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The effectiveness of chlorine dioxide gas in portable personal disinfectants to inhibit bacterial growth

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ABSTRACT

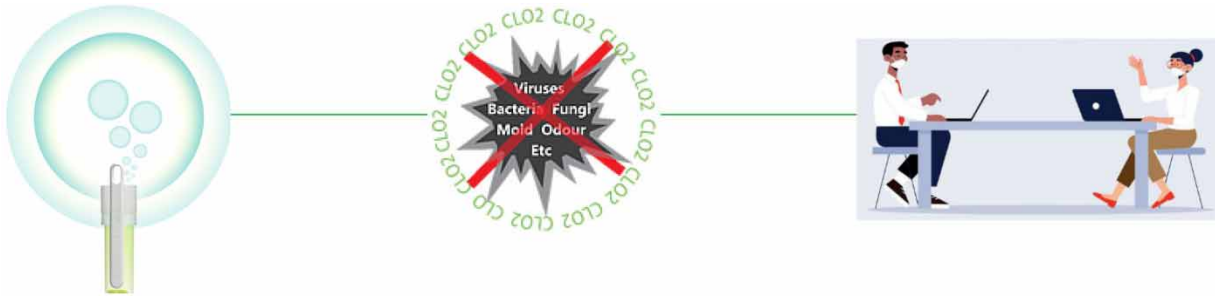
Disinfectants, especially air disinfectants, are necessary to prevent the potential spread of pathogens (bacteria and viruses) in the pandemic era and minimize the spread of pathogens. Some of the commercial disinfectant products that are often used generally contain chlorine dioxide (ClO_2) gas. This study tested the effectiveness of two different commercial disinfectants, a liquid stick disinfectant and a powder disinfection card, to carry out the disinfection of pathogenic bacteria in the environment. These two disinfectants were used as a medium for releasing chlorine dioxide gas which has a much stronger bactericidal effect. In the form of liquid stick, ClO_2 is more effective in the disinfection process rather than in the form of powder. The effectiveness of the liquid disinfectant in inhibiting the growth of pathogenic bacteria is influenced by the temperature and the area of the open space covered. Considering that the release from both disinfectants used is very small (0.002 ppmv/h), it takes a small area to ensure that the disinfection process runs effectively.

Key words: air disinfectant, chlorine dioxide gas, COVID-19 (SARS-CoV-2), new normal activities, pathogens

HIGHLIGHTS

- The effectiveness of chlorine dioxide-based portable disinfectant products.
- Disinfection of pathogenic bacteria in the environment to support the new normal era.
- Pathogen bacteria were evaluated with the Gram staining procedure.
- Growth of pathogenic bacteria is influenced by temperature and the disinfectant's coverage area.
- Both disinfectants used very small dosages of chlorine dioxide gas referring to FDA's regulation.

GRAPHICAL ABSTRACT



INTRODUCTION

Outbreaks of airborne infectious diseases, such as influenza, tuberculosis, and the current outbreak, namely the SARS-CoV-2 or COVID-19 pandemic, pose a very serious threat to humans and civilization, especially if the scale is a pandemic. Special

attention is needed to overcome or control the spread of viruses and (including) pathogenic bacteria. In addition to implementing the health protocols recommended by the government, the need for disinfectants, especially air disinfectants, is important to prevent the potential spread of pathogens (bacteria and viruses) that can cause a pandemic. All pathogenic microorganisms have been shown to survive in the environment from hours to days (Kramer *et al.* 2006). The disinfectants used are generally disinfectants that have been registered with the Food and Drug Authority (FDA), hence are proven to be effective in eliminating pathogens in medical devices. Various efforts are being taken to narrow the spread of the COVID-19 virus in order to support activities in this new normal era. One of the efforts that can be done to break the chain of transmission to maintain cleanliness and kill viruses or bacteria before infecting humans besides washing hands is spraying disinfectants for various inanimate objects that may be exposed to viruses or bacteria.

