

Propylene glycol maintains the virucidal efficacy of disinfectants at sub-zero temperatures: implications for African swine fever biosecurity

Małgorzata Juskiewicz^{ID} · Marek Walczak · Grzegorz Woźniakowski · Sandra Lasota · Katarzyna Podgórska

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Abstract: African swine fever (ASF) is a highly contagious viral disease that continues to threaten global pig production. In the absence of an effective vaccine, strict biosecurity and disinfection remain the primary measures limiting virus transmission. However, in temperate climates winter conditions may significantly compromise disinfection efficacy, because many liquid formulations freeze at sub-zero temperatures, preventing proper surface coverage and virus–disinfectant contact.

Background: The present study evaluated the role of propylene glycol as a cryoprotective additive in disinfectant formulations used against African swine fever virus (ASFV). Selected active substances recommended for ASF control were tested in a suspension assay based on the PN-EN 14675 standard using ASFV Ba71V. Experiments were conducted at 21 °C, −10 °C and −20 °C under low and high levels of soiling conditions. **Results:** Preliminary experiments demonstrated that 30% propylene glycol prevented the freezing of disinfectant formulations without compromising their virucidal activity. Sodium hypochlorite, glutaraldehyde, potassium peroxymonosulfate, caustic soda and acetic acid retained strong virucidal activity across all temperatures, achieving $\geq 4 \log_{10}$ reductions in virus titer. In contrast, phenol, benzalkonium chloride and formaldehyde showed limited measurable activity, mainly due to cytotoxic effects or reduced performance under the tested conditions. **Conclusions:** These findings indicate that incorporation of propylene glycol can maintain disinfectant stability at sub-zero temperatures without compromising the efficacy of highly active compounds. Glycol-stabilized formulations may therefore improve the reliability of winter biosecurity protocols aimed at limiting ASFV transmission.

Keywords African swine fever virus · Disinfection · Biosecurity · Sub-zero temperatures · Propylene glycol · Cryoprotectant

Abbreviations

ASF	African swine fever
EU	European Union
ASFV	African swine fever virus
EURL	European Union Reference Laboratory

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FBS	Fetal bovine serum
TCID ₅₀ /mL	Tissue culture infectious dose
PEG	Polyethylene glycol
WOAH	World Organisation for Animal Health
BSA	Bovine Serum Albumin
BSA+YE	Bovine Serum Albumin with Yeast Extract
CPEs	Cytopathic effects
SD	Standard deviation

Introduction

African swine fever (ASF) is a highly contagious and often lethal viral disease affecting both domestic pigs and wild boar. With no approved vaccine or effective treatment currently licensed in the European Union (EU), ASF represents one of the most significant ongoing threats to global swine production [1]. Since its re-emergence in Eastern Europe, the disease has spread rapidly throughout Asia and parts of Western Europe, driven by a combination of natural transmission routes and human-mediated factors [2–4]. The virus's exceptional environmental resistance, coupled with its ability to persist in diverse matrices, including contaminated feed, fomites, and carcasses, greatly contributes to its complex epidemiology and renders disease control extraordinarily difficult [5–7].

The consequences of ASF outbreaks are profound, encompassing not only animal health impacts but also wide-reaching socio-economic disruptions [8–11].

Quantitative losses from ASF are substantial, with millions of pigs culled globally in an effort to contain the disease. These losses have deeply affected the agricultural sector, particularly in countries where pig farming represents a significant component of the economy [8]. In addition to direct animal losses, countries affected by ASF often face trade restrictions, severe disruptions in pork supply chains, and increases in pork prices [12]. The economic burden is further compounded by the costs associated with disease control measures, including enhanced biosecurity, surveillance programs, and compensation schemes for affected farmers [8]. As a result, ASF poses a multifaceted threat, impacting not only animal health but also food security, rural livelihoods, and broader economic stability across entire regions [9, 12].

Among available control measures, disinfection constitutes a cornerstone of ASF biosecurity. Effective inactivation of ASFV on contaminated surfaces, vehicles, equipment, and within animal housing facilities is essential to interrupt transmission pathways [7, 13–15]. However, environmental conditions, particularly low temperatures characteristic of temperate and continental climate zones during winter, represent a critical yet often underestimated challenge to disinfectant performance [5, 16].

