

# Efficacy evaluation of commercial disinfectants by using *Salmonella enterica* serovar Typhimurium as a test organism

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The efficacies of six commercial disinfectants were evaluated by using *Salmonella enterica* serovar Typhimurium under simulated natural conditions such as sub-zero temperature, short disinfecting time, and surface type (uneven or smooth). We used a suspension model test to determine the disinfecting efficacy under varying contact times (1, 5, 10, and 30 min) and temperatures (25°C, 4°C, 0°C, and -10°C). The bactericidal effect according to surface structure was measured by using a carrier model test at 25°C and -10°C. The effective concentrations of each disinfectant were fixed to give a disinfecting effect within a short time (< 1 min) at 25°C and -10°C. The suspension model results revealed that bactericidal efficacy significantly dropped at low temperature for most of the disinfectants used; a sodium dichloroisocyanurate product showed the strongest efficacy. In the carrier test, bacterial load on a wooden surface was more difficult to remove than that on a stainless-steel surface. The results show that commercial disinfectant products vary in their disinfecting efficacy, which is affected by several field factors including temperature, contact time, and carrier material. Environmental conditions and surface type for disinfection should be considered prior to selecting an optimal disinfectant in the field.

**Keywords:** *Salmonella*, carrier-model test, disinfectant, surface structure, suspension-model test

## Introduction

Salmonellosis is an important public health issue. *Salmonella* (*S.*) *enterica* serovar Typhimurium and *S. enterica* serovar Enteritidis are responsible for most cases of human salmonellosis with over 80% of these infections being caused by poultry products, such as eggs and poultry meat [19,24].

Once chickens are exposed to *Salmonella*, the poultry flock is colonized quickly. The infected chickens shed *Salmonella* through feces, which leads to meat contamination during slaughtering. Adult laying hens can be asymptomatic carriers that continually produce contaminated eggs [19]. Therefore, a farm-level *Salmonella* eradication program is needed to eliminate *Salmonella* infection. Disinfection is a very important component of the biosecurity program designed by the poultry industry [20].

Adequate and correct disinfection procedures can reduce the incidence of diseases and their transmission. The kinetics of disinfection are altered by several environmental factors,

including organic load, temperature, and contact time [16]. Hence, when a disinfection program is adopted in a farm or industrial setting, many factors must be considered for successful decontamination [4].

In this study, changes in the efficacy, induced by different environmental factors such as temperature, contact time, and surface type, of disinfection of six disinfectants used in Korean farms were evaluated. Four contact times (1, 5, 10, and 30 min) and four temperature conditions (25°C, 4°C, 0°C, and -10°C) were investigated by using suspension tests. Bactericidal efficacy by surface type (wood and stainless steel) was evaluated by using carrier tests. Moreover, the concentration of each disinfectant was set to produce a disinfectant effect within a short time (< 1 min) at 25°C and -10°C to assess disinfectants in warm and cold seasons, respectively.

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## Materials and Methods

### Bacteria preparation

*S. Typhimurium* strain ATCC 13311, used as the test organism, was incubated in nutrient broth (Oxoid, USA) overnight at 37°C. The inoculum turbidity was determined by an absorbance of 0.005 at 600 nm by using a micro-ELISA plate reader (SoftMax Pro, USA).

### Disinfectants and carriers

Six disinfectants commonly used in farms were assessed. Each disinfectant was diluted in hard water, by dissolving 0.139 g magnesium chloride hexahydrate (Junsei Chemical, Japan) and 0.305 g calcium chloride (Sigma-Aldrich, USA) in de-ionized water, diluting to 1,000 mL and autoclaving at 121°C for sterilization. The hard water was stored in the refrigerator for no longer than 1 month. The working concentrations of each disinfectant were prepared based on the manufacturers' recommendations, which were also approved by the Animal and Plant Quarantine Agency (QIA), Korea, and are detailed in Table 1.

Two carriers with different surface structure types were used, birch wood piece (1.5 cm × 1.5 cm, square) and stainless steel (2 cm diameter, AISI 304; Posco, Korea). Before use, carriers were washed twice in deionized water and autoclaved at 121°C for 15 min.