The definition of a disinfectant itself is a chemical used to inhibit or kill microorganisms (e.g., bacteria, viruses, and fungi except for bacterial spores) on inanimate surfaces, such as furniture, rooms, floors, and others (Agustina *et al.* 2021). Disinfectants are not used on the skin or mucous membranes, because of the risk of irritating the skin and potentially triggering cancer. This is different from antiseptics which are intended for disinfection on the surface of the skin and mucous membranes. One type of disinfectant studied in this study is chlorine dioxide (ClO_2), a chlorine class material (for example, ClO_2, sodium hypochlorite, and hypochlorous acid) that can kill viruses by entering through the virus wall and destroying the inside of the virus. The mechanisms of inactivation of the virus by ClO_2 include the disruption of the virus protein or damage to the genome (Ge *et al.* 2021). From the study by Ge *et al.* (2021), it is reported that heterogeneity of the virus population or virus attachment to other (virus) particles could be responsible for the tailing behaviour. The inactivation rates increase with increasing ClO_2 dosages, when the contact time is longer, the lower dose of ClO_2 can also effectively penetrate the surface structure of viruses and lead to their death (Lin *et al.* 2014).

ClO_2 is an unstable gas that dissociates into chlorine gas (Cl_2), oxygen gas (O_2), and heat. When ClO_2 is photo-oxidized by sunlight then it falls apart. The end-products of ClO_2 reactions are chloride (Cl^-), chlorite (ClO^-), and chlorate (ClO_3^-) (Comeskey & Smith 2009). At −59 °C, solid ClO_2 becomes a reddish liquid or greenish yellow to orange gas at room temperature with a distinctive pungent odour like chlorine. At 11 °C, ClO_2 turns into gas (Lenntech Water Treatment Solutions 2021). ClO_2 is 2.4 times denser than air, as a liquid ClO_2 has a bigger density than water. ClO_2 can be used in gas or liquid form and can be used effectively as a disinfectant against pathogenic microorganisms such as fungi, bacteria, viruses, and spore-forming bacteria and biofilms (Chen *et al.* 2020). The ClO_2 gas form is a strong oxidizing agent and can explode if the concentration is more than 10% v/v at atmospheric pressure and explodes easily on exposure to sunlight or heat (Budavari *et al.* 1996). It processes efficiently at temperatures from 25 to 30 °C, which does not lead to the formation of trihalomethanes or chloramines and is not mutagenic or carcinogenic for humans (Kowalski & Morrissey 2004).

There are several studies reviewing ClO_2 as a disinfectant. From the research by Hatanaka *et al.* (2021), ClO_2 is more potent as a disinfectant or antiviral agent against COVID-19 virus than sodium hypochlorite although the results strongly suggest that both are strong antiviral agents. There is a study conducted by Jefri *et al.* (2022) that reviewed ClO_2 as a disinfectant and came to the conclusion that ClO_2 even at low concentrations is effective against microorganisms tested although not all of the concentrations used in the study were the same. The formulation effects and concentration of disinfectant used, the presence of an organic load, exposure times, temperature, test method, and many other factors can influence the antimicrobial activity (Xiao *et al.* 2022). The resulting study by Trinh *et al.* (2021) indicated that gaseous ClO_2 can be effectively and rapidly generated with a fully controlled physical process.

The ClO_2 gas has been implemented as a disinfectant, sterilizer and oxidizer in various fields and can be used for antimicrobial decontamination in medical, food processing, and odour mitigation due to its high penetration and oxidizing ability (Chen *et al.* 2020). The presence of ClO_2 gas is expected to increase the permeability of the outer membrane and cytoplasm which plays a role in inhibiting the activity of the intracellular enzyme β-galactosidase (Ofori *et al.* 2017). One of the currently available disinfection systems for indoor use is Puristic, which contains ClO_2 gas. Ogata *et al.* (2016), reported that very low concentrations of ClO_2 (30 ppb, volume/volume) were able to prevent influenza virus infection with no unwanted side effects in an experimental model of infection in mice.

To use ClO_2 gas indoors, a ClO_2 gas-producing device is needed that can ensure that the dose used is kept low. The device is commercially available in several countries, some of which even produce ClO_2 gas which can be used directly without requiring special equipment, such as Puristic, a liquid stick disinfectant, which is a trademark widely used in South Korea, Canada, and the United States. However, there is always a challenge to ensure the effectiveness of the use of ClO_2 in the Puristic form in relation to the dose presented. Besides Puristic, a powder disinfection card product (Virus Shut Out) is also stealing

attention in the midst of a pandemic. Both disinfectant products have been sold in the market and the public can buy these products easily. From the packaging inside the Virus Shout Out, it is written that it can protect its users from pathogenic viruses for 30 days per product. The product is used by draping it around your neck and a low concentration of ClO_2 will be released from the product to remove germs and viruses in the air at a distance of 0.5 m.

