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Evaluation of hydrogen peroxide virucidal efficacy against yellow fever virus 17DD vaccine strain for application in a vaccine manufacturing industry

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ABSTRACT

The aim of this study was to evaluate the inactivation performance of hydrogen peroxide to the yellow fever virus 17DD vaccine strain, used for the production of attenuated yellow fever vaccine, in two matrixes: formulated yellow fever vaccine (FYV) and yellow fever viral suspension - active pharmaceutical ingredient (API). The samples were dried on stainless steel and exposed to hydrogen peroxide liquid (HPL) at concentrations of 30, 10, 3 and 1% for 20 and 60 min; and to hydrogen peroxide vapour (HPV) in an isolator. The exposure to HPL 30 and 10 %, within 20 min, reduced the virus titre at least 3.85 $\log_{10}$ PFU/mL (74.8 %). During 60 min of exposure, the HPL 30, 10 and 3% reduced the virus titre by at least 3.18 $\log_{10}$ PFU/mL (62.6 %). HPV exposure resulted in complete virus inactivation in FYV ($\geq$ 4.42 $\log_{10}$ PFU/mL reduction) and for API samples 3.17 $\log_{10}$ PFU/mL (64.3 %) reduction. Hydrogen peroxide showed to be a promising disinfectant for elimination of yellow fever virus. However, the optimum concentration and contact time will vary depending on the type of application, and as such may complement individual risk assessments of biological production processes.

1. Introduction

The Immunobiological Technology Institute / Oswaldo Cruz Foundation (Bio-Manguinhos/Fiocruz) located in Brazil is internationally recognized as a manufacturer of the yellow fever vaccine (YFV) [1,2]. The attenuated YFV is prepared from the seed lot of yellow fever virus 17DD vaccine strain grown in chicken embryos, according to the standards established by the World Health Organization (WHO) [1,3]. Bio-Manguinhos/Fiocruz produce vaccines to supply the Immunization Program of Brazilian Ministry of Health and also to World Health System. In 2019, the Institute produced 27,779,000 doses of YFV and 743,700 doses were exported: 1300 to Antigua, Curaçao and Cayman Islands, through Pan American Health Organization, 692,400 to the Democratic Republic of Congo, through United Nations International Children's Emergency Fund, and 50,000 doses to India by WHO [2].

The production of sterile biological products, as YFV, is a complex process that requires the implementation of Good Manufacturing Practices (GMP). GMP is the aspect of quality assurance that ensures that medicinal products are consistently produced and controlled based on appropriate quality standards for intended use and as required by the product specification (WHO, 2016). Cleaning validation plays an important role in reducing the possibility of product contamination from biopharmaceutical manufacturing equipment/system. It demonstrates that the cleaning process adequately and consistently removes product residues, process residues and environmental contaminants from the equip-

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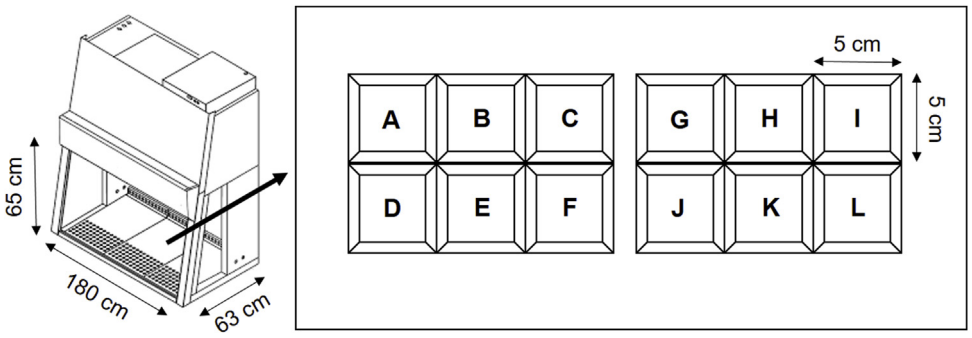

Fig. 1. Schematic diagram of sample spiking in the stainless steel surface of a Class II A1 biosafety cabinet. Samples locations are indicated by letters: A and G with water for injection (negative control); and B, C, D, E, F, H, I, J, K, and L with formulated yellow fever vaccine. (For interpretation of the references to colour in the Figure, the reader is referred to the web version of this article).

ment/system, so that this equipment/system can be safely used for the manufacture of defined subsequent products [4].