Under sub-zero conditions, many conventional disinfectant formulations lose fluidity and freeze [17]. Even though virucidal properties of the active substance remain preserved, loss of the liquid state prevents adequate coating, surface wetting, and penetration into microstructures and organic residues. Consequently, a frozen or partially crystallized agent may not ensure sufficient contact between the active compound and viral particles, thereby compromising practical biosecurity effectiveness despite maintained chemical activity [16–18]. This physical limitation represents a major gap in winter disinfection strategies. In addition, low temperatures promote viral persistence, as demonstrated in several studies, thereby increasing the risk of environmental contamination [5].

To address this issue, the incorporation of cryoprotective additives into disinfectant formulations has been proposed as a means to maintain liquid-phase stability under freezing conditions [19, 20]. By lowering the freezing point and preserving formulation homogeneity, such additives may ensure sustained contact between active substances and viral particles [21]. Propylene glycol, a widely used cryoprotectant with favorable physicochemical properties and low toxicity, represents a promising candidate for winter biosecurity applications [21–23].

The present study aimed to evaluate the role of propylene glycol as a cryoprotective additive in disinfectant formulations against ASFV under sub-zero temperature conditions. By assessing both its freeze-preventing properties and its influence on virucidal efficacy, this work seeks to provide a scientifically grounded approach to improving seasonal biosecurity strategies in cold climates (Fig. 1).

Methods

Cells and viruses

The Vero-adapted ASFV strain Ba71V was supplied by the European Union Reference Laboratory (EURL, Spain). Cell culture procedures for the Vero line (ATCC® CCL-81™) involved propagation in Minimum Essential Medium (Gibco, Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Billings, MT, USA) and 1% antibiotic–antimycotic solution (100×; Sigma-Aldrich, St. Louis, MO, USA). Environmental conditions within the incubator were strictly maintained at 37 °C under a humidified 5% CO₂ atmosphere, following previously described cultivation protocols [17].

Virus stock preparation

To generate viral stocks, subconfluent Vero cell monolayers were inoculated with ASFV at a dose of 10⁶ TCID₅₀/mL and maintained at 37 °C until a complete cytopathic effect (CPE) emerged. To achieve a baseline titer sufficient to demonstrate a minimum 4 log₁₀ reduction (at least 10^{5.5} TCID₅₀/mL), the virus was concentrated via precipitation using a 20% polyethylene glycol (PEG) and 2.5 M NaCl buffer system, as detailed in our previous study [17]. Briefly, the virus-buffer mixture (3:2 ratio) was stirred overnight at 4 °C, after which the viral particles were pelleted by centrifugation at 13,000 × g for 90 min. The resulting pellet was resuspended in 1/10th of the