### Suspension tests

Four different reaction temperatures (25°C, 4°C, 0°C, and -10°C) and contact times (1, 5, 10, and 30 min) were investigated. For low-temperature application, diluted disinfectants were kept on ice for 30 min at 0°C and then maintained in a refrigerator set at -10°C for 20 min.

Suspension (4 mL) containing the test organism ( $> 10^7$  CFU/mL, absorbance with ELISA reader = 0.005) was added to 96 mL of 5% yeast extract (Oxoid, USA) solution, and 2.5 mL of this mixture was then inoculated with 2.5 mL of each disinfectant. After the appropriate contact time, 1 mL of the solution was neutralized with 9 mL of Dey-Engley neutralizing

solution (Difco, USA) for 5 min. The number of residual viable organisms was determined by determining plate counts on 3M Petrifilm Aerobic Count Plates (3M, USA). The concentration establishment test was performed in the same way as the suspension test, except that the contact times used were 30 sec, 1 min, and 5 min.

### Carrier tests

Equivalent volumes of 3% bovine serum albumin (Sigma, USA) solution and test inoculum suspensions were mixed, and 100 µL of this mixture were inoculated onto each carrier. Each carrier in 6-well plates (Nunc, Denmark) was placed on a clean bench with airflow on and light off for 60 to 80 min. In the 25°C group, carriers and disinfectants were maintained at room temperature. In the -10°C group, dried carriers were placed in a refrigerator at -10°C for 10 min. Disinfectants were kept on ice before use. Each carrier was inoculated with 200 µL of each disinfectant, and after 1 and 5 min, the carriers were transferred to 50-mL tubes containing 10 mL of Dey-Engley neutralizing broth (Difco). The bacterial cells from the carriers were detached by full-speed vortexing for 3 min. The residual surviving bacteria were determined by plate counts on 3M Petrifilm aerobic count plates (3M).

### Statistical analysis

The bacterial enumeration data were expressed as mean and standard deviation values of the log<sub>10</sub> reduction value (n = 3). Statistical analysis (*t*-test) was performed by using SigmaPlot 8.0.2 (SPSS, USA). Significant differences were present when  $p < 0.05$ .

## Results

### Suspension tests

We considered a 5 log<sub>10</sub> reduction (99.999%) compared to the control which was not treated by disinfectants to quantify the recovered *Salmonella* by using the plate count method. At 25°C, 2.5% citric acid (CA) was effective ( $> 5$  log<sub>10</sub> reduction) after a 10 min contact; however, it did not inactivate *S. Typhimurium*

**Table 1.** List of disinfectants used in the study

| Classification  | Active ingredient | Concentration of active ingredient | Used concentration |
|-----------------|-------------------|------------------------------------|--------------------|
| Acid            | CA                | 400 g/L                            | 1:16               |
|                 | CA+QACs           | 200 g/L + 100 g/L                  | 1:600              |
| Oxidizing agent | MPS               | 500 g/kg                           | 1:100              |
|                 | MPS+NaDCC         | 500 g/kg + 50 g/kg                 | 1:50               |
|                 | NaDCC             | 1,000 g/kg                         | 1:300              |
| Aldehyde        | GA                | 100 g/L                            | 1:100              |