Viral contaminations in cell culture-based biotech manufacturing can result in substantial plant downtimes, financial losses, and also in delay in delivery of life-saving drugs or vaccines to patients [5]. For an effective risk mitigation strategy precautionary measures including disinfection treatments for process equipment and facility surfaces should be implemented and validated [4,6]. The capability of disinfectants to inactivate viruses is critical for any biological manufacturing and should be assessed from risk mitigation to (re)establishing post-virus-reduction status of manufacturing areas [5]. However, knowledge about the activity of disinfectants against viruses is still limited, particularly with respect to differences in efficacy between application to surfaces, suspensions, and even in the air [7]. The information on virucidal efficacy of disinfectants does typically not cover all target viruses relevant for the biologicals industry, as such as e.g. the yellow fever virus.

The use of disc-based carrier assays for standardized virus disinfection studies is well established for public health purposes [8–10]. However, such systems have not been widely used to investigate the virucidal efficacy of disinfection procedures in biotechnological process equipment and facility surfaces [7,11].

Hydrogen peroxide is considered an environmentally safe chemical according to United States of America Food and Drug Administration due to its decomposition to oxygen and water [12]. Hydrogen peroxide vapour (HPV) 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 [9,11,13,14]. It has been used for environmental decontamination in various types industries, including foods and pharmaceuticals industries [7,11,13,15].

Studies conducted to evaluate the efficacy of HPV technology to eliminate pathogenic human viruses from hospital environmental presented promising results [8–10,15]. However, few studies were realized concerning the application of HPV for virus disinfection in cleaning validation programs in biotechnology industries [7]. When dried on inanimate surfaces, viruses are less susceptible to disinfection than when hydrated in suspension [11,16]. The resistance to disinfection is also increased in the presence of soil, blood, stool and others organic matrixes [9,16,17]. So, it is important to study the hydrogen peroxide virucidal effect in the presence of interfering substances that can be present in the different intermediary products that are manufactured during the biological production chain [9,10].

The aim of this study was to evaluate the performance of hydrogen peroxide to inactivate the yellow fever virus 17DD vaccine strain, used in the production of attenuated YFV in Bio-Manguinhos/Fiocruz, in different matrixes for application in cleaning validation in the facilities.

2. Materials and methods

2.1. Viruses samples and reference material

Yellow fever virus 17DD vaccine strain was used for inoculation experiments. The vaccine strain was tested in two presentations: 1) formulated yellow fever vaccine (FYV) titre 6.25 $\log_{10}$ PFU/mL; 2) yellow fever viral suspension - active pharmaceutical ingredient (API) titre 6.94 $\log_{10}$ PFU/mL. One batch of YFV produced by Bio-Manguinhos/Fiocruz (lot: VR004- BIOMANGUINHOS) titre 5.21 $\log_{10}$ PFU/mL was used as reference material (RM) to validate the assays. This RM possess inferior (5.05 $\log_{10}$ PFU/mL) and superior (5.36 $\log_{10}$ PFU/mL) confidentiality limits previously established in a control chart. Water for injection (WFI, Bio-Manguinhos/Fiocruz, Rio de Janeiro, Brazil) was used as negative control.

2.2. Yellow fever virus titration

The titres of FYV, API, RM and samples were determined using the plaque forming unit (PFU) methodology described in Brazilian Pharmacopeia [18].

Undiluted and serial 4-fold dilutions of the samples were prepared in 199 medium supplemented with 0.1 % of gentamicin sulphate, 0.1 % of B amphotericin, 5.0 % of sodium bicarbonate, and 5.0 % of inactivated fetal bovine serum (Gibco, New York, USA) followed by inoculation of 0.2 mL into 6-well plates (using three wells/dilution) containing 3.0 mL of Vero ATCC® CCL-81TM (3×10^5 cells/mL) and incubated at 37 ± 1 °C with 5 ± 2% CO_2 for 1 h. For negative control, WFI used in the assays and 199 medium were inoculated into six wells. The inoculum was aspirated and 3.0 mL of carboxymethylcellulose medium 3 % (Sigma, São Paulo, Brazil) were added and the plates were incubated at 37 ± 1 °C with 5 ± 2% CO_2 for 7 days. The plates were fixed with 2% formaldehyde solution (Merck, Darmstadt, Germany) and stained with 1% violet crystal (Merck, Darmstadt, Germany). The inoculum containing PFU ranging from 10 to 60 were selected and counted with a negatoscope (Konex, São Paulo, Brazil) and the $\log_{10}$ PFU/mL was calculated using the following equation:

$$\text{Titre}(\log_{10}\text{PFU/mL}) = \log_{10}(\text{averageofthePFUcountintothethree wellsx5xdilutionfactor})$$

When no PFU was found in any dilution, the assay detection limit was calculated considering 1 PFU in the total volume of lowest dilution inoculated that presented intact monolayer.