Through this study, a trial is conducted on the effectiveness of both products to carry out the disinfection of pathogenic bacteria in the environment or space. This study only carried out an effectiveness test that focused on pathogenic bacteria. This study also analyzed the efficacy of the use of disinfectants based on the temperature and the type of disinfectant used.

METHODS

In this study, Puristic and Virus Shut Out were used as a medium for releasing ClO_2 gas which has a much stronger bactericidal effect. Puristic is a liquid ClO_2 in a plastic stick with a dimensions of height 13.97 cm $\times$ length 1.27 cm $\times$ width 3.81 cm and the concentration release of ClO_2 in the air is 0.002 ppmv. The product is made from plastic with a protection diameter of up to 243.84 cm and can last up to 28 days. Meanwhile, Virus Shut Out is a sterilization card neck type with dimensions of width 5.5 cm $\times$ height 8 cm $\times$ depth 0.5 cm. The product contains ClO_2 generator (sodium chlorite and natural zeolite) and is made from plastic with a protection area of up to 500 cm and can last up to 30 days.

Bacterial preparation

In this study, to evaluate the effect of ClO_2 gas, clinical procedures for handling bacteria were applied. However, we did not determine the release of ClO_2 gas component since we did not use a gas detector and further examination is needed for the next research. For this research, four Gram-positive and five Gram-negative bacteria were used, consideration of the selection of bacteria based on cases of infection in humans that often occur in Indonesia. The bacteria to be used are as follows:

1. Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 9027, *Klebsiella pneumonia* ATCC 10031, and *Enterobacter aerogenes* ATCC 13048.
2. Gram-positive bacteria: *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, and *Enterococcus faecalis* ATCC 29212.

For the preliminary test, the identified target bacteria both Gram-negative and Gram-positive were grown in tryptic soy agar (TSA) and incubated at 36 $\pm$ 1 °C for 24 $\pm$ 4 h. Then, bacteria were suspended in sterile NaCl with turbidity according to standard 1 McFarland. The single colonies used in the form of TSA at 37 °C were re-examined through the Gram staining procedure. For accurate culturing of colonies, single colonies were diluted with 0.85% NaCl and adjusted to 0.5 of McFarland's turbidity (Jung 2019), resulting in colonies ranging from 1.5×10^3 to 1.5×10^6 CFU/mL. In the case of the microorganism enumeration technique, the spread plate method was used, where the principle of this method is to grow pathogenic microorganisms (aerobic bacteria) from a solution to the surface of solid media using a spreading spatula, L-rod, or a Drigalsky spatula. Colonies are expected to grow on the agar surface by utilizing nutrients from the growth medium as described in the following. The advantages of this method are that it allows for a higher amount of the same volume compared to the pour plate method and makes it easier to observe colony morphology which is clearer and suitable for the growth of aerobic microorganisms.

Disinfection test

The adjusted bacteria were then inserted into the TSA plate for further experimental application. Puristic and Virus Shut Out were applied to the incubator along with several plates that are already filled with bacteria so that it is expected to release ClO_2 in the incubator at a temperature of 30 $\pm$ 1 °C with dimensions 60 $\times$ 40 $\times$ 50 cm and the second incubator at a temperature of 36 $\pm$ 1 °C with dimensions 100 $\times$ 50 $\times$ 80 cm. In order to observe and culture the bacteria, the Petri dish was put into the incubator for the incubation process at temperatures 30 and 37 °C. Bacterial growth was observed periodically for up to 24 h and compared with the group of bacteria that were not given Puristic and Virus Shut Out as a control. To analyze the ability of Puristic and Virus Shut Out in inhibiting bacterial growth, Puristic and Virus Shut Out were incubated together with bacteria, therefore this research used three plates for each bacterium and one plate for control colonies.

