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SHORT COMMUNICATION

Conditions for the disinfectant efficacy test under subzero temperatures

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Abstract: To establish appropriate conditions for a disinfectant efficacy test at subzero temperatures, this study examined mixtures of frozen foot-and-mouth disease virus or avian influenza virus solutions and disinfectant diluents at −5ºC and monitored temperature and freezing status of an anti-freezing diluent (AFD, 15% ethanol + 30% propylene glycol + 55% distilled water) over time at various subzero temperatures. Viral solutions and disinfectant diluents froze before the mixtures reached −5ºC, whereas the AFD was not frozen at −30ºC. The times taken for the AFD to reach −10, −20, −30, and −40ºC from room temperature were 36, 39, 45, and 48 min, respectively.

Keywords: foot-and-mouth disease virus, avian influenza virus, disinfectant efficacy test, subzero temperature, anti-freezing diluent

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Occurrences of foot-and-mouth disease (FMD) and highly pathogenic avian influenza (HPAI) have been reported in Korea since 2000, and such infections have almost exclusively arisen during the period from the end of November to February [1, 2]. During that period in Korea, difficulties in disinfection arise due to freezing of the liquid disinfectants used for biosecurity. The disinfectants used for domestic animals marketed in Korea are products whose efficacies have been confirmed in accordance with the regulation on the veterinary disinfectant efficacy test guidelines (2017-29) [3]. However, most commercially available animal disinfectants are prepared by reacting a bacterial or viral solution with a disinfectant diluent at 4ºC for 30 min, based on the standard test conditions (STC) in the Korean regulation [3]. Therefore, there is a difference between laboratory and field conditions under which such disinfectants are used. In accordance with the selective test conditions (SEC) described in the Korean regulation [3], disinfectant efficacy under field conditions where organic matters such as feces and urine are high, and there are various temperature changes, can be confirmed by reacting the bacterial (or viral) solution and the disinfectant diluent for 15 min under different temperature conditions.

Recently, livestock epidemics such as FMD and HPAI have occurred mainly during the winter season, and several studies on disinfectants that are effective without freezing in the winter season, have been reported [4-6]. However, some problems with the experimental methods of the previous studies tested under subzero temperature conditions have arisen because the SEC presented in the Korean regulation are unclear. Therefore, this study was carried out with the aim of establishing precise conditions for a disinfectant efficacy test under subzero temperatures.

In this study, FMD virus (FMDV) and avian influenza virus (AIV) solutions and nine commercially available disinfectants (Table 1) were confirmed to be frozen at −5ºC. Additionally, the temperature and freezing status of an anti-freezing diluent (AFD, 15% ethanol + 30% propylene glycol + 55% distilled water) were measured and monitored over time at various subzero temperatures (−10, −20, −30, and −40ºC).

In the present study, the FMDV and AIV solutions and all disinfectant diluents froze before reaching −5ºC (data not shown). Figure 1 shows the time

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.

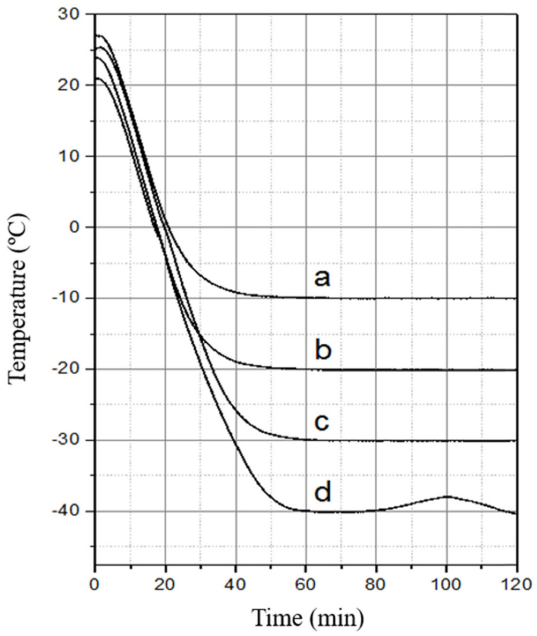

Fig. 1. Temperature-time dependence of an anti-freezing diluent (15% ethanol + 30% propylene glycol) in a chamber adjusted to −10°C (a), −20°C (b), −30°C (c), and −40°C (d).

required for the temperature of the AFD to reach various subzero temperatures and the freezing status of the AFD at those temperatures. The AFD was not frozen at −30°C, but it began to freeze after more than 15 min at −40°C. In addition, when exposed to temperatures of −10, −20, −30, and −40°C, respectively, the durations until the temperature of the AFD reached −10, −20, −30, and −40°C, respectively, were approximately 36, 39, 45, and 48 min, respectively.