2.3. Inoculation experiments

2.3.1. Hydrogen peroxide liquid exposure

HPL disinfection was performed in one experiment using FYV as inoculum and exposure time of 20 and 60 min were tested. A stainless steel surface of a Class II A1 biosafety cabinet (Kendro Laboratory Products, Type HS18, Herasafe™, Hanau, Germany) was divided into 12 areas (A–L) of 25 cm^2 and were spiked with 0.5 mL of: A and G with WFI (negative control); and B, C, D, E, F, H, I, J, K, and L with FYV (Fig. 1). The FYV used for inoculation was kept inside the biosafety cabinet under the same conditions. After 180 min, the inoculums were completely dry and the residue of areas A, B, G and H were collected with sterilized swabs (TX715 Alpha Sampling Swab Texwipe, Kernersville, USA) and added to tubes containing 5.0 mL of 199 medium supplemented as previously described (item 2.2). The solutions were sterilized using 0.22 μm filter (Merck Millipore, Carrigtwohill, Ireland), transferred to new tubes, vortexed for 10 s and titrated as previously described (item 2.2). Two vials of RM were analyzed in this moment to validate the assay.

Hydrogen peroxide 30 % (Merck, Darmstadt, Germany) and dilutions in WFI in the concentrations of 10, 3 and 1% were prepared in the moment of the assay. One mL of each specific hydrogen peroxide solutions was added as following: area C and I - 30 %, area D and J – 10 %, area E and K - 3%, and area F and L- 1% (Fig. 1). After 20 min the residue of the areas C, D, E and F were collected and titrated to determine the amount of viable viruses. Two vials of RM were analyzed in this moment to validate the assay. After 60 min the residue of the areas I, J, K and L were collected and titrated to determine the amount of viable viruses. Two vials of RM were analyzed in this moment to validate the assay. The log_{10} PFU/mL virus reduction was calculated by comparing the titres of the exposed samples with each HPL solution and the unexposed samples.

2.3.2. Hydrogen peroxide vapour exposure

HPV disinfection was performed using duplicate samples of formulated YFV 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 cm^2 and were added 0.5 mL of: A, D, G and J with WFI (negative control); B, C, E and F with FYV; and H, I, K and L with API (Fig. 2). The isolator was kept in circulation mode (similar as a biosafety cabinet) and the FYV and API used for inoculation were kept inside the isolator under the same conditions. After 3 h, the inoculums were completely dry and the residue of areas A, B, C, G, H and I were collected and titrated (item 2.2). The FYV and API used for inoculation were withdraw of the isolator and one aliquot of each was taken and titrated (item 2.2). The vials were kept closed in room temperature, but outside the isolator, for further analysis. Two vials of RM were analyzed 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 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 for 20 min, followed by gassing dwell of 0.5 g/min 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.

After the decontamination cycle (~11 h), the residue of the areas D, E, F, J, K and L were collected and titrated (item 2.2). The FYV and API used for inoculation and kept in room temperature outside the isolator were also titrated (item 2.2). Two vials of RM were analyzed 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 difference in log_{10} PFU/mL between the FYV and API after three and 11 h was calculated and the value was discounted in the titre of the respectively unexposed sample. The percentage of reduction in log_{10} PFU/mL was calculated by comparing the titre of the unexposed with the HPV exposed samples, using the following equation:

$$\%\text{ofviralreduction}(\log_{10}\text{PFU/mL}) = 100 - [(\text{averageofHPVexposed samplesx100})/(\text{averageofunexposedsamples} - \text{difference betweenthesampleafter3and11hinroomtemperature})]$$

3. Results

3.1. Hydrogen peroxide liquid exposure

The results of virus inactivation using HPL are summarize in Table 1. It was not possible to calculate the titre of the samples exposed to HPL 30 and 10 % for 20 min and 30, 10 and 3% for 60 min due to the death of the monolayer, probably associated to the presence of hydrogen peroxide residues in the samples. No cytopathic effect or PFU were observed in the negative controls and the RMs presented satisfactory results according to the control chart.

