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Note

Inactivation of SARS-CoV-2 by Commercially Available Disinfectants and Cleaners

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The recent emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a major burden for health care systems worldwide, and is a threat to our daily lives. Various effective ingredients against SARS-CoV-2 were already reported, however, since products contain various ingredients, it is also important to evaluate the effectiveness of commercially available disinfectants per se. In this study, the virucidal efficacy of forty-eight commercially available products were evaluated according to the standardized suspension method EN 14476 and the following results were obtained: Alcohol-based disinfectants, hand soaps, wet wipes, alkaline cleaners, quaternary ammonium compound sanitizers and oxygen bleach had great virucidal efficacy against SARS-CoV-2. Enveloped viruses such as SARS-CoV-2 are among the most susceptible of pathogens to formulated microbicidal actives and detergents, but as the results of this study showed, it is also necessary to pay attention to the concentration at the time of use and the required contact time.

Key words : SARS-CoV-2 / COVID-19 / Virucidal effect / EN 14467.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), belonging to a family of enveloped RNA virus, Coronaviridae, is a novel virus that was first identified in Wuhan, China (Lai et al., 2020). This virus had quickly spread all over the world, and was responsible for causing coronavirus infection disease 2019 (COVID-19). The predominant route of human-to-human transmission of SARS-CoV-2 is through respiratory droplets and close contact, and is likely to be an airborne infection (Kumar et al., 2020). It is also confirmed that SARS-CoV-2 infection is transmitted through contaminated hands and high-touch surfaces (Pope et al., 2022). Therefore, it is important to continue to practice proper hand hygiene and environmental hygiene.

Many researchers have already reported about the effective ingredients for inactivating SARS-CoV-2 (Ogilvie et al., 2021; Xiling et al., 2021). However, commercially available products consist of various ingredients and there is possibility that some combinations may decrease virucidal efficacy against SARS-CoV-2, so that it is also important to evaluate the virucidal efficacy of commercially available products themselves.

In this study, we evaluated the virucidal efficacy of forty-eight commercially available products against SARS-CoV-2, including eighteen alcohol-based hand sanitizers, thirteen alcohol-based environmental disinfectants, five hand soaps, six wet wipes, one alkaline cleaner, four quaternary ammonium compounds (QAC) sanitizers, and one oxygen bleach (Tables 1 and 2), according to European standard, EN 14476 (European Committee for Standardization, 2019). Some products were diluted with hard water designated to EN14476 as needed, and as for wet wipes evaluation, the squeezed solution from each wet wipes was prepared.

In this study, all experiments were carried out at the

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TABLE 1. Summary of alcohol-based products examined in this study.

