela, M.C. (2020) COVID-19 hospitalizations in Brazil's Unified Health System (SUS). *PLoS One* **15**, e0243126.

Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Farmacopeia Brasileira. 6ª edição - produtos biológicos. *Diário Oficial da República Federativa do Brasil*. Brasília, 12 de agosto de 2019.

Castro, M.C., Kim, S., Barberia, L., Ribeiro, A.F., Gurzenda, S., Ribeiro, K.B., Abbott, E., Blossom, J. *et al.* (2021). Spatiotemporal pattern of COVID-19 spread in Brazil. *Science*. Apr 14:eabh1558. Epub ahead of print.

Castro, M.C., Massuda, A., Almeida, G., Menezes-Filho, N.A., Andrade, M.V., de Souza Noronha, K.V.M., Rocha, R., Macinko, J. *et al.* (2019) Brazil's unified health system: the first 30 years and prospects for the future. *Lancet* **394**, 345–356.

Chewins, J. (2018) Standard test method for automated airborne disinfection systems. *J Hosp Infect* **100**, e67.

Domingues, C.M.A.S., Maranhão, A.G.K., Teixeira, A.M., Fantinato, F.F.S. and Domingues, R.A.S. (2020) The Brazilian National Immunization Program: 46 years of achievements and challenges. *Cad. Saude Publica* **36**(Suppl 2), e00222919.

Faria, N.R., Mellan, T.A., Whittaker, C., Claro, I.M., Candido, D.D.S., Mishra, S., Crispim, M.A.E., Sales, F.C. *et al.* (2021) Genomics and epidemiology of a novel SARS-CoV-2 lineage in Manaus, Brazil. *medRxiv* [Preprint] **3**:2021.02.26.21252554.

Fundação Oswaldo Cruz – FIOCRUZ (2021) Instituto de Tecnologia em Imunobiológicos – Bio-Manguinhos/ Fiocruz. Rio De Janeiro - RJ 32 p. Balanço da Gestão de Bio-Manguinhos de 2017 a 2021.

Goyal, S.M., Chander, Y., Yezli, S. and Otter, J.A. (2014) Evaluating the virucidal efficacy of hydrogen peroxide vapour. *J Hosp Infect* **86**, 255–259.

Gradini, R., Chen, F., Tan, R. and Newlin, L. (2019) A summary on cutting edge advancements in sterilization and cleaning technologies in medical, food, and drug industries, and its applicability to spacecraft hardware. *Life Sci Space Res (Amst)* **23**, 31–49.

Kindermann, J., Karbiener, M., Leydold, S.M., Knotzer, S., Modrof, J. and Kreil, T.R. (2020) Virus disinfection for biotechnology applications: different effectiveness on surface versus in suspension. *Biologicals* **64**, 1–9.

L'Association Francaise de Normalisation. *NF T 72–281. Procedes de disinfection des surfaces par voie aerienneedetermination de l'activite bactericide, fongicide, levuricide, mycobactericide, tuberculocide, sporicide et virucide incluant les bacteriophages*. Paris: AFNOR.

Otter, J.A., Yezli, S., Perl, T.M., Barbut, F. and French, G.L. (2013) The role of 'no-touch' automated room disinfection systems in infection prevention and control. *J Hosp Infect* **83**, 1–13.

Parenteral Drug Association – PDA (2012) *Technical report no. 29: points to consider for cleaning validation*. Bethesda, MD: PDA—Parental Drug Association. ISBN: 978-0-939459-48-3

Tuladhar, E., Terpstra, P., Koopmans, M. and Duizer, E. (2012) Virucidal efficacy of hydrogen peroxide vapour disinfection. *J Hosp Infect* **80**, 110–115.

United States of America Food and Drug Administration – U.S. FDA. (2020). Code of federal regulations CITE: 21CFR184.1366, Sec. 184.1366 hydrogen peroxide.

Vannier, M. and Chewins, J. (2019) Hydrogen peroxide vapour is an effective replacement for Formaldehyde in a BSL4 Foot and mouth disease vaccine manufacturing facility. *Lett Appl Microbiol* **69**, 237–245.

World Health Organization – WHO (2016) *WHO good manufacturing practices for biological products*. WHO Team: Health Product Policy and Standards, Technical Standards and Specifications. WHO Technical Report Series, vol. 999. Geneva: World Health Organization. Annex 2, 2016.

World Health Organization – WHO. (2021) COVID-19 Vaccine Explainer. COVID-19 Vaccine ChAdOx1-S [recombinant] - Developed by Oxford University and AstraZeneca. https://www.who.int/publications/m/item/chadox1-s-recombinant-covid-19-vaccine (accessed 16 April 2021).