The FYV used for inoculation after 180 min in room temperature contained 5.95 log_{10} PFU/0.5 mL, indicating that the loss of viruses because of drying was very low (0.80 log_{10} PFU). The exposure to HPL 30 % and 10 %, within 20 min, reduced the virus titre at least 3.85 log_{10} PFU / mL (74.8 %). This value is probably higher, since it was not possible to observe the plaques on the lower dilution due to the monolayer death. Exposure to VPL 3% for 20 min led to a reduction of 2.02 log_{10} PFU / mL (39.2 %) of the viral titre. During 60 min of exposure, the HPL 30 %, 10 % and 3% reduced the virus titre by at least 3.18 log_{10} PFU/mL (62.6 %) but this value is also probably higher. The exposure to HPL 1% for 20 and 60 min led to decrease of 0.90 log_{10} PFU/mL (17.5 %) and 0.93 log_{10} PFU/mL (18.3 %) in the viral titre respectively.

3.2. Hydrogen peroxide vapour exposure

The results of virus inactivation using HPV inside the isolator are summarize in Table 2. No cytopathic effect or PFU were observed in the negative controls and the RMs presented satisfactory results according to the control chart. 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.

No significant difference was observed in the FYV samples in room temperature between 3 and 11 h, and no adjustment was necessary to be applied. It was not possible to use the dilution 10° to calculate the titre of the FYV samples exposed to HPV due to the death of the monolayer, probably associated to the presence of hydrogen peroxide residues in the samples. Excluding this dilution, HPV exposure resulted in complete inactivation of yellow fever virus 17DD vaccine strain present in FYV presenting a reduction $\geq$ 4.42 log_{10} PFU/mL. For API samples, the difference between 3 h (6.94 log_{10} PFU/mL) and 11 h (6.75 log_{10} PFU/mL) was 019 log_{10} PFU/mL and an adjustment in the titre of the unexposed API samples was applied, resulting in virucidal effect presenting 3.17 log_{10} PFU/mL (64.3 %) against yellow fever virus in API.

4. Discussion

The use of solutions of hydrogen peroxide for surface disinfection in biotechnology industries is common [7,15]. In the present

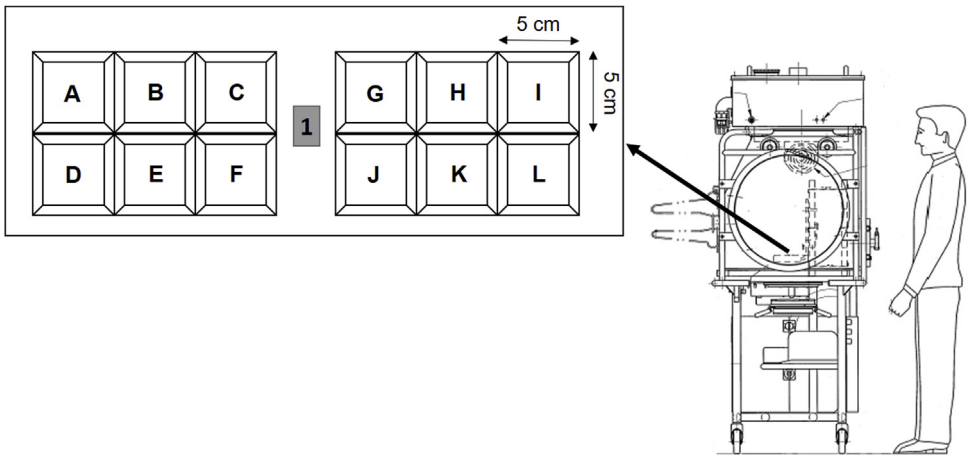

Fig. 2. Schematic diagram of sample spiking in the stainless steel AISI316 surface of 3-gloves isolator (La Calhène, Vélizy Cedex, France). Samples locations are indicated by letters: A, D, G and J with water for injection (negative control); B, C, E and F with formulated yellow fever vaccine; and H, I, K and L with active pharmaceutical ingredient. Gray square indicate with number 1 was the position of *Geobacillus stearothermophilus* biological indicator (Bioquell, Andover, UK). (For interpretation of the references to colour in the Figure, the reader is referred to the web version of this article).

Table 1
Virucidal efficacy of exposure for 20 and 60 min of 30, 10, 3 and 1% hydrogen peroxide liquid solution against yellow fever virus 17DD vaccine strain dried in stainless steel surface.