Type of disinfectant	Product name	Active ingredient (concentration)	Solution form	Use dilution	pH (in use)
Alcohol-based hand sanitizer	A	Ethanol (76.9-81.4 v/v%), Phosphoric acid	Liquid	×1	2.8
	B	Ethanol (76.9-81.4 v/v%), Phosphoric acid	Gel	×1	2.8
	C	Ethanol (71.8 w/w%), Phosphoric acid	Liquid	×1	2.8
	D	Chlorhexidine Gluconate (1.0 w/v%), Ethanol (about 80 v/v%)	Liquid	×1	6.0
	E	Chlorhexidine Gluconate (0.5 w/v%), Ethanol (about 80v/v%)	Liquid	×1	6.0
	F	Chlorhexidine Gluconate (0.2 w/v%), Ethanol (about 80 v/v%)	Liquid	×1	6.5
	G	Chlorhexidine Gluconate (0.1 w/v%), Ethanol (about 80 v/v%)	Liquid	×1	6.8
	H	Chlorhexidine Gluconate (0.1 w/v%), Ethanol (about 63 v/v%)	Liquid	×1	6.7
	I	Chlorhexidine Gluconate (0.1 w/v%), Ethanol (about 39 v/v%)	Foam	×1	6.0
	J	Chlorhexidine Gluconate (0.2 w/v%), Ethanol (about 80 v/v%)	Gel	×1	7.5
	K	Chlorhexidine Gluconate (0.1 w/v%), Ethanol (about 80 v/v%)	Gel	×1	7.4
	L	Chlorhexidine Gluconate (0.2 w/v%), Ethanol (about 20 v/v%)	Liquid	×1	6.2
	M	Ethanol (76.9-81.4 v/v%)	Gel	×1	6.7
	N	Ethanol (76.9-81.4 v/v%)	Gel	×1	6.6
	O	Ethanol (76.9-81.4 v/v%)	Gel	×1	6.5
	P	Ethanol (76.9-81.4 v/v%)	Gel	×1	6.7
	Q	Ethanol (76.9-81.4 v/v%)	Foam	×1	7.5
	R	Ethanol (72.3 w/w%)	Liquid	×1	7.2
Alcohol-based environmental disinfectant	S	Ethanol (67.1 w/w%)	Liquid	×1	6.9
	T	Ethanol (58.8 w/w%)	Liquid	×1	6.9
	U	Ethanol (67.1 w/w%)	Liquid	×1	6.9
	V	Ethanol (54.1 w/w%), Lactic acid (0.2 w/w%)	Liquid	×1	5.0
	W	Ethanol (67.1 w/w%), Citric acid (0.7 w/w%)	Liquid	×1	2.6
	X	Ethanol (50.0 w/w%), Citric acid (0.5 w/w%)	Liquid	×1	3.9
	Y	Ethanol (58.8 w/w%), Citric acid (1.5 w/w%), Malic acid (0.5 w/w%)	Liquid	×1	2.8
	Z	Chlorhexidine Gluconate (0.1 w/v%), Ethanol (46.2 w/w%)	Liquid	×1	7.0
	AA	Ethanol (50 w/w%), Quaternary ammonium chloride	Liquid	×1	7.3
	BB	Ethanol (50 w/w%), Arginine	Liquid	×1	10.8
	CC	Benzalkonium chloride (0.1 w/v%), Sodium carbonate, Ethanol (54 w/w%)	Liquid	×1	10.8
	DD	Ethanol (72.6 w/w%), Quaternary ammonium chloride	Liquid	×1	7.8
	EE	Isopropyl alcohol (58.6 w/w%), Quaternary ammonium chloride	Liquid	×1	7.7

Research Institute for Microbial Diseases, Osaka University in Japan. SARS-CoV-2 JPN/TY/WK-521 was provided by National Institute of Infectious Diseases in Japan. SARS-CoV-2 was propagated in African green monkey kidney epithelial cells expressing transmembrane serine protease TMPRSS2 (VeroE6/TMPRSS2 JCRB1819). VeroE6/TMPRSS2 cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum (FBS) at 37℃ under 5% CO_2 atmosphere. For preparing SARS-CoV-2 stock, sub-confluent VeroE6/TMPRSS2 cells in 75 cm^2 tissue culture flask were infected with SARS-CoV-2 at a multiplicity of infection (MOI) of 0.1-1. After 1 hour of incubation at 37℃, 8 ml of DMEM supplemented with 2% FBS was added and the culture was incubated for 2 days. After that, culture supernatants containing SARS-CoV-2 were clarified by centrifugation at 3,000 rpm for 10 min and stored at -80℃ until use.

The experimental procedure was done according to quantitative suspension test EN 14476. The virus suspension was added to the solution of alcohol-based hand sanitizers under clean condition [0.3 g/L bovine

TABLE 2. Summary of other products examined in this study

Type of disinfectant	Product name	Active ingredient (concentration)	Use dilution	pH (in use)
Hand soap	a	Isopropylmethylphenol, KOH soap (36 w/w%)	×7	10.6
	b	Isopropylmethylphenol, KOH soap (7 w/w%)	×1	10.3
	c	Isopropylmethylphenol, KOH soap (3.9 w/w%)	×1	9.5
	d	Isopropylmethylphenol, Amine soap (7 w/w%)	×1	8.5
	e	Isopropylmethylphenol, KOH soap (5.3 w/w%)	×1	9.6
Wet wipes (Solution squeezed from each product)	f	Sodium hypochlorite (>0.1%), Sodium hydroxide	×1	9.8
	g	Ethanol (80%)	×1	7.2
	h	Ethanol (54 w/w%), Quaternary ammonium chloride, Alkaline agent	×1	10.5
	i	Ethanol (50 w/w%), Citric acid	×1	4.1
	j	Ethanol, Quaternary ammonium chloride	×1	7.3
	k	Quaternary ammonium chloride, Alkaline agent	×1	11.4
Alkaline cleaner	l	Alkaline agent (4.4 w/w%), Anionic surfactant (6 w/w%)	×20	12.1
			×50	11.7
QAC sanitizer	m	Benzalkonium chloride (0.1w/w%), Alkylamine oxide (0.1w/w%), Alkylglucoside (0.1 w/w%)	×1	6.8
	n	Quaternary ammonium chloride (10 w/w%)	×100	8.9
	o	Quaternary ammonium chloride (10 w/w%)	×600	6.8
	p	Quaternary ammonium chloride (5 w/w%)	×300	8.6
Oxygen bleach	q	Sodium percarbonate, Sodium carbonate	×20	11.6
			×100	10.7