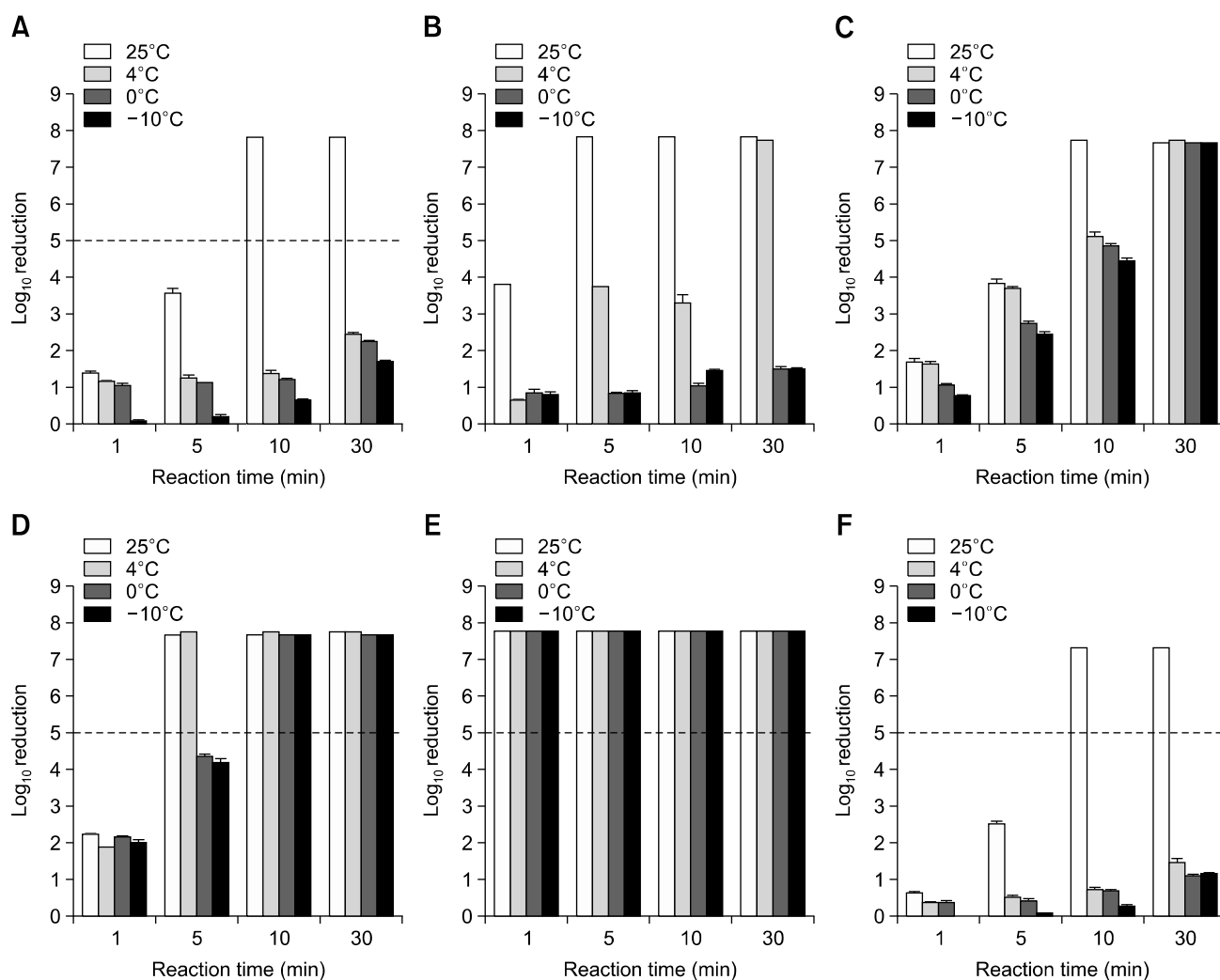
CA, citric acid; QACs, quaternary ammonium compounds; MPS, potassium peroxymonosulfate; NaDCC, sodium dichloroisocyanurate; GA, glutaraldehyde.

at low temperatures (4°C, 0°C, or -10°C) despite a 30 min contact time. At a reaction time of 30 min, 2.4, 2.24, and 1.7 reductions were recorded at 4°C, 0°C, and -10°C, respectively (panel A in Fig. 1). Although disinfection was slightly more rapid than that of CA at 25°C and 4°C, a similar pattern was observed in 0.03% CA + 0.02% quaternary ammonium compounds (QACs) at 0°C and -10°C. At 4°C, the CA + QAC combination showed 3.31 and 3.76 reductions in the 5 and 10 min reactions; however, 7.8 log<sub>10</sub> reduction (complete bactericidal activity) occurred in the 30 min reaction (panel B in Fig. 1). In 0.5% potassium peroxydisulfate (MPS), 7.7, 5.1, 4.87, and 4.45 reductions were observed at 25°C, 4°C, 0°C, and -10°C, respectively, at the 10 min contact time (panel C in Fig. 1). At the 30 min contact time, 7.8 log<sub>10</sub> was observed in all temperature conditions, while 1% MPS + 0.1% sodium dichloroisocyanurate

(NaDCC) showed faster efficacy (over 5 log<sub>10</sub> reduction attained at 5 min) than MPS only (panel D in Fig. 1). In suspension tests, 0.3% NaDCC was the most effective and rapid-acting agent, with 100% bactericidal activity at all temperature conditions and within a short time (1 min) (Fig. 1). In contrast, 0.1% glutaraldehyde (GA) was the most temperature-sensitive agent. With the exception of 25°C at 10 and 30 min, bacteria were hardly inactivated (panel F in Fig. 1). The levels of reduction by 0.1% GA were 1.47, 1.12, and 1.18 at 4°C, 0°C, and -10°C, respectively, after 30 min.