Time of exposure	HPL	Dilution / n.º of PFU								Titre (log_{10} PFU/mL)
		10°	1:4	1:16	1:64	1:256	1:1,024	1:4,096	1:16,834	
20 min	NE	>60	>60	>60	>60	>60	23-30-29	0	0	5.15
	30 %	*	*	*	*	*	0	NR	NR	>2.63
	10 %	*	*	*	*	0	0	NR	NR	>2.03
	3 %	*	*	17–15-19	0	0	0	NR	NR	3.13
	1 %	*	*	>60	51-53-57	15-15-13	0	NR	NR	4.25
60 min	NE	>60	>60	>60	>60	>60	25-26-20	0	0	5.08
	30 %	*	*	*	*	*	0	NR	NR	>2.63
	10 %	*	*	*	*	0	0	NR	NR	>2.03
	3 %	*	*	*	0	0	0	NR	NR	>4.36
	1 %	*	*	>60	45-44-42	10-12-12	0	NR	NR	4.15

HPL - Hydrogen peroxide liquid; PFU – Plaque-forming unit; NE – Not exposed; * - The plaques could not be counted due to the monolayer death; NR – Not realized; NC – Not calculated.

Table 2
Virucidal efficacy of exposure to hydrogen peroxide vapour against yellow fever virus 17DD vaccine strain dried in stainless steel surface.

Samples	Dilution / n.º of PFU									Titre (log_{10} PFU/mL)
	10°	1:4	1:16	1:64	1:256	1:1,024	1:4,096	1:16,384	1:65,536	1:262,144	
Without exposure to hydrogen peroxide vapour											
FYV sample 1	NR	NR	>60	>60	>60	32–31-31	<10	<10	NR	NR	5.21
FYV Sample 2	NR	NR	>60	>60	>60	36–32-33	<10	<10	NR	NR	5.24
API Sample 1	NR	NR	NR	NR	>60	24–20-20	<10	<10	<10	<10	5.04
API Sample 2	NR	NR	NR	NR	>60	28–29-34	<10	<10	<10	<10	5.19
After exposure to hydrogen peroxide vapour											
FYV sample 1	*	0	0	0	0	0	0	0	NR	NR	<0.82
FYV Sample 2	*	0	0	0	0	0	0	0	NR	NR	<0.82
API Sample 1	2–2-2	4–5-4	0	0	0	0	0	0	0	0	1.94
API Sample 2	3–3-1	4–6-7	0	0	0	0	0	0	0	0	2.05

PFU – Plaque-forming unit; FYV - formulated yellow fever vaccine; NR – Not realized; API - active pharmaceutical ingredient (yellow fever viral suspension) - * - The plaques could not be counted due to the monolayer death.

study, the efficacy of four concentrations of HPL ranging from 1 to 30 % to eliminate yellow fever virus 17DD vaccine strain in contact times of 20 and 60 min was evaluated. Even thought it was not possible to determine the titre in higher concentrations of HPL due to presence of hydrogen peroxide residues that interfering with the titration assay, it was observed that 20 min of exposure at low concentrations (1 and 3 %) was not sufficient to the complete inactivation of the viruses. But using 60 min of contact time, HPL at concentrations ≥3 % seems to be sufficient to complete inactivation of the viruses dried in the stainless steel surface (Table 1).

Other studies evaluated the virucidal performance of HPL but using different viruses and matrixes, and varying the HPL concentrations, contact time, and viruses inoculums, which difficult the comparison of results. Rutala et al. [19] observed that 3% hydrogen peroxide did not demonstrate at least a 3 log_{10} reduction in titrers of adenovirus with 5 min contact time. Kindermann et al. [7] reported that treatment with the 0.80 % hydrogen peroxide and 0.06 % peroxyacetic acid led to complete inactivation of bovine viral diarrhea virus, minute virus of mice and enteric respiratory orphan virus type III in suspension and in stainless steel metal disc. According

to Kindermann et al. [7], the use of hydrogen peroxide based disinfectants seem to be an attractive choice for wipe-down different types of surfaces as part of a preventive risk mitigation concept. The results obtained in the present study indicate that HPL seems to be an alternative for yellow fever virus disinfection in surfaces. However, to assure effectiveness sufficiently, long and continuous wetting of the respective surfaces may be required.

The isolator is commonly used for evaluation of HPV efficacy on viruses' elimination [9,14]. In the preset study, a validate cycle using HPV was used to disinfect yellow fever virus in different matrices. *Geobacillus stearothermophilus* spores (6 log) was used as control since it is used to validate HPV based medical device sterilization equipment and aseptic processing environments, where drugs intended to be directly introduced into a patient are manufactured [20].

