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Efficacy test of commercially available disinfectants against foot-and-mouth virus under subzero temperature using anti-freezing diluents

Mi Young Kang[1)], Su Yeon Han[1)], Wooseog Jeong[1)], Jaegoo Jung[2)], Won-Seok Chae[2,*)] and Hu-Jang Lee[3,*)]

[1)] Animal and Plant Quarantine Agency, Gimchun 39660, Korea
[2)] Division of Life-Science & Chemistry, Daejin University, Pocheon 11159, Korea
[3)] Institute of Animal Medicine and College of Veterinary Medicine, Gyeongsang National University, Jinju 52828, Korea

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Abstract

This study was carried out to establish the composition of an anti-freezing diluent (AFD) which can prevent the freezing of liquid disinfectants during winter. Freezing points of AFDs were measured to −30°C by varying the composition ratio of ethanol (EtOH) and propane-1,2-diol (PG), and the AFD composited with 15% EtOH and 30% PG did not freeze at −30°C. Additionally, the virucidal efficacy of nine commercially available disinfectants against foot-and-mouth disease virus (FMDV) was evaluated at three different reaction temperatures and two contact times. At 4°C for 30 min, seven disinfectants were effective against FMDV ($>$ 4 $\log_{10}$ reduction), while only one disinfectant belonging to the oxidizing agents was effective against FMDV at both −10 and −20°C for 15 minutes.

Key Words: foot-and-mouth disease virus, anti-freezing diluent, subzero temperature

Foot-and-mouth disease (FMD), which produces blisters in the mouth and on the udder and feet, is an acute viral disease that affects cloven-hoofed animals[7)]. The occurrence of the disease causes tremendous economic loss due to high morbidity and a decrease of productivity[2)]. Since 2010, there have been six outbreaks in Korea, four of which have occurred during the winter season. In Korea, the average temperature in winter ranges from −6°C to 7°C, and the daily low temperature often goes down to approximate −20°C in some northern or mountainous regions[6)]. During that time, most liquid disinfectants were not used due to freezing. Therefore, in order to solve this problem in winter, it is necessary to consider the addition of antifreezes to disinfectants to lower the freezing point.

In this study, anti-freezing diluents (AFDs) were prepared and their freezing points tested between temperatures ranging from 0 to −30°C. In addition, the virucidal effects against the

*Corresponding author:
Hu-Jang Lee, College of Veterinary Medicine, Gyeongsang National University, 501 Jinju-daero, Jinju 52828, Korea
Phone: +82-55-772-2352. Fax: +82-55-772-2308. E-mail: hujang@gnu.ac.kr
Won-Seok Chae, Division of Life-Science & Chemistry, Daejin University, Pocheon 11159, Korea
Phone: +82-31-539-1844. Fax: +82-31-539-1840. E-mail: wschae@daejin.ac.kr
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Table 1. List of disinfectants used in this study

Samples	Classification	Active ingredient	Concentration of active ingredient	Used concentration
A	Oxidizing	KMPS + NaDCC	500 + 50 g/kg	1 : 1,100
B	Oxidizing	KMPS + MA	500 + 100 g/kg	1 : 1,500
C	Oxidizing	HP + CA	110 + 620 g/L	1 : 400
D	Acid	CA + BKC	200 + 100 g/L	1 : 1,000
E	Acid	DDAC + CA + PA	100 + 200 + 100 g/L	1 : 1,200
F	Acid	BKC + CA + PA	100 + 200 + 60 g/L	1 : 400
G	Acid	BKC + CA + PA	200 + 200 + 60 g/L	1 : 480
H	Aldehyde	GA + DCBAC	150 + 100 g/L	1 : 64
I	Oxidizing	NaDDC	5 g/ tablet (13 g)	1 : 190

KMPS, potassium monopersulfate; NaDCC, sodium dichloroisocyanurate; MA, malic acid; HP, hydrogen peroxide; CA, citric acid; BKC, benzalkonium chloride; DDAC, didecyldimethylammonium chloride; PA, phosphoric acid; GA, glutaradehyde; DCBAC, dodecyldimethylammonium chloride.

FMDV were confirmed at −10 and −20°C by adding the AFD composited with 15% ethanol (EtOH) and 30% propane-1,2-diol (PG) to nine commercially available disinfectants.