#### Carrier tests

Most disinfectants were more effective on the stainless-steel surface than on the wooden surface ( $p < 0.05$ ). Complete reduction of bacteria was defined as 5.1 log<sub>10</sub> reduction on

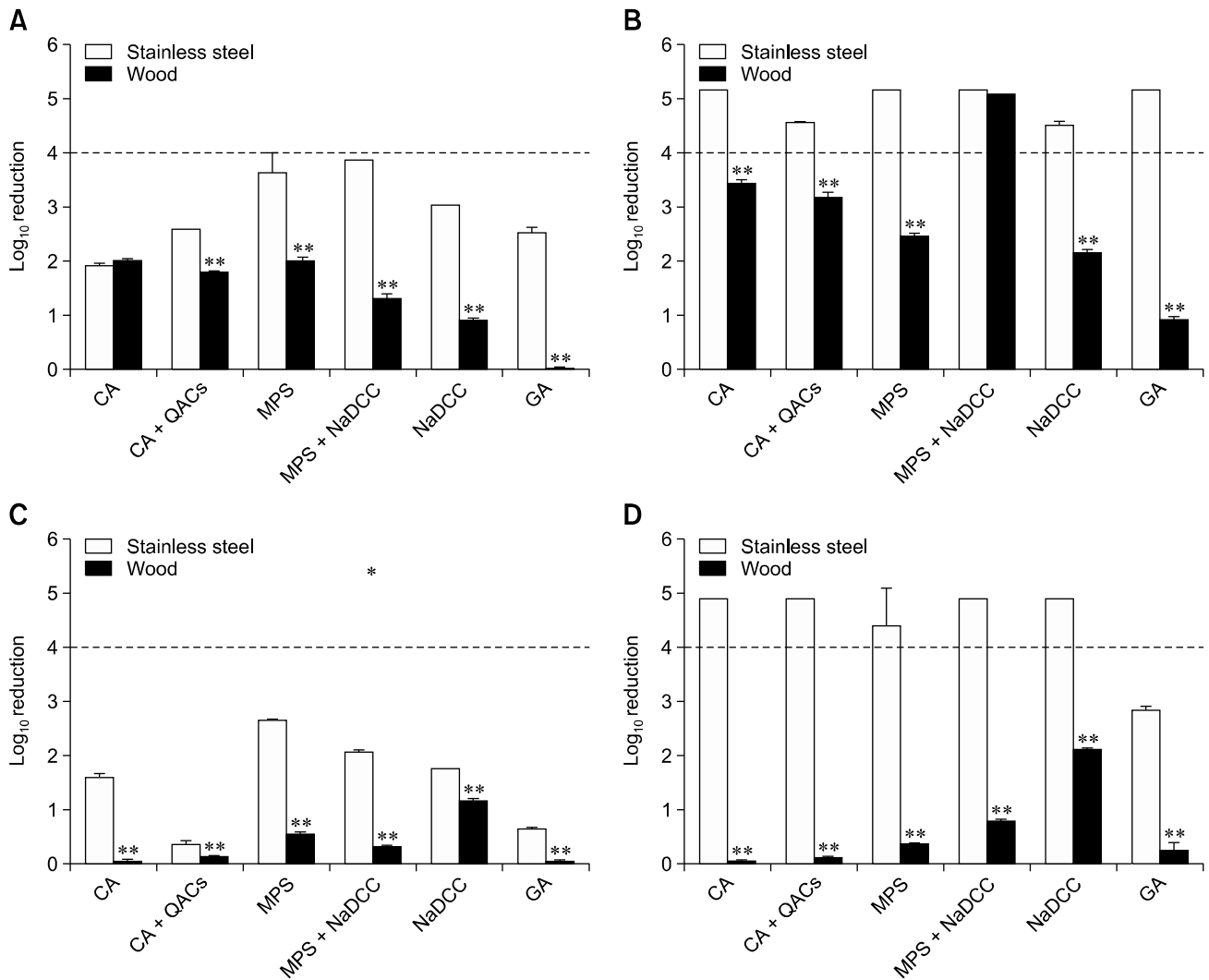


**Fig. 1.** Evaluation of *Salmonella* Typhimurium bactericidal activity of six disinfectants by suspension testing at four different contact times and temperatures. Complete reduction of bacteria was defined as  $\geq 7.8$  log<sub>10</sub> reduction, while a  $> 5$  log<sub>10</sub> reduction was considered effective. (A) 2.5% citric acid. (B) 0.03% CA + 0.02% QACs. (C) 0.5% MPS. (D) 1% MPS + 0.1% NaDCC. (E) 0.3% NaDCC. (F) 0.1% GA.

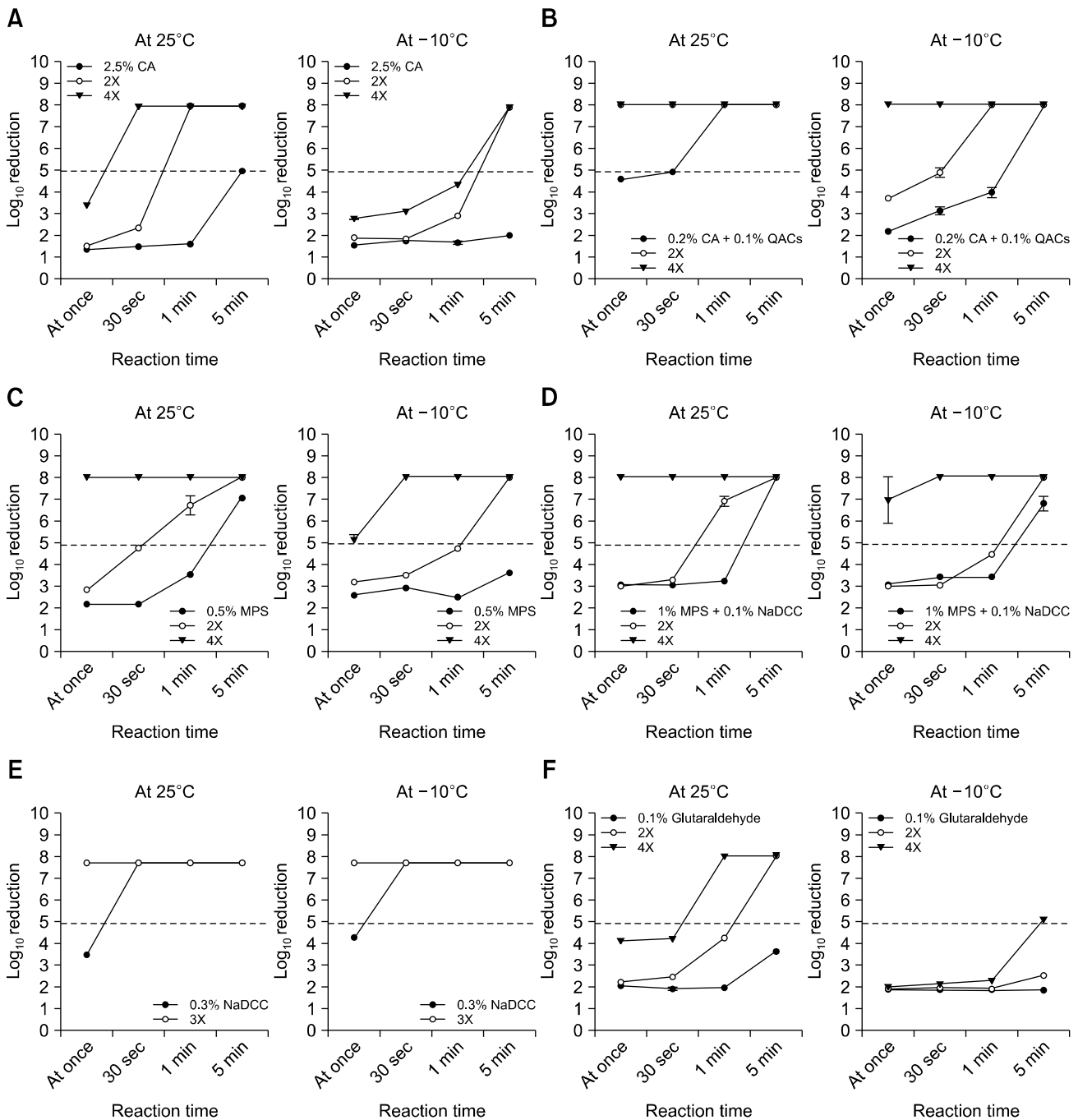
stainless steel and 5.09 log<sub>10</sub> reduction on wood, while a 4 log<sub>10</sub> reduction was defined as effective. For adequate disinfection of stainless steel at 25°C, a 5 min contact time was needed; whereas a > 5 min contact time was required for wood disinfection at the same temperature. At -10°C, the stainless-steel carrier test revealed that most disinfectants were able to completely decontaminate. *S. Typhimurium*; the exception being 0.1% GA with a 5 min contact time. However, not all disinfectants were effective against dried *S. Typhimurium* on wood at -10°C. Similar to the suspension test results, 0.1% GA showed 2.84 and 0.2564 log<sub>10</sub> reductions on stainless steel and wood, respectively, at -10°C. The oxidizing agents, namely, MPS, MPS + NaDCC, and NaDCC, were generally more effective than acid agents (Fig. 2).