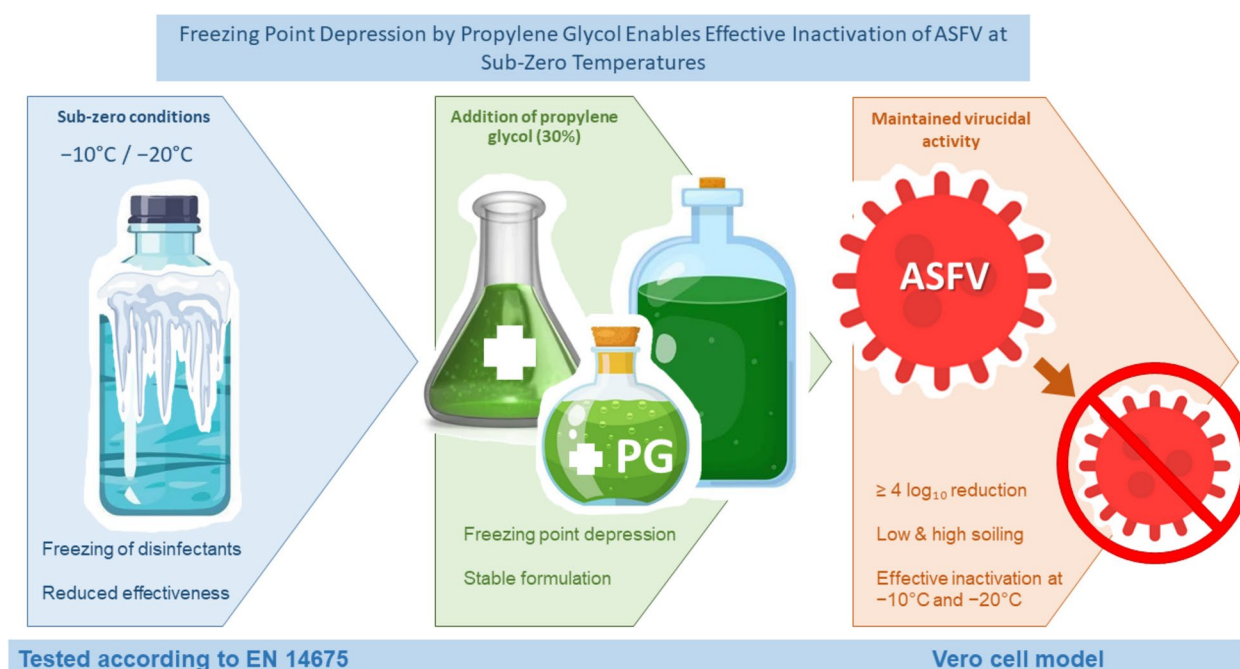


Fig. 1 Effect of propylene glycol on freezing point depression and preservation of virucidal efficacy of disinfectants against African swine fever virus at -10 °C and -20 °C

original medium volume. Aliquots of the viral stock were stored at -80°C following titration. Final viral infectious titers were calculated via the Spearman-Kärber method based on end-point dilutions [24, 25].

Disinfectants

Working solutions of each disinfectant were freshly prepared prior to each experimental run by dilution in standardized hard water. Baseline concentrations were chosen based on efficacy recommendations against ASFV outlined by the World Organisation for Animal Health (WOAH) [6] and backed by research [17, 26].

All tested chemical formulations and baseline working concentrations evaluated in this study represent the final active substance concentrations (w/v or v/v) within the final test matrix, rather than simple dilutions of commercial products. The working solutions were prepared from analytical-grade laboratory stocks or concentrated commercial formulations to achieve the following precise active criteria: sodium hypochlorite (prepared from a 15% active chlorine aqueous stock; Stanlab, Lublin, Poland) at a final concentration of 1% (v/v) active chlorine, glutaraldehyde (diluted from 25%; Carl Roth, Karlsruhe, Germany) at 0.1% (v/v), potassium peroxymonosulfate (Envolab, Długomiłowice, Poland) at 0.5% (w/v), caustic soda (sodium hydroxide; POCH) at 2% (w/v), acetic acid (Chempur) at 3% (v/v), formaldehyde (POCH, Gliwice, Poland) at 0.4% (v/v), phenol (Chempur, Piekary Śląskie, Poland) at 1% (v/v), and benzalkonium chloride (Pol-Aura, Olsztyn, Poland) at 1% (w/v). Each chemical agent was meticulously dissolved or diluted in hard water or the designated cryoprotectant matrix to ensure that the stated percentages reflect the true, exposure concentration of the active molecule during the virucidal assay.

Diluent and interfering substances

Standardized hard water (pH 7.0) containing defined concentrations of mineral ions (Mg^{2+} , Ca^{2+} , Cl^{-} , and HCO_3^{-}) was prepared according to the PN-EN 14675:2015 standard and used for all disinfectant dilutions. To evaluate virucidal efficacy under varying organic loads, suspension assays incorporated clean and dirty soil models as specified by the PN-EN 14675:2015 guidelines: low-level soiling was simulated with bovine serum albumin (BSA, 3.0 g/L), while high-level soiling relied on a mixture of BSA (10 g/L) and yeast extract (YE, 10 g/L) [17]. Furthermore, 30% propylene glycol was integrated into the system to serve as a cryoprotectant for the active ingredients under sub-zero testing regimes.

Cryoprotective additive

Prior to incorporating propylene glycol into disinfectant formulations, a preliminary assessment was conducted to determine its intrinsic virucidal activity and cryoprotective potential at different concentrations (30%, 60%, and 80%). This step was essential to distinguish between a true cryoprotective effect and a potential synergistic antiviral contribution of glycol itself. The results demonstrated that higher concentrations (60% and 80%) exhibited marked virucidal activity against ASFV, leading to substantial reductions in viral titer. In contrast, the 30% concentration did not produce a measurable reduction in ASFV titer and showed no independent virucidal effect under the tested conditions (Fig. 2). At the same time, 30% propylene glycol effectively prevented freezing at -10°C and -20°C . Therefore, this concentration was selected for subsequent experiments to ensure adequate antifreeze properties while allowing a reliable evaluation of the virucidal efficacy of the tested active substances without confounding antiviral interference from glycol itself.

To maintain the final intended concentration of each active substance after incorporation of 30% (v/v) propylene glycol, disinfectant formulations were prepared at appropriately increased pre-mix concentrations, calculated according to the final dilution factor, ensuring that the effective concentration of each active substance remained consistent with standard test conditions.