For the disinfection test at incubation temperatures of 37 and 30 °C, the target bacteria that have been grown in TSA media and incubated at 36 $\pm$ 1 °C and 30 $\pm$ 1 °C for 24 $\pm$ 4 h. Then the microbial suspension was prepared in sterile NaCl with turbidity according to the standard 0.5 of McFarland; sufficient number of dilution tubes have been prepared, each of which has

been filled with 9 mL of NaCl. 1 mL of the 10^{-1} dilution is pipetted into the second NaCl tube and then shaken homogeneously until a 10^{-2} dilution is obtained. Then, the next dilution is made in the same way as the required dilution level. 0.1 mL of each dilution is pipetted onto the surface of the Petri dish which already contains TSA and is carried out as the duplicate. The inoculum is spread evenly using a sterile spreading rod and repeated until the required dilution. Let the suspension stand for any time until it is absorbed in the TSA plate; then the Petri dish was incubated at 36 ± 1 °C and 30 ± 1 °C for 24 ± 4 h with Puristic and Virus Shut Out with the lid of the cup opened and the other Petri dish used as a control plate was incubated without the disinfectant. The same procedure was followed in the next day with a different type of bacteria. The number of colonies was observed and counted using a Total Plate Count (TPC) calculation method. The TPC calculation method is the number of bacteria on a Petri dish × 1/dilution factor. After that amount is multiplied by the dilution obtained (Palawe & Antahari 2018), the calculation of the percentage bacterial growth inhibition rate is as follows:

$$\text{Percentage growth inhibition} = \frac{\text{Numbers of Bacteria Control Plate} - \text{Numbers of Bacteria Test Plate}}{\text{Numbers of Bacteria Control Plate}} \times 100\% \quad (1)$$

After carrying out the test according to the procedure described previously, the number of colonies on the control plate and the plate that had been exposed to the Puristic and Virus Shut Out were calculated. The study was conducted at incubation temperatures of 37 and 30 °C to see the effect of temperature on the optimal effectiveness of Puristic and Virus Shut Out. Puristic and Virus Shut Out were applied to a plate that is already filled with bacteria at room temperature 30 ± 1 °C in the incubator with dimensions 60 × 40 × 50 cm and 36 ± 1 °C with dimensions 100 × 50 × 80 cm with a product distance of less than 1 m as shown in Figure 1.

In the initial experiment, bacterial exposure was tested on the plate and Puristic by placing it directly in the incubator at a temperature of 36 ± 1 °C for 24 ± 4 h, as depicted in Figure 1(a) and 1(b). Considering that the release of ClO_2 in Puristic used is very small, which is <0.002 ppmv, for a more effective exposure, a re-test using plastic is needed to minimize the contamination and hoped that Puristic exposure will directly affect the bacteria on the plate, as shown in Figure 1(a). As for the Virus Shut Out product, the concentration value of ClO_2 is not stated but it is stated that the product releases ClO_2 at ultra-low concentration. The disinfection test of Virus Shut Out in the incubator at a temperature of 36 ± 1 °C is shown in Figure 1(c) and in the incubator at a temperature of 30 ± 1 °C is shown in Figure 1(d).

Figure 1 | Disinfection test for Puristic and Virus Shut Out. (a) Disinfection test using plastic; (b) disinfection test using Puristic; (c) disinfection test using Virus Shut Out at 37 °C; and (d) disinfection test using Virus Shut Out at 30 °C.

Disinfection test in the room

Observation of the use of Puristic in the room was carried out in the National Centre for Drug and Food Testing Development (PPPOMN) laboratory room, where tests were carried out when the room was used and when the room was not used. This test aims to determine the effectiveness of Puristic when used indoors. Considering that Puristic's range is only 1-m radius, the plates in the room will be placed 1 m apart.

The open Petri dish was placed in a room for 15 min in the morning, then the Petri dish was incubated. During the day, different open Petri dishes were placed in the same room for 15 min and then incubated. After that, Puristic is placed in the room for approximately 18–24 h. Then in the next day, the same method was repeated to see the effectiveness of Puristic in disinfecting the room.

RESULTS AND DISCUSSION

In this study, we used Puristic and Virus Shut out with the aim to evaluate the effectiveness of ClO_2 gas in killing pathogenic bacteria: the effectiveness of Puristic as a disinfectant when used in a room with a high enough activity, and to evaluate the effectiveness of the ClO_2 gas that comes out of the Virus Shut Out product. Puristic is a stick-shaped liquid ClO_2 that is practically used daily as a disinfectant, while Virus Shut Out is a product shaped like lanyards or tags, which contains ClO_2 in a powder form.