**Establishment of effective concentrations under low temperature**

We evaluated the efficacy of using 2× and 4× the manufacturers' recommended concentrations of each disinfectant at 25°C and -10°C for 0 sec, 30 sec, 1 min, and 5 min. A complete bactericidal effect was observed after 30 sec of 10% CA (4×) treatment at 25°C, whereas 4× CA required 1 to 5 min for adequate efficacy (> 5 log<sub>10</sub> reduction) at -10°C (panel A in Fig. 3). The 2× and 4× concentrations of CA + QACs showed complete bactericidal effect at the time of treatment at 25°C, whereas only the 4× concentration of CA + QACs (0.8% CA + 0.4% QACs) reduced *S. Typhimurium* completely at the time of treatment at -10°C. Adequate bactericidal efficacy from 2× CA + QACs (0.4% CA + 0.2% QACs) required > 30 sec (panel



**Fig. 2.** The effect of each disinfectant against dried *Salmonella Typhimurium* on wood and stainless steel at 25°C or -10°C for 1 min or 5 min. Complete reduction of bacteria was defined as 5.1 log<sub>10</sub> reduction on stainless steel and 5.09 log<sub>10</sub> reduction on wood, while a 4 log<sub>10</sub> reduction was defined as effective. Error bars indicate standard deviations of the means. (A) 25°C for 1 min. (B) 25°C for 5 min. (C) -10°C for 1 min. (D) -10°C for 5 min. \*\*Significant differences between the results from stainless steel and wood (*p* < 0.01).



**Fig. 3.** Evaluation of bactericidal activity for short contact times by different concentrations of each disinfectant at 25°C and -10°C. Complete reduction of bacteria was defined as  $\geq 7.8 \log_{10}$  reduction and  $> 5 \log_{10}$  reduction was considered effective reduction. (A) Citric acid. (B) Citric acid + QACs. (C) MPS. (D) MPS + NaDCC. (E) NaDCC. (F) Glutaraldehyde.

B in Fig. 3). *S. Typhimurium* was adequately inactivated by 1% MPS administered for 0.5 to 1 min at 25°C. At -10°C, 1% MPS showed sufficient bactericidal effect after 1 to 5 min (panel C in Fig. 3). However, 2× MPS + NaDCC (2% MPS + 0.2% NaDCC) failed to provide  $> 5 \log_{10}$  reduction within 1 min at -10°C (panel D in Fig. 3). *S. Typhimurium* was inactivated

completely and immediately by 1% NaDCC (3×) at -10°C (panel E in Fig. 3). Although it was ineffective at -10°C, even after 5 min at 0.1% and 0.2% glutaraldehyde (2×), 0.4% glutaraldehyde (4×) showed  $> 5 \log_{10}$  reductions at 1 min at 25°C and at 5 min at -10°C (panel F in Fig. 3).