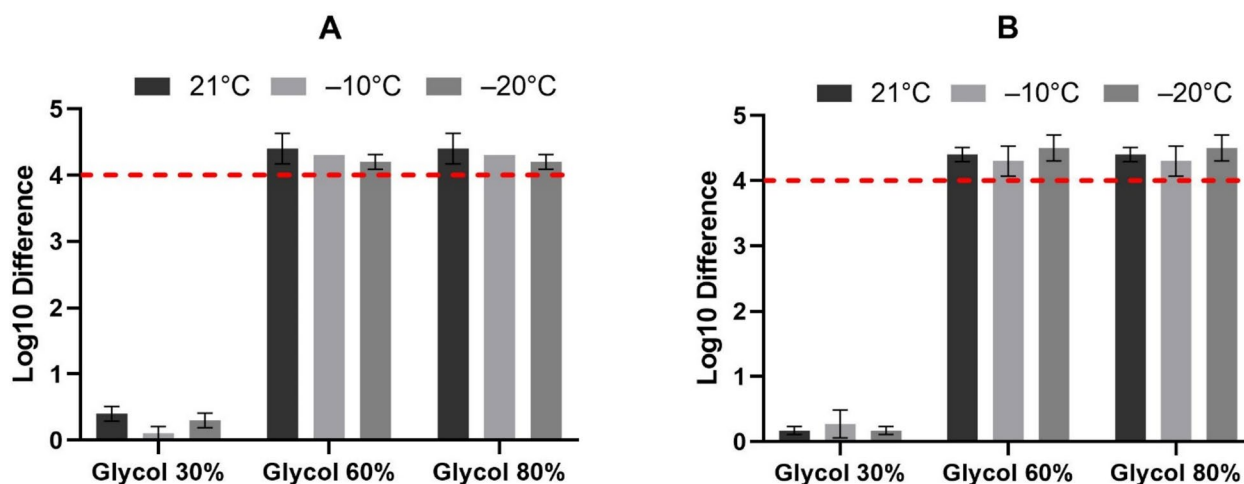


Fig. 2 Logarithmic differences of ASFV titers in the presence of various glycol concentrations with (A) a low soiling level (Bovine Serum Albumin—BSA) and (B) high soiling level (Bovine Serum Albumin with Yeast Extract—BSA+YE). Contact time, 30 min. Incubation temperature, 21 °C, -10 °C, -20 °C. Each data point is the mean of three experiments, $\pm$ SD

Temperature conditions

Virucidal suspension assays were executed in triplicate across three target temperatures (21 ± 1 °C, -10 ± 1 °C, and -20 ± 1 °C) adapting the core framework of the PN-EN 14675 standard as described previously [17]. Briefly, one part of the ASFV suspension was combined with one part of the respective organic soil (BSA or BSA+YE) and pre-incubated at the designated exposure temperature for 2 min (± 10 s). Next, eight parts of the 1.25-fold concentrated disinfectant (containing 30% propylene glycol) were added to the matrix. Following a 30-min (± 10 s) exposure at the specified temperature, the reaction was halted by transferring the test tubes to ice water (4 °C), in strict accordance with the validation and suppression protocols mandatory for the PN-EN 14675 standard. Both experimental and control mixtures were added to 96-well plates containing culture medium, where tenfold serial dilutions were performed in quadruplicate. Immediately following the dilutions, the plates were filled with a freshly prepared, uniform Vero cell suspension, ensuring immediate viral interaction during the cell sedimentation phase. The cultures were incubated at 37 ± 2 °C with 5% CO₂ for 7 days, with daily light microscopic evaluations to monitor CPE progression. Virucidal capability was determined based on the PN-EN 14675 pass-fail threshold, where a reduction in viral titer of ≥ 4 log₁₀ compared to the virus control is mandatory. To statistically validate this reduction, a baseline control titer of at least 5.5 log₁₀ TCID₅₀/mL was maintained throughout the trials.

Test controls

In the control samples, the chemicals were replaced with hard water. The controls were processed in the same manner as the chemical compounds tested. The mean titers of the virus controls were 5.97 ± 0.44 and 6.22 ± 0.53 log₁₀ TCID₅₀/mL for low level and high level soiling conditions, respectively.