Research conducted by Akamatsu *et al.* (2012) showed that exposure to ClO_2 gas at a concentration of 0.1 ppm v/v did not have a toxic effect on rats during and after gas exposure within six months. Ogata's research (2013) also confirmed that exposure to ClO_2 gas at a concentration of 1.0 ppm did not have a toxic effect on rats during and after the exposure period of 5 h per day for 5 days within 10 weeks. The American Occupational Safety and Health Administration also states that the exposure limit for ClO_2 gas for humans is 0.1 ppm with an average exposure time of 8 h. Based on these data, it is concluded that exposure to very low concentrations of ClO_2 gas (<0.1 ppm) will not cause harmful effects on humans but has the potential to kill bacteria or viruses that are transmitted in the air. In this study, the PPPOMN Laboratory did not have a gas detector or analyzer, so the concentration of ClO_2 released from Puristic cannot be detected, hence further research is needed in other laboratories that can detect the ClO_2 gas released by Puristic and Virus Shut Out.

Table 1 shows that the effectiveness of reducing bacterial growth by Puristic is not significant and tends to increase. This can be caused by the possibility of contamination from around the incubator and the ability of bacteria can grow or inactivate

Table 1 | Results of the disinfectant at an incubator temperature of 37 °C

				Puristic		Virus Shut Out	
Temperature	Gram staining	Bacteria	Groups	Numbers (CFU/mL)	Growth inhibition rate (%)	Numbers (CFU/mL)	Growth inhibition rate (%)
37 °C	+	*Staphylococcus aureus*	Disinfectant (ClO_2)	5.0×10^3	99	1.1×10^7	9
			Control	1.1×10^7		1.0×10^7	
	+	*Staphylococcus epidermidis*	Disinfectant (ClO_2)	3.5×10^6	29	3.0×10^6	–
			Control	4.9×10^6		3.2×10^6	
	+	*Bacillus subtilis*	Disinfectant (ClO_2)	6.7×10^2	99	2.7×10^6	30
			Control	7.0×10^5		1.9×10^6	
	+	*Enterococcus faecalis*	Disinfectant (ClO_2)	2.9×10^6	83	2.4×10^7	–
			Control	1.7×10^7		2.5×10^7	
	–	*Escherichia coli*	Disinfectant (ClO_2)	8.5×10^4	99	2.8×10^6	64
			Control	1.9×10^7		1.0×10^6	
	–	*Salmonella typhimurium*	Disinfectant (ClO_2)	1.2×10^4	99	2.7×10^7	7
			Control	8.1×10^6		2.5×10^7	
	–	*Pseudomonas aeruginosa*	Disinfectant (ClO_2)	2.1×10^7	5	4.0×10^7	22
			Control	2.2×10^7		3.1×10^7	
	–	*Klebsiella pneumonia*	Disinfectant (ClO_2)	1.3×10^7	–	4.1×10^6	17
			Control	1.0×10^7		3.4×10^6	
	–	*Enterobacter aerogenes*	Disinfectant (ClO_2)	$>3.0 \times 10^7$	–	1.1×10^7	9
			Control	$>3.0 \times 10^7$		1.0×10^7	

in a room environment. Table 1 is a resume of the results of the Puristic test at 37 °C which has been carried out in the laboratory and has been facilitated without plastic as can be seen in Figure 1(b).

It can be seen in Table 1 that Puristic testing was carried out at 37 °C which is the optimal temperature for bacterial growth. There is a significant decrease in the number of bacterial colonies occurring in *S. aureus*, *B. subtilis, S. typhimurium, E. coli, and E. faecalis* bacteria. The effectiveness of reducing the number of bacteria consecutive bacterial colonies is 99.95, 99.90, 99.85, 99.55, and 82.94%. As for the Virus Shut Out, the greatest effectiveness occurred in *E. coli* bacteria along with a decrease in the number of bacteria by 64.29%. ClO_2 itself claimed to be able to kill various kinds of bacteria, viruses, or fungi. These five types of bacteria live in human and animal bodies, soil, and water, and can also be found in the decomposition of materials. These bacteria are classified as pathogenic bacteria that can cause various diseases such as the bacteria used in this study.