## Discussion

Disinfectants are used for disease prevention and control in a variety of fields [13]. Many livestock and poultry farms have introduced farm hazard analysis and critical control point (HACCP) programs that deem it mandatory to install foot dips in front of premises and disinfection facilities (including wheel dips) at farm entrances [20].

The efficacy of disinfectants is affected by disinfectant type, mode of application, exposure time, natural microbial population, surfaces, and temperature. Thus, we evaluated changes in the efficacy of six disinfectants under different conditions, including four contact times (1, 5, 10, and 30 min), four temperatures (25°C, 4°C, 0°C, and -10°C), and two surface types to reflect field environments.

Although foodborne salmonellosis outbreaks mainly occur in summer (> 20°C), *Salmonella* are able to survive > 550 days at -20°C and 4°C with no significant reduction [6]. Further, dried *Salmonella* cells on paper discs can survive for 2 years at 4°C [22]. The efficacy of most chemical disinfectants is negatively affected at low temperatures [4]. Due to the lack of scientific literature on the efficacy of disinfectants at extreme cold temperature, we conducted suspension tests under various temperatures, including below zero conditions, with *S. Typhimurium*, a representative test microorganism for disinfectant efficacy standards in QIA [1]. The disinfectants used in this study completely inactivated *S. Typhimurium* in 30 min at 25°C. However, modes of application, such as dipping, spraying, misting, or fumigation are not generally performed in the field for 30 min. because of evaporation and inactivation of the disinfectants by organic matter and soil [15]. Hence, an ideal disinfectant should be effective within a very short time, especially at low environmental temperatures. This study found that most disinfectants were unable to inactivate *S. Typhimurium* within 5 min at 25°C; hence, when disinfectants are applied at 25°C, the disinfection process should last at least 5 to 10 min and include soaking the surface being disinfected with the disinfectant solution. The 2.5% CA and 0.03% CA + 0.02% QACs showed inadequate efficacies at 4°C, 0°C, and -10°C, even with a prolonged contact time of 10 min. These two chemicals are commonly used in farms and food processing industries because of their safety levels compared to those of other chemicals [2]. The environmental temperature in milk or meat processing factories are maintained under 15°C in the work areas and under 4°C in the storage areas [8]. Moreover, the human infectious dose of *S. Typhimurium* is around 2 log<sub>10</sub> [7]. Therefore, it is necessary to determine the optimum concentration and contact time of disinfectants for the actual ambient temperature in the work area in order to achieve complete decontamination of *S. Typhimurium*. The activity of CA is enhanced by the presence of anionic detergents like QACs. Therefore, combination agents can be applicable in different

fields, including processing industries, after establishing the appropriate concentrations at low temperatures or by expanding the exposure times [12].

At 0°C and -10°C, 0.5% MPS and 1% MPS + 0.1% NaDCC were effective against *S. Typhimurium* at a > 10 min contact time, while 0.3% NaDCC showed complete *S. Typhimurium* inactivation, regardless of contact time or temperature. Although the liquid disinfectant is effective after 10 min at low temperatures, the temperature of the liquid is rapidly lowered in the field immediately after spraying during the cold season. Therefore, the disinfectant exposure time ends up being significantly shorter [15]. Thus, we conducted additional experiments to determine the effective concentrations for immediate disinfection at below zero temperatures.