Statistical analysis

Statistical analyses and data visualisation were performed using the GraphPad Prism software (version 8.4.3, La Jolla, CA, USA). Viral titers were determined using the Spearman-Kärber method and are expressed as the mean of three independent experimental replicates $\pm$ standard deviation (SD).

Results

Physical stabilization and baseline cryoprotectant evaluation

The addition of 30% (v/v) propylene glycol successfully prevented the freezing of all tested chemical formulations at sub-zero temperatures (-10°C and -20°C), maintaining proper liquid phase fluidity throughout the 30-min exposure time. Preliminary baseline controls demonstrated that 30% propylene glycol alone exhibited no independent virucidal activity against ASFV (Fig. 2), confirming that any observed viral inactivation is solely attributable to the active ingredients.

Active substances retaining robust virucidal efficacy

Among the evaluated disinfectants, sodium hypochlorite (1%), glutaraldehyde (0.1%), potassium peroxymonosulfate (0.5%), caustic soda (2%), and acetic acid (3%) demonstrated robust and stable virucidal performance across the entire temperature range (21°C , -10°C , and -20°C). In the presence of 30% glycol, these compounds consistently met the PN-EN 14675 pass threshold under both low (BSA) and high (BSA+YE) soiling levels. The viral titer reductions for this highly effective group remained within a high range of 4.1 ± 0.23 to $5.5 \pm 0.2 \log_{10}$ steps. Notably, acetic acid (3%) displayed the highest overall clearance, maintaining a stable reduction of 4.8 to $5.5 \log_{10}$ regardless of organic load or sub-zero thermal conditions. Complete numerical values and graphical comparisons are systematically compiled in Table 1 and Fig. 3, respectively.

Compounds incompatible with glycol-stabilization or limited by cytotoxicity

In contrast, formaldehyde (0.4%), phenol (1%), and benzalkonium chloride (1%) failed to demonstrate a measurable or reliable virucidal effect under glycol-stabilized conditions. Phenol (1%) showed negligible activity, with titer reductions failing to exceed $1.3 \log_{10}$ at room temperature and dropping below $0.3 \log_{10}$ at sub-zero levels. For formaldehyde (0.4%) and the quaternary ammonium compound—benzalkonium chloride (1%), measurable $\log_{10}$ differences hovered consistently low between 1.2 and $1.8 \log_{10}$ steps across all tested environments. However, these low scores do not necessarily indicate an absence of virucidal potential. Rather, they reflect severe host-cell cytotoxicity on the Vero line, which could not be technically bypassed via microfiltration in the presence of glycol. Consequently, these three active agents cannot be recommended for use within glycol-supplemented winter biosecurity matrices.

Discussion

Environmental temperature may be a critical determinant of disinfectant performance against ASFV, particularly under conditions relevant to winter biosecurity in temperate and continental climate zones. Low temperatures represent a severe biosecurity vulnerability in external transport disinfection sectors, vehicle wheel baths, as well as slaughterhouses, and cold-chain meat processing units. In the absence of cryoprotective additives, all evaluated disinfectant formulations freeze at sub-zero temperatures (-10°C and -20°C), which may effectively prevent adequate contact between active substances and viral particles [20]. This crystallization and phase transition behavior was previously demonstrated and empirically validated under identical thermal profiles in our study [17]. This physical limitation alone underscores a significant vulnerability of conventional disinfection protocols during winter months, when ASFV persistence in the environment remains epidemiologically relevant.

The use of antifreeze additives represents a rational strategy to maintain disinfectant performance under freezing conditions. Compounds such as propylene glycol, methanol, or certain inorganic salts have been shown to preserve formulation stability at low temperatures and, in some cases, to enhance virucidal effectiveness in cold

Table 1 Logarithmic reduction of ASFV titers in the presence of tested active substances with glycol addition. Contact time, 30 min. Temperature of incubation, 21 °C, −10 °C, −20 °C. Each data point is the mean of three experiments, ± SD

