Evaluating the Virucidal Efficacy of Hydrogen Peroxide against SARS-CoV-2 on Different Types of Fabrics

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Article

Keywords:

Posted Date: August 17th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1944446/v1

Abstract

SARS-CoV-2 has been shown to remain infectious on surfaces from hours to days depending on the surface type and environmental factors. The presence of virus on the cloth surface may pose a risk of disease transmission during use and handling. This study aimed to investigate the virucidal effect of hydrogen peroxide against SARS-CoV-2 virus on several types of fabrics. The SARS- CoV-2 virus at a concentration similar to viral load from a throat swab of a positive COVID patient was used to test the virucidal effect of hydrogen peroxide at different concentrations and different time points on different types of fabrics using VERO E6 cell culture. This study showed that the SARS-Cov2 soaked on different types of fabrics was killed within 30 s of exposure to hydrogen peroxide. Hydrogen peroxide showed a potent virucidal effect on SARS CoV-2 on different types of fabrics.

Introduction

Coronavirus disease 2019 (COVID-19) is a disease caused by SARS-CoV-2 which started in December 2019 and now has become a pandemic that affected almost all countries in the world. The virus is transmitted through respiratory droplets from an infected patient during coughing, sneezing, or talking. The transmission of the virus can also occur through touching contaminated surfaces followed by touching the eyes, mouth, and nose. The SARS-CoV-2 virus can survive on materials such as plastics, cupboards, and metal for up to 72 hours which aided the transmission further [1]. On clothes and wood, the SARS-CoV-2 can survive up to 48 hours [2].

The transmission of COVID 19 can be prevented through hand washing, social distancing, wearing a mask, and using a disinfectant. An enveloped virus including SARS-CoV-2 is sensitive to the majority of disinfectants. Disinfectant is a chemical used to eliminate pathogenic organisms. There are many types of disinfectants including alcohol, chlorine and chlorine compounds, formaldehyde, glutaraldehyde, ortho-phthalaldehyde, hydrogen peroxide, iodophors, peracetic acid, phenolics, and quaternary ammonium compounds [3]. Most of these disinfectants are toxic to humans and used for inanimate objects except for hydrogen peroxide.

Hydrogen peroxide is less toxic to humans if used at a lower concentration. Analysis of 22 studies revealed that human coronavirus such as SARS-CoV and MERS-CoV can be efficiently inactivated by 70% ethanol, 0.5% hydrogen peroxide, or 0.1% sodium hypochlorite within one minute [4]. Hydrogen peroxide is a high-level disinfectant that can also be used as an antiseptic. Hydrogen peroxide vapour has shown a virucidal effect on SARS-CoV surrogates, avian influenza virus, and swine influenza virus [5].

To date, there is no study available on the effectiveness of disinfectants in eliminating pathogens from body surfaces and clothes. There is also no study to date, looking into the effect of hydrogen peroxide on the SARS-CoV-2 virus on cloth material. Therefore we would like to conduct a virucidal study to investigate the effect of hydrogen peroxide on the SARS-CoV-2 virus on several types of clothes.

Material And Methods

Cells and viruses

The SARS-CoV-2 used was isolated, propagated and maintained in Vero E6 cells at Tropical Infectious Diseases Research & Education Centre (TIDREC), University of Malaya. The Vero E6 cells were cultured in DMEM (Gibco, Grand Island, NY, USA) supplemented with 10% FBS. The cells were maintained at 37°C with 5% CO_2. Virus titers were determined by microtitration using the Vero E6 cells and expressed in $TCID_{50}$/mL. When cytopathic effects (CPE) were evident under the microscope, the supernatant was harvested, clarified by centrifugation and stored at -80ºC until needed.

Cytotoxicity assay

A cytotoxicity assay was performed to determine the concentration at which the test product was non-cytotoxic to the host cells. Briefly, different dilutions of H_2O_2 (3% and 10x dilution) were added to a confluent monolayer of the Vero E6 cells cultured in a 96 well plate. The plate was then incubated for 72 hours and crystal violet dye was used to check for cell viability.

Virucidal effects of H_2O_2 against infected fabrics with SARS-CoV2

Testing for H_2O_2 against infected fabrics was done by exposing the fabrics and their respective control to SARS-CoV-2 inoculum according to the ISO18184-2019 standard protocol. The fabrics were exposed to 100 µl of SARS-CoV-2 suspension with a virus titer of $5x10^5$ and washed using 800 µl of 3% H_2O_2 30s and 60s contact time. After 30s and 60s of contact time, H_2O_2 activity was neutralized by immediate serial dilution in DMEM supplemented with 2% fetal bovine serum. The mixture was then added to Vero E6 cells maintained in tissue culture plates and the formation of virus cytopathic effects was monitored daily. A mixture of paraformaldehyde and crystal violet was used to fix and stain the infected cells. The virus titers were determined using the Spearman-Karber method and expressed as a tissue culture infectious dose of 50% ($TCID_{50}$/ml). The virucidal activity was determined by the difference of the logarithmic titer of the virus control minus the logarithmic titer of the test virus (Δ log10 $TCID_{50}$/ml). A reduction in virus titer of ≥ 4 log10 (corresponding to inactivation of ≥ 99.99%) was necessary for claiming virucidal activity of the product.