As for other bacteria such as *S. epidermidis, P. aeruginosa, K. pneumonia*, and *E. aerogenes*, the decrease in bacterial growth was not very effective or even showed no effect from exposure to Puristic and Virus Shut Out. The effectiveness of Puristic itself is seen for 2–3 days after ClO_2 gas is activated. In addition, the smell of chlorine was felt on the second day after Puristic is activated, and it got reduced in the following days. It can be said that there is a possibility that the effectiveness of Puristic decreased after the third day of testing, so the reduction in the number of bacteria was not effective.

The disinfection test for Virus Shut Out was carried out in an incubator with a temperature of 37 °C, the decrease in the number of bacteria occurred but was not very significant. The effectiveness of reducing the number of bacteria was highest in *E. coli*, which was a growth inhibition rate of 64.29% while the other bacteria did not see the effect of ClO_2 release on Virus Shut Out products. The characteristics of *E. coli* bacteria for their life cycle are strongly influenced by temperature (Anggraeni 2012), thus the use of high temperatures is considered effective to weaken or kill these bacteria (Kurniati *et al.* 2020). Therefore, for *E. coli*, the effectiveness of the reduction is greater at the incubator temperature of 37 °C. As seen in Table 1, the reduction of bacteria due to exposure to Puristic is more effectively carried out by a liquid ClO_2 disinfectant rather than a powder one. To find out the optimum temperature for using Puristic and Virus Shut Out as inhibitors of bacterial growth, a re-test was carried out at a temperature of 30 °C, where the temperature is not the optimum temperature for bacterial growth. As seen in Table 2 above, the results of the Puristic and Virus Shut Out disinfection test at a temperature of 30 °C.

From Table 3, it can be seen that bacterial growth is inhibited if it does not reach the optimum temperature (37 °C). This growth is further hampered by the presence of Puristic and Virus Shut Out that act as disinfectants. Even though the Puristic's

Table 2 | Results of the disinfectant at an incubator temperature of 30 °C

				Puristic		Virus Shut Out	
Temperature	Gram staining	Bacteria	Groups	Numbers (CFU/mL)	Growth inhibition rate (%)	Numbers (CFU/mL)	Growth inhibition rate (%)
30 °C	+	*Staphylococcus aureus*	Disinfectant (ClO_2)	3.5×10^6	41	1.1×10^7	–
			Control	5.9×10^6		1.1×10^7	
	+	*Staphylococcus epidermidis*	Disinfectant (ClO_2)	4.0×10^6	–	4.1×10^6	22
			Control	3.6×10^6		3.2×10^6	
	+	*Bacillus subtilis*	Disinfectant (ClO_2)	1.5×10^6	66	2.6×10^6	23
			Control	4.4×10^6		2.0×10^6	
	+	*Enterococcus faecalis*	Disinfectant (ClO_2)	3.5×10^6	35	2.7×10^7	22
			Control	5.4×10^6		2.1×10^7	
	–	*Escherichia coli*	Disinfectant (ClO_2)	2.0×10^5	96	1.8×10^6	22
			Control	4.9×10^6		1.4×10^6	
	–	*Salmonella typhimurium*	Disinfectant (ClO_2)	1.6×10^7	20	3.4×10^7	38
			Control	2.0×10^7		2.1×10^7	
	–	*Pseudomonas aeruginosa*	Disinfectant (ClO_2)	2.6×10^7	–	3.1×10^7	13
			Control	1.9×10^7		2.7×10^7	
	–	*Klebsiella pneumonia*	Disinfectant (ClO_2)	8.8×10^6	27	4.1×10^6	17
			Control	1.2×10^7		3.4×10^6	
	–	*Enterobacter aerogenes*	Disinfectant (ClO_2)	1.7×10^7	–	1.1×10^7	–
			Control	1.5×10^7		1.1×10^7	

Table 3 | Comparison of growth inhibition rates

	Puristic		Virus Shut Out	
Bacteria	**37 °C**	**30 °C**	**37 °C**	**30 °C**
Staphylococcus aureus	99%	41%	9%	–
Staphylococcus epidermidis	29%	–	–	22%
Bacillus subtilis	99%	66%	30%	23%
Enterococcus faecalis	83%	35%	–	22%
Escherichia coli	99%	96%	64%	22%
Salmonella typhimurium	99%	20%	7%	38%
Pseudomonas aeruginosa	5%	–	22%	13%
Klebsiella pneumonia	–	27%	17%	17%
Enterobacter aerogenes	–	–	9%	–

disinfection process was not as significant as it was at 37 °C, the decrease in bacterial growth in this experiment occurred in almost all the bacteria used. From this experiment, it can also be seen that the performance of Puristic and Virus Shut Out as a disinfectant was affected by the temperature and the content of the disinfectant itself.