A 4× of the manufacturer's recommended concentration of CA (10%) there was incomplete reduction of bacteria at 1 min at -10°C, whereas 4× concentrations of CA + QACs (0.8% CA + 0.4% QACs) showed immediate, complete reduction (*i.e.*, 7.8 log<sub>10</sub> reduction) at -10°C. *S. Typhimurium* was also inactivated by treatment with 2× concentrations of CA + QACs (0.4% CA + 0.2% QACs) for > 30 sec at -10°C. With the exception of 0.1% GA, the MPS-based disinfectants were able to show immediate, adequate bactericidal efficacy at 4× concentrations, which was the recommended concentration for use at -10°C. Among the six disinfectants, GA was unable to sufficiently inactivate *S. Typhimurium* at -10°C, and *S. Typhimurium* was only effectively reduced by 4× GA (0.4%) at -10°C after 5 min. Although most disinfectants were able to quickly (1 min) inactivate *S. Typhimurium* when their concentrations were increased, high concentrations of chemicals increase the risk of environmental pollution and risks to the person applying the disinfectant [12]. Aldehydes (formaldehyde and glutaraldehyde) are generally considered effective disinfectants at the farm level [5,10,11]. However, glutaraldehyde fumes are notably irritating and toxic to the mucous membranes, particularly those of the respiratory tract. Respiratory tract irritation is observed at 0.3 ppm concentrations [2]. In addition, a cross-sectional study of Dutch pig farmers showed that the use of disinfectants, including aldehydes, was an important etiological factor in chronic respiratory health effects [17]. In addition, high concentrations of or long exposure times to oxidizing agents and acids can be corrosive to metal surfaces [2]. Therefore, temperature range and exposure time should be considered when choosing an appropriate disinfectant.

Suspension tests are easy to perform; however, they have certain limitations. Pathogens adhere to equipment surfaces through organic or cellular debris [14,18]. Carrier tests are more relevant for predicting the activity of chemical disinfectants under field conditions [9]. Two kinds of carriers with different surface types were selected, namely, stainless steel (2 cm diameter, AISI 304B; Posco) and wood (1.5 cm × 1.5 cm square), to represent farm environments. The results for the

stainless-steel carrier at 25°C showed that disinfectant efficacy depended on the disinfectant's property, such as whether it was a fast-acting agent or not. Although most disinfectants did not inactivate to a  $> 4 \log_{10}$  reduction within 1 min, oxidizing agents such as MPS, MPS + NaDCC, and NaDCC can act quickly. At both 25°C and -10°C, stainless steel showed a  $> 4 \log_{10}$  reduction at 5 min contact time with most disinfectants except glutaraldehyde. With a 5 min contact time on wood at 25°C, most disinfectants, except MPS + NaDCC, showed a  $< 4 \log_{10}$  reduction. The difference in disinfectant efficacy between stainless steel and wood was greatest at -10°C. On wood, all six disinfectants were ineffective against dried *S. Typhimurium* after 5 min. A study by Yilmaz *et al.* [25] revealed that disinfection of porous surfaces is more difficult than that of non-porous surfaces, as porous surfaces impede the removal of the particles through cleaning or resuspension in the disinfectant solution. In this study, we found similar patterns of disinfection differences between dried bacteria on wood and the less porous stainless steel. Thus, cleaning should be performed prior to disinfection to overcome the efficacy difference of surface materials. Cleaning is regarded as a crucial procedure to eliminate infectious pathogens. After a single wipe with water and liquid soap, influenza virus has shown an approximate 4  $\log_{10}$  reduction [21]. However, successful elimination of *Salmonella* from poultry by cleaning and disinfection is labor intensive and costly, as well, it requires attention with respect to selection and application of the disinfectant [23]. A field trial showed that cleaning and disinfection programs have failed in 60% of 60 commercial laying houses due to cleaning difficulty associated with structures intrinsic to laying houses [3]. Therefore, appropriate disinfectants that will penetrate into organic and porous material in farms should be chosen.

This study aimed to evaluate efficacy changes among six disinfectants under various conditions including temperature, contact time, and surface type. The efficacy of most disinfectants was decreased by low temperatures, short contact times, and porous surfaces. Thus, environmental temperature, disinfection duration, and target surface should be considered for successful disinfection infield situations. Disinfectants that are intended for veterinary applications are assessed for efficacy by standard testing methods supported by national bodies, such as the Animal and Plant Quarantine Agency in Korea and our study used standard testing methods. As stated above, the practice of effective disinfection varies considerably according to the method of application, such as spray, mist, or fumigation; thus, various test protocols are required to reflect field conditions. Further studies are required to determine the ideal length of disinfectant contact time at low temperatures.

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## Conflict of Interest

The authors declare no conflicts of interest.

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