Results

Cytotoxicity effect of H_2O_2

Different dilutions of medium consisting of H_2O_2 were added to Vero cells for 72 hours. Crystal violet dye was used to check for cell viability. H_2O_2 showed cytotoxicity effect at 3%, 1.5%, 0.75% concentration on VERO E6 cell line.

Virucidal effects of H_2O_2 against infected fabrics with SARS-CoV2

H_2O_2 was tested against infected fabrics by exposing the fabrics to 100 µl of SARS-CoV-2 inoculum at one contact time. The fabrics were disinfected by washing the fabrics with 3% H_2O_2 and further diluted to neutralize the H_2O_2 activity. The $TCID_{50}$ method was used to determine the virucidal activity of H_2O_2. Results obtained suggested ≥ 4 log10 reductions in virus titers was observed in all infected fabrics after 30s contact time (Table 1). This finding suggests that the H_2O_2 (3%) killed 99.99% SARS-CoV-2.

Table 1
Virucidal effects of 3% H_2O_2 against infected fabrics with SARS-CoV2

Fabrics Samples	**Log10 Reduction in viral titers compared to control**	
	Contact time	
	30s	60s
1. Cotton	≥4.00	≥4.00
2. Knit cotton	≥4.00	≥4.00
3. Polyester	≥4.00	≥4.00
4. Jersey/Lycra	≥4.00	≥4.00
5. Satin	≥4.00	≥4.00
6. Linen	≥4.00	≥4.00

Discussion

Hydrogen peroxide produced a hydroxyl free radical which can destroy lipid, DNA and other cellular materials of microorganisms. SARS-CoV-2 is an enveloped virus that consist of a lipid layer which may be killed through disruption of the envelope layer through oxidisation by hydrogen peroxide. This study compared the effectiveness of hydrogen peroxide against SARS-CoV-2 virus on different types of fabrics at a clinically suggested concentration of 3% and contact times of 30s and 60s. The result of this study have shown that 3% hydrogen peroxide is very potent in killing 99.9% SARS-CoV-2 within 30s and it is no difference between the types of fabric used.

Apart from having potent antimicrobial properties, hydrogen peroxide at a concentration of 3% or less is safe. In terms of application, hydrogen peroxide is suitable to be used as a disinfectant for fabrics material such as cloth face masks and clothes. It can also be used to disinfect shared equipment that is difficult to be washed for example sofa in the waiting room, curtains and soft toys in nurseries. A study by Jenni Virtanen et al showed that the SARS-CoV-2 virus can survive on cotton and polyester fabric for less than a day [6]. Our study showed that hydrogen peroxide had a similar virucidal effect on cotton, polyester and other types of fabrics. Even though the survival of the virus is less than a day it still poses a great risk of contamination and transmission of COVID-19 in public places. It is important to disinfect the

clothes as soon after coming back from public places using 3% hydrogen peroxide which is safer for the user and environment.

Compared to other disinfectants commonly used for fabric such as chlorine, hydrogen peroxide is a better option to be used as a disinfectant. Hypochlorite is commonly used as a disinfectant and is easily available in local stores. Nevertheless, it bleaches fabrics and causes irritation of skin or mucous membrane, aside from the potential adverse effects of chlorine odour on persons who are sensitive to it, such as asthmatics [7]. Meanwhile, 60 to 95% alcohol is also widely used as hand sanitizer and disinfectant. However, it is flammable and also prolonged used may cause skin irritation and dryness. On the other hand, quartenary ammonium compound (QAC) such as 0.2% benzalkonium has also been used widely as disinfectants. However, QAC has been demonstrated to have an impact on cleaning materials. The use of cotton towels to wipe surfaces sprayed with QAC disinfectants, for example, has been found to deactivate QACs, reducing the disinfection efficacy of these compounds [8].

Disinfectants should be applied with a cloth or wipe that has been soaked in the disinfectant as suggested by the WHO [9]. Routine disinfectant spraying or fogging on environmental surfaces has been demonstrated to be ineffective in eliminating contaminants outside of direct spray zones [10].

Conclusion

Hydrogen peroxide with a concentration of 3% was able to kill 99.99% of SARS-CoV-2 virus on different types of fabrics in less than a minute. Based on our study 3% of hydrogen peroxide can be used as a disinfectant to curb the spread of COVID-19 on fabrics.

Declarations

Funding

This study was supported in part by a grant from Universiti Sains Islam Malaysia COVID-19 Fund (PPPI/COVID19_0120/FPSK/051000/15420) and the Ministry of Education, Malaysia for niche area research under the Higher Institution Centre of Excellence (HICoE) program (Project MO002-2019).

Author contributions

P.H and V.T. performed the viral inhibition studies and drafted the methodology section, S.A.B provide advice on laboratory methods and reviewed the final draft, W.S.W.S contributed to the idea, planning and conceptualization of the study, N.A.M and M.D.M.R drafted the manuscript, M.S.M.A supervised the whole project and reviewed the final draft.

Data Availability Statement

All relevant data are within the manuscript.

Competing interests

The authors declare no competing interests.

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