Under the STC of the Korean regulation [3], the bacterial (or viral) solution and the disinfectant diluent adjusted to 4°C are allowed to react for 30 min. However, it would be consistent with the purpose of the SEC of the Korean regulation if the pathogen solution and the disinfectant diluent, when adjusted to the desired temperature, be allowed to react for 15 min.

In a previous study [4], an FMDV solution stored in a refrigerator was mixed in the same amount of a mixture of disinfectant diluent and deicer, adjusted to −20°C, and reacted in a freezer at −20°C for 5 and 30 min. However, it was overlooked that the temperature of the mixed solution became higher than −20°C when the viral solution at 4°C and the disinfectant and deicer mixture at −20°C were mixed in equal amounts. Additionally, although it takes time for the temperature of a mixed solution at higher than −20°C to reach to −20°C, the study's reaction times were fixed and did not consider the time taken for the mixture to cool to −20°C.

In other previous studies [5, 6], six commercially available disinfectants were diluted with hard water to the manufacturer's recommended dilution, and after the diluent was placed on ice at 0°C for 30 min, the disinfectant diluents were placed in a freezer set at −10°C for 10 min to reduce the temperature of the diluent to −10°C. Equal amounts of bacterial or AIV solutions at 4°C and the disinfectant diluent at −20°C were mixed and reacted for a set time in a freezer set to −10°C. The authors reported that the temperature of the disinfectant diluent dropped from 0°C to −10°C after 10 min in the freezer set to −10°C. However, in the present study, the disinfectant dilutions were completely frozen before cooling to −5°C, and the AFD took more than 25 min to cool to −10°C (Fig. 1). In the above studies [5, 6], although the initial temperature of the mixture of bacterial or AIV solutions at 4°C and the disinfectant diluent at −10°C was higher than −10°C, the mixture was placed in a freezer adjusted to −10°C and reacted for various set times. As pointed out for the study of Hong *et al.* [4], the authors of the above studies [5, 6] did not consider the time taken for the mixture to cool to −10°C.

Furthermore, Tsujimura *et al.* [7] reported on mixtures of 20 µL of equine herpes virus diluted four-fold with 20% methanol and 180 µL of one of six disinfectants (5 commercial disinfectants and 1 anionic surfactant) that were reacted at −10°C for 10 min. In their study, the disinfectant diluent and the viral solution were mixed without first adjusting them to −10°C, and the mixtures were reacted in a freezer

adjusted to −10°C for 10 min without considering the time taken for them to cool to −10°C; an approach that is inconsistent with the Korean regulation [3]. Thus, it is inappropriate to state that the reaction was carried out at −10°C for 10 min.

Despite the widespread use of astronomical disinfectants to control the spread of FMD and HPAI in Korea during winter periods, the disinfectants have not been effective in blocking the transmission of FMD and HPAI because the disinfectants are less than effective due to the freezing. In addition, the SEC for freezing conditions in the Korean regulation [3] were not clearly defined.

According to the results obtained in the present study, when the AFD was not added to the pathogen and disinfectant diluent mixture prior to the mixture attaining a subzero temperature (< −5°C), as per the SEC in the Korean regulation [3], the pathogen and disinfectant diluent mixture froze before reaching to −5°C. Thus, it was impossible to effectively perform the disinfectant efficacy test under subzero temperatures.

Therefore, there is a need to establish a new evaluation rule for the use of AFD for disinfectants. In addition, there is a need to specify in the SEC of the Korean regulation [3] that the bacterial (or viral) solution and disinfectant diluent (or mixture of disinfectant and deicer) mixture should be reacted for a set time at the same subzero temperature after the solutions and diluents are established at the appropriate subzero temperature.

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