Unfortunately, it was not possible to evaluate the PFU in the dilution 10° in FYV samples due to the death of the monolayer (Table 2). Moreover, uncommon results were found in API samples exposed to VPH, because more PFU were found in the dilution 1:4 than 10°, which is in disagreement with logic of the titration assay since the number of PFU must be consistent with the inoculated dilutions (Table 2). This result may be associated with the fact that in dilution 10°, the concentration of residual hydrogen peroxide was higher and may have affected the monolayer (similar to observed in the FYV) in a way that inhibited viral infection/replication, and consequently the formation of a greater number of PFU, but not in a way to kill the monolayer. Thus, using the 1:4 dilution to perform the titre calculation of API samples exposed to HPV, the reduction was 3.17 $\log_{10}$ PFU/mL (64.3 %). This reduction percentage was lower when compared to the FYV, and may be associated with the differences between the composition of the API and FYV.

Strategies to remove residual hydrogen peroxide have already been described, as the addition of catalase in the culture medium [6] or including an 1 h aeration step to wait the conversion of the hydrogen peroxide into water and oxygen [9]. However, the effect of adding these steps in viral titre must evaluated before the implementation. Inferring that the death of the monolayer would not have occurred in 10° dilution of FYV samples exposed to HPV, probably no PFU would be found and the viral titre reduction would be $\geq$ 5.01 $\log_{10}$ PFU/mL ($\geq$ 96.0 %). These results suggest that the HPV decontamination process performed seems to be effective to inactivate yellow fever virus 17DD vaccine strain in FYV. According to NF T72-281:2014 standard for surface disinfection processes by air, a >4-log reduction is recommend for viruses decontamination [21]. In the present study, the exposure to HPV was sufficient to reach this target for YFV but not for API. However, some researchers 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 [22]. According to Ali et al. [23], when evaluating hydrogen peroxide decontamination systems, organisms of local concern and relevant matrixes challenges should be used to show in-use efficacy. With increasing use of automated whole-room disinfection devices, novel testing standard needs to be designed with relevant reduction targets to facilitate the comparison between studies [23].

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 [24]. So, if the matrix that the viruses are present possess organic matter, the hydrogen peroxide reacts and reduces its efficacy [17]. In the present study, yellow fever virus 17DD vaccine strain presented in API showed greater resistance to HPV exposure compared to FYV. API contains a great amount of organic matter, since it is prepared with chicken embryos [1,3]. During the preparation of the FYV, the API is used as one of the inputs and it is diluted approximately 10 times. This can explain the virucidal effect reduction of HPV due to the interaction with API organic components. Another point is that catalase is present in the peroxisome organelle in chicken embryos cells [25] and, during YFV manufacturing, the infected embryos are macerated and catalase is released in bulk [1]. The higher concentration of catalase in API probably cause greater degradation of hydrogen peroxide and, consequently, decreased the virucidal effectiveness of HPV. Pottage et al. [17] reported that HPV was effective against MS2 bacteriophage, but extended decontamination time was necessary when house blood was added in the matrix due to the presence of catalase. As the HPV decontamination process performed in the present study was not sufficient to completely eliminate the viruses in the API, more studies are necessary, as extending the HPV contact time of the cycle and/or add a pre-cleaning step to remove organic matter in the API.

The virucidal performance of HPV using others viruses in different matrixes/surfaces has been already described but none using yellow fever virus. Otter and Budde-Niekiel [13] demonstrated that the ability of HPV to achieve >6 $\log_{10}$ reduction in two lactococcal phages after 50 min of exposure in a flexible film isolator. Berrie et al. [8] observed an >8 $\log_{10}$ reduction of adenovirus after 45 min exposed to HPV in a biosafety cabinet. Tuladhar et al. [9] reported that HPV disinfection on stainless steel, gauze carriers, and framing panel carriers resulted in complete inactivation of poliovirus, rotavirus, adenovirus and murine norovirus (>4 $\log_{10}$); and influenza A virus (>2 $\log_{10}$). HPV was virucidal (>3,8 log) against feline calicivirus, adenovirus, transmissible gastroenteritis coronavirus of pigs, swine influenza virus, and avian influenza virus [10]. HPV was effective in complete inactivation of bovine viral diarrhea 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 exposure [7].

Vannier and Chewins [11] reported that HPV reduced 7.5 $\log_{10}$ of foot and mouth disease virus strain obtained from a vaccine manufacturing facility but the viral inactivation was not repeatable, and associated this variation due to occlusion of viral particles by the dried support matrix. These results were similar to the obtained in the present study, and confirm the recommendations of HPV technology manufacturers that surfaces should be cleaned, and visibly clean, prior to decontamination [10,11]. In the production areas of biological industries, this step is generally realized with water and neutral detergents ([4] [26];). So, it is recommend the inclusion of a cleaning step in the YFV manufacturing areas before HPV decontamination in order to reduce organic matter and increase the virucidal effect of HPV.