serum albumin (BSA)] and the solution of other products under dirty condition (3.0 g/L BSA + 3.0 ml/L erythrocytes as interfering substances). Shortly, 100 μL of virus suspension was added to 100 μL of the interfering substance and 800 μL of the product test solution at 20.0±1.0℃. A virus control mixture was also assessed using hard water in place of the test product. After the specified contact time, the specimens were immediately 10-fold diluted in DMEM containing 10% FBS to neutralize test sample activity, then serially diluted ten folds. For each test suspension, eight wells of a microtiter plate containing a confluent monolayer of VeroE6/TMPRSS2 cells were inoculated with 50 μL of test suspension, and the plates were incubated for 3 days at 37℃ under 5% CO_2 atmosphere to observe cytopathic effect. After incubation, the plates were stained with methylene blue solutions, and the $TCID_{50}$ was calculated by the Behrens-Kärber method.

All alcohol-based hand sanitizers, regardless of their ethanol concentrations (20-80%), pH (acidic, alkaline, neutral) or solution type (liquid, gel, foam), significantly reduced the infectivity of SARS-CoV-2 by at least 4.00 $\log_{10}$ within 15 seconds of exposure time under clean conditions, and all alcohol-based environmental disinfectants also reduced the infectivity of SARS-CoV-2 below the detection limit (>3.75 $\log_{10}$ reduction) within 30 seconds of exposure time under dirty conditions (Table 3). As SARS-CoV-2 is sensitive to alcohol, and minimal ethanol concentration of 30% ethanol is sufficient for SARS-CoV-2 inactivation (Kratzel et al., 2020; Xiling et al., 2021), SARS-CoV-2 was mainly inactivated by sufficient ethanol efficacy of the alcohol-based hand sanitizers. On the other hand, "Product L", which contains about 20 v/v% ethanol and 0.2 w/v% chlorhexidine gluconate, showed high virucidal efficacy. It was reported that both 20% ethanol and 0.2% chlorhexidine gluconate had low virucidal efficacy against SARS-CoV-2 (Hirose et al., 2021). However, it was also reported that the combination of ethanol and chlorhexidine gluconate achieved additive effect against some bacteria and foot-and-mouth disease virus (Oie and Koshiro, 1984; Harada et al., 2015), so that it is presumed that this result is of the same mechanism.

Five hand soaps had high efficacy against SARS-CoV-2 after 30 seconds exposure under dirty conditions (Table 4). Commercially hand soaps are comprised of some surfactants which play a key role in disrupting the lipid bilayer of enveloped viruses (Falk, 2019). It was reported that these hand soaps had bactericidal efficacy and virucidal efficacy against enveloped viruses (Furuta et al., 2009), and hand soaps also showed high virucidal efficacy for SARS-CoV-2 (Mukherjee et al., 2021).

TABLE 3. The effects of alcohol-based products against SARS-CoV-2

Type of disinfectant	Product No.	Condition	Time (second)	Reduction in titer (log_{10} $TCID_{50}$)
Alcohol-based hand sanitizer	A	Clean	15	>4.50
	B			>4.00
	C			>4.00
	D			>4.25
	E			>4.25
	F			>4.38
	G			>4.63
	H			>4.13
	I			>4.25
	J			>4.63
	K			>4.50
	L			>4.63
	M			>4.38
	N			>4.13
	O			>4.38
	P			>4.38
	Q			>4.13
	R			>4.25
Alcohol-based environmental disinfectant	S	Dirty	30	>4.75
	T			>4.63
	U			>4.63
	V			>4.00
	W			>4.50
	X			>4.50
	Y			>3.38
	Z			>4.75
	AA			>4.75
	BB			>4.13
	CC			>3.75
	DD			>3.75
	EE			>4.00