The disinfectants used in this study are the products that represent each category (oxidizing agents (4), acidic (4), and aldehyde (1)) and have sold well in the market over the past few years. The components and form of the disinfectants are listed in Table 1. The main components of these disinfectants are potassium monopersulfate (KMPS), citric acid (CA), sodium dichloroisocyanurate (NaDCC), and quaternary ammonium. These disinfectants consist of multi-active ingredients rather than a single ingredient, which helps create a high virucidal efficacy on viruses via synergistic effects[8].

The AFDs used in this study are prepared by using various ratios of EtOH (Daejung, Korea) and PG (Duksan, Korea) which were mixed with distilled water without purification. Each solute of the AFDs was used to minimize the environmental load and danger of fire due to the solutes. Freezing points of the AFDs were measured to −30°C by varying the composition ratio of EtOH and PG using the Korea Standard[9].

The virucidal efficacy of disinfects against FMDV of serotype O (Jincheon KOR2014) was evaluated in a previous study[6] and in accordance with the Korean guideline of disinfectant efficacy tests[13] and the US EPA guideline[14]. The experiment was carried out at three different reaction temperatures (4°C, −10°C and −20°C) and two contact times (30 and 15 min). Briefly, FMDV stock was diluted with hard water (HW; 0.305 g $CaCl_2$, 0.139 g $MgCl_2 \cdot 6H_2O$ in 1 l distilled water), and the titer of the virus solution was adjusted to $10^{7.5}$ 50% tissue culture infective doses ($TCID_{50}$)/ml. In addition, the disinfectants were diluted with the HW containing 5% fetal bovine serum (FBS; Sigma-Aldrich, USA) to one-half of the recommended dilution rate. Continually, 2.5 ml of the virus solution was added to the same amount of the diluted disinfectant in the tube. After reacting for 30 min at 4°C, 1 ml of this solution was mixed with the same amount of the neutralizing medium (Dulbecco modified Eagle medium (DMEM; Thermo Fisher Scientific, USA) containing 10% FBS), followed by 10-fold serial dilutions, and 200 µl of the diluted solution were infected with bovine kidney cell line (LFBK) suspensions seeded in 96-well microtiter plates. After incubation for 48 h at 37°C in 5% CO_2 atmosphere, the disinfectant-induced cytopathic effect (CPE) was checked and a $TCID_{50}$ was determined based on the method of Reed and Muench[11]. For subzero-temperature application, FMDV stock was diluted with the

AFD composited with 15% EtOH and 30% PG, and the titer of the virus solution was adjusted to $10^{7.5}$ $TCID_{50}$/ml. Also, the disinfectants were diluted with the AFD which did not freeze at −30°C to one-half of the recommended dilution rate. After the temperature of the virus solution and the diluted disinfectants were adjusted to −10 or −20°C in the self-made freezing chamber, 2.5 ml of the virus solution was added to the same amount of the diluted disinfectant in the tube. After reaction for 15 min at −10 or −20°C, the following experimental procedures were carried out in the same order as the above-mentioned test procedure at 4°C and the AFD-induced CPE was checked.

Fig. 1 shows a raw result set and how the temperature changes as the freezing process proceeds. When a solute is added to a solvent, the freezing point of the solution is lower than that of the pure solvent alone. This change in temperature is equal to the freezing point depression (ΔT) constant multiplied by the van't Hoff factor of the solute and the molality of the solution. $\Delta T = K_f \cdot m \cdot i$, where K_f is depression constant, m is molality of solution, and i is van't Hoff factor[12]. The AFD in a chilled chamber is cooled down to below the freezing point of itself, a process known as a supercooling phenomenon[4]. The supercooling occurs when the AFD is cooled, but ice does not form due to a lack of nucleation sites. As the water freezes, the temperature increases slightly due to the heat evolved during the freezing process as shown in Fig. 1. After that, the temperature slowly decreases to equilibrium with the chilled chamber to the setting temperature. Fig. 1 showed the temperature changes of the AFDs consisted with various concentration of EtOH and PG in a chamber adjusted to −30°C. The AFD containing 15% EtOH and 30% PG in the chamber did not show the supercooling phenomenon. In other words, it did not freeze at −30°C. From that result, the AFD containing 15% EtOH and 30% PG was chosen to test the virucidal efficacy of the disinfectants at subzero temperatures.