In conclusion, hydrogen peroxide showed to be a promising disinfectant for elimination of yellow fever viruses. Our study is limited by its small scale, and future decontamination studies scaling up the process to the YFV manufacturing areas are necessary to evaluate if the HPV will have the same or better virucidal effectivity for yellow fever viruses elimination in the different intermediary and final products that are manufactured in each specific area. Hydrogen peroxide showed to be a promising disinfectant for elimination of yellow fever virus 17DD vaccine strain. However, the optimum concentration and contact time will vary depending on the type of application, and as such may complement individual risk assessments of biological production processes.

Author contributions

We are informing the individual contribution of each author to this paper:

Ana Carolina Ferreira Ballestê Ajorio - Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Roles/Writing - original draft.

Vinícius Pessanha Rhodes - Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Roles/Writing - original draft.

Anderson Peclat Rodrigues - Data curation; Investigation; Validation; Writing - review & editing.

Filipe Mercês Moreira - Data curation; Investigation; Validation; Writing - review & editing.

Vanessa Alvaro Diniz - Conceptualization; Supervision; Writing - review & editing.

Josiane Machado Vieira Mattoso – Conceptualization; Supervision; Writing - review & editing.

Igor Barbosa da Silva – Conceptualization; Supervision; Writing - review & editing.

Daniel da Silva Guedes Junior – Conceptualization; Supervision; Writing - review & editing.

Marcelo Luiz Lima Brandão - Conceptualization; Data curation; Formal analysis; Supervision; Validation; Visualization; Roles/Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] T.P. Monath, Yellow fever vaccine, Expert Rev. Vaccines 4 (4) (2005) 553–574, http://dx.doi.org/10.1586/14760584.4.4.553.

[2] Fundação Oswaldo Cruz – FIOCRUZ, Relatório de gestão do exercício de 2019, in: Coordenação-Geral De Planejamento Estratégico – Cogeplan/Fiocruz Rio De Janeiro – RJ, 2019, pp. 138 (Accessed 7 January 2021) https://portal.fiocruz.br/sites/portal.fiocruz.br/files/documentos/20200826_rg2019_atualizado_com_correcoes_finais.pdf.

[3] R.S. Marchevsky, M. da Luz Leal, A. Homma, E.S. Coutinho, L.A. Camacho, A.V. Jabor, R. Galler, M.S. Freire, Molecular and phenotypic analysis of a working seed lot of yellow fever virus 17DD vaccine strain produced from the secondary seed lot 102/84 with an additional passage in chicken embryos, Biologicals 34 (3) (2006) 191–197, http://dx.doi.org/10.1016/j.biologicals.2005.09.005.

[4] Parenteral Drug Association – PDA, Technical Report No. 29: Points to Consider for Cleaning Validation, PDA. Parental Drug Association, 2012, ISBN: 978-0-939459-48-3.

[5] A.S. Rosenberg, B. Cherney, K. Brorson, K. Clouse, S. Kozlowski, P. Hughes, R. Friedman, Risk mitigation strategies for viral contamination of biotechnology products: consideration of best practices, PDA J. Pharm. Sci. Technol. 65 (6) (2011) 563–567, http://dx.doi.org/10.5731/pdajpst.2011.00820.

[6] A.A. Abd-Elghaffar, A.E. Ali, A.A. Boseila, M.A. Amin, Inactivation of rabies virus by hydrogen peroxide, Vaccine 34 (6) (2016) 798–802, http://dx.doi.org/10.1016/j.vaccine.2015.12.041.

[7] J. Kindermann, M. Karbiener, S.M. Leydold, S. Knotzer, J. Modrof, T.R. Kreil, Virus disinfection for biotechnology applications: different effectiveness on surface versus in suspension, Biologicals 64 (2020) 1–9, http://dx.doi.org/10.1016/j.biologicals.2020.02.002.

[8] E. Berrie, L. Andrews, S. Yezli, J.A. Otter, Hydrogen peroxide vapour (HPV) inactivation of adenovirus, Lett. Appl. Microbiol. 52 (5) (2011) 555–558, http://dx.doi.org/10.1111/j.1472-765X.2011.03033.x.

[9] E. Tuladhar, P. Terpstra, M. Koopmans, E. Duizer, Virucidal efficacy of hydrogen peroxide vapour disinfection, J. Hosp. Infect. 80 (2) (2012) 110–115, http://dx.doi.org/10.1016/j.jhin.2011.10.012.