Active substance containing 30% glycol	Temperature (C)	Log ₁₀ Difference** (± SD) (TCID ₅₀ /mL)		Virucidal effect (Difference ≥ 4 Log ₁₀)	
		BSA	BSA+YE	BSA	BSA+YE
Sodium hypochlorite (1%)	21	4.2 (± 0.11)	4.1 (± 0.23)	Yes	Yes
	−10	4.3 (± 0.0)	4.5 (± 0.2)	Yes	Yes
	−20	4.2 (± 0.11)	4.8 (± 0.2)	Yes	Yes
Glutaraldehyde (0.1%)	21	4.2 (± 0.11)	4.6 (± 0.11)	Yes	Yes
	−10	4.3 (± 0.0)	4.4 (± 0.23)	Yes	Yes
	−20	4.2 (± 0.11)	4.8 (± 0.2)	Yes	Yes
Potassium peroxymonosulfate (0.5%)	21	4.4 (± 0.23)	4.6 (± 0.11)	Yes	Yes
	−10	4.3 (± 0.2)	4.4 (± 0.23)	Yes	Yes
	−20	4.3 (± 0.2)	4.8 (± 0.2)	Yes	Yes
Formaldehyde (0.4%)*	21	1.2 (± 0.51)	1.3 (± 0.54)	N/A	N/A
	−10	1.3 (± 0.2)	1.4 (± 0.23)	N/A	N/A
	−20	1.3 (± 0.2)	1.8 (± 0.2)	N/A	N/A
Caustic soda (2%)	21	4.2 (± 0.11)	4.6 (± 0.11)	Yes	Yes
	−10	4.3 (± 0.0)	4.5 (± 0.2)	Yes	Yes
	−20	4.2 (± 0.11)	4.7 (± 0.11)	Yes	Yes
Phenol (1%)	21	1.3 (± 0.2)	0.3 (± 0.3)	No	No
	−10	0.3 (± 0.3)	0.1 (± 0.11)	No	No
	−20	0.2 (± 0.11)	0.3 (± 0.2)	No	No
Benzalkonium chloride (1%)*	21	1.3 (± 0.31)	1.3 (± 0.11)	N/A	N/A
	−10	1.5 (± 0.20)	1.5 (± 0.20)	N/A	N/A
	−20	1.3 (± 0.11)	1.2 (± 0.11)	N/A	N/A
Acetic acid (3%)	21	5.0 (± 0.0)	4.8 (± 0.11)	Yes	Yes
	−10	4.8 (± 0.11)	5.5 (± 0.2)	Yes	Yes
	−20	5.0 (± 0.2)	5.5 (± 0.2)	Yes	Yes

**Difference between control and tested sample, *—Cytotoxic effect, N/A – virucidal effect could not be evaluated due to cytotoxicity. BSA – bovine serum albumin 3.0 g/L (low soiling level simulation), BSA + YE – bovine serum albumin 10.0 g/L + yeast extract 10.0 g/L (high soiling level simulation)

environments [21, 27]. By preventing crystallization and ensuring sustained contact between the active substance and viral particles on surfaces they were used on, these additives may mitigate the decline in efficacy observed in subzero temperature conditions [27, 28]. Therefore, incorporating appropriate cryoprotective agents can be considered a supportive modification for disinfectants whose activity is otherwise reduced at low temperatures, particularly in seasonal biosecurity applications [21].

From a biological and environmental perspective, propylene glycol represents a favorable additive due to its established toxicological safety profile and widespread used in pharmaceutical, veterinary, and food applications [22, 23, 28]. Importantly, preliminary experiments confirmed that 30% propylene glycol did not exhibit independent virucidal activity against ASFV, allowing a clear distinction between cryoprotective and antiviral effects. Higher concentrations (60% and 80%) demonstrated virucidal properties, which justified the selection of 30% for subsequent experiments to avoid confounding synergistic effects.

The incorporation of 30% propylene glycol fully prevented freezing at both tested sub-zero temperatures, thereby ensuring maintenance of the liquid phase and enabling reliable assessment of virucidal efficacy under cold-climate conditions. These observations extend and complement our previously published findings evaluating disinfectant efficacy at sub-zero temperatures without cryoprotective additives [17]. In that earlier study, freezing of formulations significantly limited practical applicability under cold conditions [17]. The current work builds on the previous findings by incorporation of 30% propylene glycol, which effectively prevents freezing while preserving, and in some conditions enhancing, virucidal performance.