TABLE 4. The effects of commercially available products against SARS-CoV-2

Type of disinfectant	Product No. (use dilution)	Condition	Time (second)	Reduction in titer (log_{10} $TCID_{50}$)
Hand soap	a	Dirty	30	>3.25
	b			>3.75
	c			>3.75
	d			>3.13
	e			>3.75
Wet wipes (Solution squeezed from each product)	f	Dirty	15	>3.13
	g			>4.00
	h			>3.13
	i			>4.00
	j			>3.13
	k			>3.00
Alkaline cleaner	l (×20)	Dirty	30 60	>4.75 >4.75
	l (×50)		30 60	1.88 2.32
QAC sanitizer	m	Dirty	30	>3.38
	n			>3.75
	o			>4.75
	p			>3.25
Oxygen bleach	q (×20)	Dirty	30 60	>3.38 >3.38
	q (×100)		30 60 600	1.50 1.50 2.00

In addition to the physical removal efficacy of hand washing, hand washing with hand soaps is very important for control of SARS-CoV-2.

All wet wipes significantly reduced the infectivity of SARS-CoV-2 below detection limit (at least >3.00 log_{10} reduction) within 15 seconds of exposure time under dirty conditions (Table 4). For the evaluation of virucidal efficacy of wet wipe, the solution was often prepared before impregnating the wipes. Due to the reports that the components of the impregnating solution are absorbed in the wipes and the virucidal efficacy was decreased (Song et al., 2019), in the previous study, instead of preparing the impregnating solution separately, a solution squeezed from the test product was prepared instead for use. These results were achieved because squeezed solution included sufficient concentration of chlorine, ethanol and QAC. Since wet wipes also have the effect of physically removing viruses from environmental surfaces, it seemed that the wet wipes, which used in this test, are effective for control of SARS-CoV-2 on environmental surfaces.

An alkaline cleaner, "Product l", significantly reduced the infectivity of SARS-CoV-2 by at least 4.00 log_{10} within 30 seconds of exposure time under dirty conditions when it was diluted to 20-folds, while 50-fold diluted solution reduced the infectivity of this virus only about 2.00 log_{10} within 60 seconds of exposure time, both diluted solution having almost the same pH (12.1 and 11.7, respectively) (Table 4). This cleaner also contains 6% anionic surfactant, such as linear alklbenzene sulfonate, which was confirmed to have high virucidal activity for SARS-CoV-2 by National Institute of Technology and Evaluation (NITE) in Japan (National Institute of Technology and Evaluation, 2020). From these results, it was presumed that SARS-CoV-2 is relatively resistant to alkaline conditions in a short exposure time, and that the virucidal efficacy may differ depending on the concentration of the anionic surfactant contained in "product I".

All four QAC sanitizers, regardless of their pH (mildly alkaline, neutral), had high virucidal efficacy against SARS-CoV-2 after 30 seconds of exposure time under dirty conditions (Table 4). Quaternary ammonium compounds were used widely as disinfectants, and enveloped viruses were considered to be sensitive to these compounds (Lin et al., 2020). In fact, it is reported that benzalkonium chloride and other QAC compounds have high virucidal efficacy against SARS-CoV-2 (Ogilvie et al., 2021; Xiling et al., 2021; Ijaz et al., 2022). It is presumed that the virucidal ability of QAC against enveloped viruses is due to their ability to disrupt the virus' phospholipid membranes (Schrank et al., 2020). In addition, from this test, it is also considered that SARS-CoV-2 was also quickly inactivated by disruption of the envelope with QAC.

Oxygen bleach (active ingredient: sodium percarbonate, 20-fold diluted solution) significantly reduced the infectivity of SARS-CoV-2 below the detection limit (>3.75 $\log_{10}$ reduction) within 60 seconds of exposure time under dirty conditions. On the other hand, 100-fold diluted solution required 600 seconds (10 minutes) to reduce the infectivity of SARS-CoV-2 below the detection limit (Table 4). In the final report by NITE, it was concluded that oxygen bleach (1% sodium percarbonate, 100-fold diluted solution) was found to be effective within 5 minutes of exposure time (National Institute of Technology and Evaluation, 2020). The present study revealed that it is sufficiently effective against SARS-CoV-2 depending on the concentration and contact time.

In conclusion, for the measures against SARS-CoV-2, alcohol-based hand sanitizers and hand soaps are recommended to be effective for hand hygiene, while alcohol-based environmental disinfectants, wet wipes and QAC sanitizers, for environmental hygiene. If alkaline cleaners or oxygen bleach will be used, it is recommended to use them at higher concentration.

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