At 30 °C, the effectiveness of the disinfectant is seen in several bacteria, including *K. pneumonia, S. epidermidis, E. faecalis,* and *S. typhimurium* although the results were still not as effective as at 37 °C. From the study by Al-Sa'ady *et al.* (2020), *K. pneumonia* has a high sensitivity to ClO_2 gas thus the ability of ClO_2 to penetrate the biofilm and effectively remove it. *S. epidermidis, E. faecalis,* and *S. typhimurium* also have sensitivity in saline conditions. This can affect the performance of Virus Shut Out which contains sodium chlorite to be able to work effectively on this type of bacteria at 30 °C.

The study conducted by Zhu *et al.* (2008) developed ClO_2-based disinfectant powder in a single pack that had bactericidal efficacy, therefore, the disinfectant powder was prepared in a liquid state of ClO_2. The ingredients of ClO_2-based disinfectant powder or ClO_2 generator were mainly found to be chlorine and sodium hypochlorite. Thus, disinfectants active against SARS-CoV-2 will remain a cornerstone of control of COVID-19 globally. However, sodium hypochlorite has some disadvantages; it may produce more trihalomethane and it exhibits weak antimicrobial activity in the presence of organic matters compared to ClO_2 (Sorlini & Collivignarelli 2005; Miura & Shibata 2010; Hinenoya *et al.* 2015). The results of the study by Zhu *et al.* (2008) showed that the content of solid acid and the capacity of water solution were the key factors influencing the efficacy of ClO_2 products. Based on the study by Zhu *et al.* (2008), we can conclude that further research is needed to know and analyze the ingredients of both disinfectants.

Based on the European Patent Application for ClO_2 (2013), ClO_2 in the form of powder (Virus Shut Out) is sensitive to moisture in the air and mostly caking. It can be seen in Table 3, with the same time span and the same type of bacteria, the effectiveness of Puristic as a liquid ClO_2 disinfectant is more optimal at a room temperature of 37 °C. As for the Virus Shut Out as a powder ClO_2, on testing at a temperature of 30 °C the effectiveness of reducing bacteria was more evenly distributed for each type of bacteria but it was not significant, while at 37 °C the effectiveness of reducing bacteria was higher but only for some bacteria. The effectiveness of reducing bacteria is seen in the range of 12–39% only. This also proves that the Virus Shut Out product in the form of powder is not as effective as Puristic in the form of liquid disinfectant in reducing the number of bacteria; also, the concentration of ClO_2 contained in the Virus Shut Out is not known yet.

The main content of Virus Shut Out is a ClO_2 generator (sodium chlorite and natural zeolite). Hatanaka *et al.* (2021) suggest that ClO_2 is a much more powerful disinfectant than sodium hypochlorite, especially when organic matter is present in the contaminants. Thus, the content of ClO_2 in the Virus Shut Out which is different from the content in Puristic affects the effectiveness of the product's disinfection. This causes Virus Shut Out to have lower inhibition efficacy than Puristic.

Based on the research done by Park & Kang (2018), they said that in fact there has been no study that has considered the effect of experimental temperature on the inhibition of bacteria by ClO_2 gas. Experimental temperature can be an important factor influencing the inhibition of bacteria by ClO_2 gas because it can affect the solubility of ClO_2 gas and reactivity of the gas simultaneously, and the effect can be different in each experiment. When the temperature increases, the reactivity of ClO_2 gas also increases but the solubility of the gas decreases. Based on the study conducted by Park & Kang (2018), bacterial

inhibition occurred at a higher temperature at 15 °C than at 25 °C, which indicates that the solubility of ClO_2 gas compared to the reactivity has a greater effect on bacterial inhibition. Meanwhile, in this study, it was found that the inhibition of bacteria by ClO_2 gas at Puristic was more effective at 37 °C than at 30 °C, while for Virus Shut Out products, both at 37 and 30 °C, the decrease was not so significant. It can be said that the reactivity compared to the solubility of ClO_2 gas in this study had a greater effect on bacterial inhibition. Till now there is no scientific evidence or literature related to Virus Shut Out products as a disinfectant to ward off viruses or pathogenic bacteria. Hence, based on the article from EPA (Diaz 2020), Virus Shut Out product is not recommended for use as a disinfectant or as an antidote to viruses since the product is not registered with the EPA. Therefore, its safety and efficacy against viruses have not been evaluated.