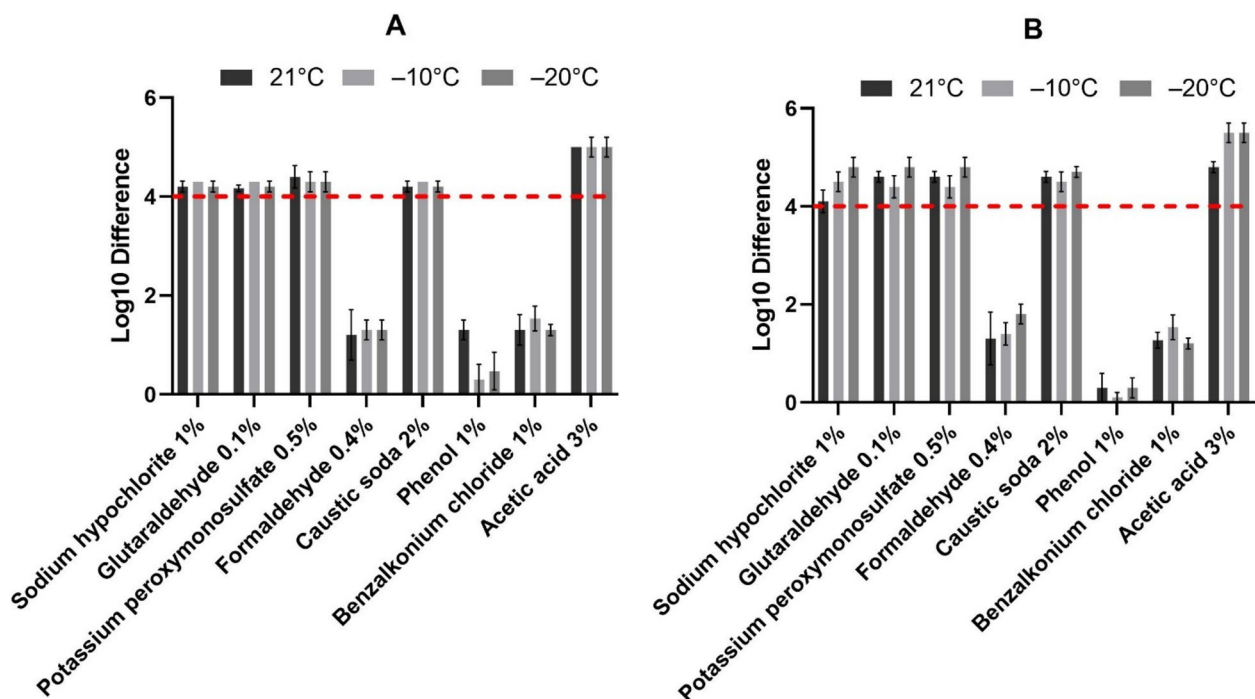


Fig. 3 The effect of temperature on the efficacy of selected disinfectants with 30% glycol added, in the presence of (A) a low level of soiling (BSA) and (B) a high level of soiling (BSA+YE), under three different temperature conditions (21 °C, -10 °C, and -20 °C). The mean detectable $\log_{10}$ differences between the control and tested samples, considering the standard deviation, are presented. The red dashed line—virucidal effect threshold

The data further indicate that for disinfectants with high antiviral activity, namely sodium hypochlorite, glutaraldehyde, potassium peroxymonosulfate, caustic soda, and acetic acid, the addition of 30% propylene glycol did not impair virucidal efficacy. In several instances, comparable or even higher $\log_{10}$ reductions were observed at -20 °C than at 21 °C, particularly under high soiling conditions. This observation is noteworthy, as organic matter is typically recognized as a major antagonistic factor reducing disinfectant efficacy [20, 26, 29, 30]. One possible explanation is that at very low temperatures, the kinetics of disinfectant neutralization reactions by organic substrates may be slowed, thereby reducing the protective effect of organic matter and prolonging effective contact time between active compounds and viral particles. Similar temperature-dependent modulation of disinfectant-organic matter interactions has been reported for other enveloped viruses, including influenza A virus, bovine herpes virus and coronaviruses, where altered reaction dynamics at reduced temperatures influenced virucidal outcomes [18, 31–33]. Although mechanistic confirmation requires further investigation, the present findings suggest that the combination of sub-zero temperature and cryoprotective stabilization may partially mitigate the inhibitory effect of organic contamination.

Interestingly, during the study, a protective effect of propylene glycol on cell cultures was observed. In selected cases, particularly for glutaraldehyde- and acetic acid-based formulations, which previously exhibited cytotoxic effects on Vero cells, the addition of propylene glycol appeared to reduce cellular damage, suggesting a modulatory effect on cell membrane interactions and chemical bioavailability. This observation may suggest a potential mitigating influence of propylene glycol on the cytotoxicity of certain disinfectant components.