[10] S.M. Goyal, Y. Chander, S. Yezli, J.A. Otter, Evaluating the virucidal efficacy of hydrogen peroxide vapour, J. Hosp. Infect. 86 (4) (2014) 255–259, http://dx.doi.org/10.1016/j.jhin.2014.02.003.

[11] M. Vannier, J. Chewins, Hydrogen peroxide vapour is an effective replacement for Formaldehyde in a BSL4 Foot and mouth disease vaccine manufacturing facility, Lett. Appl. Microbiol. 69 (4) (2019) 237–245, http://dx.doi.org/10.1111/lam.13203.

[12] United States of America Food and Drug Administration - U.S. FDA, Code of Federal Regulations CITE:21CFR184.1366, Sec. 184.1366 Hydrogen Peroxide, 2020 (Accessed 7 January 2021) https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=184.1366.

[13] J.A. Otter, A. Budde-Niekiel, Hydrogen peroxide vapor: a novel method for the environmental control of lactococcal bacteriophages, J. Food Prot. 72 (2) (2009) 412–414, http://dx.doi.org/10.4315/0362-028x-72.2.412.

[14] J.A. Otter, S. Yezli, T.M. Perl, F. Barbut, G.L. French, The role of' no-touch' automated room disinfection systems in infection prevention and control, J. Hosp. Infect. 83 (1) (2013) 1–13, http://dx.doi.org/10.1016/j.jhin.2012.10.002.

[15] R. Gradini, F. Chen, R. Tan, L. Newlin, 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 (2019) 31–49, http://dx.doi.org/10.1016/j.lssr.2019.05.002.

[16] F.G. Terpstra, A.E. van den Blink, L.M. Bos, A.G. Boots, F.H. Brinkhuis, E. Gijsen, Y. van Remmerden, H. Schuitemaker, A.B. van' t Wout, Resistance of surface-dried virus to common disinfection procedures, J. Hosp. Infect. 66 (4) (2007) 332–338, http://dx.doi.org/10.1016/j.jhin.2007.05.005.

[17] T. Pottage, C. Richardson, S. Parks, J.T. Walker, A.M. Bennett, Evaluation of hydrogen peroxide gaseous disinfection systems to decontaminate viruses, J. Hosp. Infect. 74 (1) (2010) 55–61, http://dx.doi.org/10.1016/j.jhin.2009.08.020.

[18] 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.

[19] W.A. Rutala, J.E. Peacock, M.F. Gergen, M.D. Sobsey, D.J. Weber, Efficacy of hospital germicides against adenovirus 8, a common cause of epidemic keratoconjunctivitis in health care facilities, Antimicrob. Agents Chemother. 50 (4) (2006) 1419–1424, http://dx.doi.org/10.1128/AAC.50.4.1419-1424.2006.

[20] International Standards Organization, 13408-6:2021 Aseptic Processing of Healthcare Products - Part 6: Isolator Systems, ISO, Geneva, 2021.

[21] 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, AFNOR, Paris, 2014.

[22] J. Chewins, Standard test method for automated airborne disinfection systems, J. Hosp. Infect. 100 (3) (2018) e67, http://dx.doi.org/10.1016/j.jhin.2018.05.006.

[23] S. Ali, S. Yui, M. Muzslay, A.P.R. Wilson, Test parameters for efficacy evaluations of aerial hydrogen peroxide decontamination systems, J. Hosp. Infect. 98 (4) (2018) 438–439, http://dx.doi.org/10.1016/j.jhin.2017.09.011.

[24] M. Finnegan, E. Linley, S.P. Denyer, G. McDonnell, C. Simons, J.Y. Maillard, Mode of action of hydrogen peroxide and other oxidizing agents: differences between liquid and gas forms, J. Antimicrob. Chemother. 65 (10) (2010) 2108–2115, http://dx.doi.org/10.1093/jac/dkq308.

[25] K.M.M. Saleh, A.H. Tarkhan, M.B. Al-Zghoul, Embryonic thermal manipulation affects the antioxidant response to post-hatch thermal exposure in broiler chickens, Animals (Basel). 10 (1) (2020) 126, http://dx.doi.org/10.3390/ani10010126.

[26] World Health Organization – WHO, WHO good manufacturing practices for biological products, in: WHO Team: Health Product Policy and Standards, Technical Standards and Specifications. WHO Technical Report Series, No. 999, Annex 2, 2016, World Health Organization, Geneva, 2016, 2016.