At a temperature of 30 °C, Puristic itself is classified as effective only for *E. coli* bacteria with a decrease in the number of bacteria up to 95.91%. Based on research conducted by Song & Yung (2018), with an incubation temperature of 37 °C, ClO_2 produced by Puristic can significantly inhibit the growth of several bacteria, one of which is *E. coli*, which reaches more than 99%. The study also stated that *E. coli* is a bacterium that can be applied to certain conditions and environments, and based on Banach *et al.* (2018) and Haute *et al.* (2017) the growth of this bacterium is easily inhibited in the presence of ClO_2. So, it can be said that with the concentration of ClO_2 at Puristic and at a temperature of 30 °C, *E. coli* bacteria can be killed effectively. Likewise with Virus Shut Out products, there was a decrease in *E. coli* bacteria by 64.29% at 37 °C.

For the experimental mechanism, as can be seen in Figure 2, the Puristic effectiveness test in the room is carried out in the PPPOMN laboratory room, where the test is carried out when the room has high activity and when the room is empty. Before carrying out the disinfectant test, a control plate was first placed for 24 h to see the amount of exposure to bacteria in the room. Then, Puristic is placed on a table with a plate distance of less than 1 m and it can be seen in Table 4 that the highest Puristic effectiveness occurred 24 h after ClO_2 was activated with a decrease in the number of bacteria by 69.69% from 33 colonies in the control plate to 10 colonies. It can be said that the effectiveness of Puristic in the room in inhibiting bacteria can be seen after 24 h of activation.

Figure 2 | Indoor Puristic testing.

Table 4 | Indoor test results of Puristic

Treatment	Exposure time (h)	Distance (m)	Number of colony	Growth inhibition rate (%)
Control without Puristic	24	–	33	–
With Puristic	1	<1	15	54.55
	6	<1	18	45.45
	24	<1	10	69.69
	48	<1	11	66.67

CONCLUSIONS

The effectiveness of Puristic liquid stick in a room occurs within 24–48 h after ClO_2 is activated and it can inhibit the microbial growth effectively in areas <1 m (50 cm). The effectiveness of Puristic in inhibiting the growth of pathogenic bacteria is influenced by the temperature and the coverage area where the Puristic is used. Considering that the release from the disinfectant used is very small (<0.002 ppmv/h), it requires a small area to ensure that the disinfection process runs effectively. For the bacterial types *S. aureus, B. subtilis, S. typhimurium, E. coli*, and *E. faecalis*, the growth inhibition rates, respectively, were 99.95, 99.90, 99.85, 99.55, and 82.94%. The effect of temperature on the effectiveness of Puristic is also seen in reducing the number of bacteria which is not too significant at a temperature of 30 °C. The effect of temperature and the ingredients of the disinfectant need to be studied more deeply to find the characteristics of ClO_2, so that other possibilities can be found that can inhibit or support the use of Puristic. The effectiveness of a powder disinfection card, Virus Shut Out, has not been seen significantly for all the tested bacteria because the effectiveness of reducing the number of bacteria has not reached 70–80% both at 30 and 37 °C. The effectiveness of reducing the number of bacteria at a temperature of 37 °C was high in *E. coli* by 64.29%. Meanwhile, at a temperature of 30 °C, the decrease in the number of bacteria was seen more evenly distributed than at 37 °C, although the effectiveness of the reduction was only in the range of 12–39%. Further examination is needed in another laboratory that has a gas detector or analyzer to determine the concentration of ClO_2 released from Puristic so that the effectiveness of Puristic can also be studied based on the concentration of ClO_2 contained in it. So, it can be concluded that Puristic is more effective rather than Virus Shut Out based on the results of the growth inhibition rate from the disinfection process.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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