The reduced $\log_{10}$ reductions observed for formaldehyde and benzalkonium chloride should not be interpreted as definitive evidence of limited antiviral activity. Rather, the high cytotoxicity of these compounds toward Vero cells, combined with the inability to perform post-incubation microfiltration without simultaneous removal of glycol, restricted accurate evaluation of their virucidal potential under glycol-containing conditions. Similarly, phenol exhibited diminished virucidal activity following glycol addition, suggesting possible physicochemical

interaction affecting its antiviral performance. On the basis of these findings, formaldehyde, benzalkonium chloride, and phenol cannot be recommended for use in glycol-stabilized formulations in sub-zero temperature conditions.

The practical implications of these findings are significant for winter biosecurity management in ASF-endemic regions. Disinfectants are routinely applied in vehicle disinfection stations, footbaths, equipment surfaces, and farm entry points, where freezing conditions may compromise efficacy precisely when environmental virus stability remains high. The demonstrated ability of 30% propylene glycol to prevent freezing while preserving virucidal activity suggests that glycol-stabilized formulations may provide a reliable and scientifically supported modification of winter disinfection protocols. Maintaining liquid-state integrity ensures proper surface coverage and adequate contact time, both essential for effective virus inactivation. Incorporating validated cryoprotective additives into seasonal biosecurity strategies may therefore enhance the robustness and reliability of ASF control measures during periods of negative temperatures.

Limitations of the study

While this study provides valuable experimental insights into cold-weather virus inactivation, several inherent limitations must be acknowledged. First, all evaluations were conducted using an *in vitro* suspension assay based on the standardized PN-EN 14675:2015 framework. While suspension assays are essential for determining baseline chemical parameters, they do not fully replicate the mechanical challenges of real-world porous, rough, or corroded surfaces. Second, the true virucidal capacity of formaldehyde and benzalkonium chloride remained partially masked due to unavoidable host-cell cytotoxicity, preventing an absolute assessment of their efficacy boundaries. Third, the organic soil matrices used (BSA and yeast extract), though strictly regulated by European validation standards, lack the complex biological, cellular, and lipid composition of natural field matrices such as liquid manure or decomposed organic material. Lastly, this study relied on the cell-culture adapted, avirulent ASFV strain Ba71V; while biophysically representative of the virus, future studies should incorporate virulent field strains (e.g., Genotype II) on primary alveolar macrophages to firmly translate these cryoprotective protocols into legislative field guidelines.

Conclusions

This study demonstrates that sub-zero temperatures pose a critical challenge to ASF biosecurity due to the freezing of conventional disinfectant formulations, which prevents effective virus-disinfectant contact. The incorporation of 30% propylene glycol successfully prevented freezing at -10°C and -20°C while preserving the virucidal efficacy of highly active disinfectants against ASFV. In several cases, enhanced reductions were observed under high soiling conditions at -20°C , suggesting that the combination of cryoprotection and low temperature may attenuate the antagonistic effects of organic matter. It should be noted that the slowed neutralization kinetics at -20°C remains a speculative hypothesis requiring further direct kinetic assays. Importantly, 30% propylene glycol exhibited no independent antiviral activity, ensuring that observed effects were attributable to the active disinfectant compounds.

While evaluation of results for certain agents, including formaldehyde, benzalkonium chloride, and phenol, was limited by cytotoxicity or reduced performance under glycol-containing conditions, the majority of tested disinfectants retained stable and robust activity across the evaluated temperature range. These findings provide experimental evidence supporting the implementation of glycol-stabilized disinfectants as part of winter-adapted ASF biosecurity protocols. Accounting for environmental temperature, organic load, and formulation stability is essential for ensuring consistent disinfection efficacy and reducing the risk of virus persistence and mechanical spread during cold seasons.

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Data availability All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare that they have no competing interests.

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Authors and Affiliations

Małgorzata Juskiewicz¹  · Marek Walczak¹ · Grzegorz Woźniakowski² · Sandra Lasota¹ · Katarzyna Podgórska¹

✉ Małgorzata Juskiewicz
malgorzata.juskiewicz@piwet.pulawy.pl

Marek Walczak
marek.walczak@piwet.pulawy.pl

Grzegorz Woźniakowski
grzegorz.wozniakowski@umk.pl

Sandra Lasota
slaso-ta@piwet.pulawy.pl

Katarzyna Podgórska
katarzyna.podgorska@piwet.pulawy.pl

¹ Department of Virology and Viral Animal Diseases, National Veterinary Research Institute, Par-Tyzantów 57 Avenue, 24-100, Puławy, Poland

² Department of Infectious, Invasive Diseases and Veterinary Administration, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Toruń, Lwowska 1 Street, 87-100 Toruń, Poland