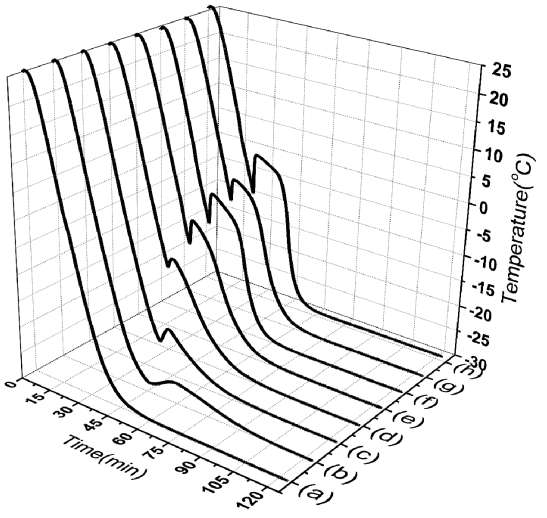

Fig. 1. Temperature changes of anti-freezing diluents prepared by various ratios of ethanol (EtOH) and propane-1,2-diol (PG) in a chamber adjusted to −30°C. (a), 15% EtOH and 30% PG; (b), 12% EtOH and 24% PG; (c), 10% EtOH and 21% PG; (d), 7% EtOH and 15% PG; (e), 5% EtOH and 10% PG; (f), 3% EtOH and 6% PG; (g), 2% EtOH and 3% PG; (h), hard water as control.

Table 2 shows the results of the efficacy test for the inactivation of FMD virus using the disinfectants at various reaction temperatures (4°C, −10°C and −20°C) and contact times (30 and 15 min). In this study, the CPE of all disinfectants and the 100 fold-diluted AFD was not observed. At the reaction temperature of 4°C for 30 min, seven (A–G) out of nine disinfectants had a virucidal effect against FMDV due to the reduction of more than 10^4 $TCID_{50}$/ml. However, the other two disinfectants (H and I) did not inactivate the FMDV. At the reaction temperature of −10 and −20°C for 15 min, only product C showed a reduction of 4.5 and 5.3 $\log_{10}$ ($TCID_{50}$/ml) at −10 and −20°C, respectively, indicating that there was a virucidal effect on the FMDV. Product C with a relatively lower dilution rate showed a virucidal efficacy against the FMDV at subzero temperature so that it could be more active in the AFD than the other oxidizing and acidic disinfectants. The AFD prepared at high concentrated organics to prevent freezing, is an organic condition, so it inhibits the virucidal activity of the disinfectant components. Therefore,

Table 2. Results of virucidcal efficacy test for nine commercial disinfectants against foot-and-mouth disease virus at various contact temperatures and contact times

Products	Dilution times	Reduction of $\log_{10}$ ($TCID_{50}$/ml)[a]		
		4°C[b]	Subzero temperature[c]	
			−10°C	−20°C
Control	–	5.8[d]	4.5	5.3
A	1,100	5.8	2.4	NT
B	1,500	5.8	0.4	NT
C	400	4.3	4.5	5.3
D	1,000	5.8	1.2	NT
E	1,200	4.3	0	NT
F	400	4.3	1.2	NT
G	480	5.1	1	NT
H	64	2.3	1.4	NT
I	190	3.3	0.4	NT

NT, not tested.
[a]This means a reduced $\log_{10}$ ($TCID_{50}$/ml) compared to the control.
[b]The reaction time of the virus solution and diluted disinfectant was 30 min.
[c]The reaction time of the virus solution and diluted disinfectant was 15 min.
[d]The median of values from three replicates.

in the case of the disinfectants with low concentration, that is, high dilution rate, they are less active against the FMDV, especially, at subzero temperature where the AFD concentration is high.

In a previous study[5,6], EtOH and PG were highly effective antifreezes for the freeze prevention of disinfectants at subzero temperatures. EtOH has many industrial and medical uses, such as in antifreeze, antiseptics and cosmetics[3] and generally degrades rapidly in the environment[10]. In addition, PG is commonly used in medications, antifreeze/engine coolant, foods, and cosmetics and is generally recognized as safe (GRAS) by the US Food and Drug Administration[1]. Therefore, the AFD containing EtOH and PG is an eco-friendly option.

In conclusion, considering the effects of the AFD tested for anti-freezing properties in some disinfectants at subzero temperature in this study, a general manual needs to be prepared to apply the AFD to commercially available disinfectants with the efficacy on the FMDV in winter.

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Conflict of Interest

The authors declare no conflicts of interest.

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