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Understanding the Bactericidal and Virucidal Capacity of Potassium Peroxymonosulfate in Varied Environments and Its Utilization in Eradicating Germs on Diverse Surfaces and Clothing

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Understanding the Bactericidal and Virucidal Capacity of Potassium Peroxymonosulfate in Varied Environments and Its Utilization in Eradicating Germs on Diverse Surfaces and Clothing

Darsaniya Punyadarsaniya [1,2], Nutnaree Kunanusont [2], Kazuaki Takehara [3] and Sakchai Ruenphet [1,2,*]

[1] Immunology and Virology Department, Mahanakorn University of Technology, Bangkok, Thailand; darsaniya_p@yahoo.de (D.P.); sakchai@mut.ac.th (S.R.)
[2] Master of Science Program in Animal Biotechnology, Mahanakorn University of Technology, Bangkok, Thailand; darsaniya_p@yahoo.de (D.P.); dr.pukpuipony@gmail.com (N.K.); sakchai@mut.ac.th (S.R.)
[3] Laboratory of Animal Health, Department of Veterinary Medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Japan; takehara@cc.tuat.ac.jp (K.T.)
* Correspondence: sakchai@mut.ac.th; +6694-871-8888

Simple Summary: The present study evaluated the disinfectant efficacy in an aqueous phase and applied it to artificial contaminated carrier surfaces and clothes, comparing potassium peroxymonosulfate's effectiveness with sodium dichloroisocyanurate and quaternary ammonium compounds. The present study demonstrated potassium peroxymonosulfate's ability to inactivate food-borne bacteria such as *Salmonella* Infantis resistant to rifampicin, *Escherichia coli* and *Salmonella* Infantis, along with enveloped viruses like Newcastle disease virus and avian influenza virus. Moreover, potassium peroxymonosulfate displayed superior effectiveness on diverse carrier surfaces and artificially contaminated clothes in comparison to sodium dichloroisocyanurate and quaternary ammonium compounds. Nevertheless, the effectiveness in killing bacteria and viruses relies on optimal concentration, organic material presence, and exposure/contact duration. Hence, potassium peroxymonosulfate represents a viable alternative disinfectant, especially for bolstering biosecurity in managing bacteria and viruses in animal farms, slaughterhouses, or hospitals.

Abstract: The study evaluated potassium peroxymonosulfate (PPMS) efficacy against rifampicin-resistant *Salmonella* Infantis (rif-SI), *Escherichia coli* (E. coli), *Salmonella* Infantis (SI), Newcastle disease virus (NDV) and avian influenza virus (AIV). The assessment encompassed varied concentrations, organic material conditions, and exposure durations to comprehensively evaluate PPMS efficacy. Moreover, PPMS's efficacy was tested on diverse carrier surfaces and clothing to evaluate its bactericidal and virucidal potential. At the recommended concentrations (1X), PPMS effectively inactivated all pathogens within 5 sec, except for NDV, which required 30 sec in the presence of organic materials. At 0.5X, PPMS successfully inactivated *E. coli*, AIV and NDV within 5 sec in the absence of organic material. However, rif-SI and SI necessitated 30 sec for inactivation. Most pathogens were inactivated within 30 sec, with the exception for AIV, which was inactivated within 5 sec. PPMS effectively eliminated rif-SI on all carriers within 30 sec. Moreover, at both concentrations, PPMS successfully inactivated AIV on all carriers within 30 sec, except on stainless steel at 0.5X. Furthermore, PPMS at both concentrations required 5 and 30 sec, respectively, to inactivate AIV on the rayon sheet. Therefore, due to its demonstrated efficacy against a wide range of pathogens across diverse surfaces, PPMS emerges as a crucial alternative disinfectant significantly enhancing biosecurity on and around chicken farms.

Keywords: bactericidal; carrier surface; cloth; disinfectant; potassium peroxymonosulfate; virucidal

1. Introduction

Food products from animals, like eggs, meat, and milk, are susceptible to contamination by various pathogens leading to foodborne illness, notably salmonellosis, colibacillosis, and campylobacteriosis [1]. Typically, bacterial transmission in chicken products primarily happens

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between flock through the transport of cages that carry faecal contamination from previously transported chickens, particularly those with unclean floor surfaces [2]. Various researchers have delineated the pathways through which salmonella enteritis spreads through direct contact, involving infected animals and fomites, among which are plastic chicken transport cages [3,4]. Viral diseases in poultry, notably Newcastle disease (ND) and avian influenza (AI), are highly contagious and significantly affect the poultry industry's economics [5]. Infected birds release substantial viral agents from their respiratory and digestive systems, leading to environmental contamination [6]. Ensuring effective disease control necessitates implementing comprehensive sanitation and biosecurity measures on and around farms. Achieving successful disease control against viral infections requires the optimal application of disinfectants within sanitation and biosecurity programs. The Ministry of Agriculture, Forestry and Fisheries in Japan has implemented a hazard analysis and critical control point (HACCP) in animal farms to ensure food safety [7]. Alongside, the Japan Good Agricultural Practice (JGAP) was established in April 2017 by the Japan GAP Foundation to monitor and regular animal farm standards [8]. Enforcement of biosecurity measures on farms is crucial for both these systems. An integral part of the HACCP strategy involves using appropriate disinfectants to enhance biosecurity in and around animal farms.

Researchers have found that bacteria and virus pathogens present in organic matter from infected birds like feces, saliva, or vomitus, exhibit high tolerance to various disinfectants [9]. Pathogens, being resilient to disinfectants, can persist and spread through footwear worn by farmers, employees, and farm visitors [4]. They can also persist on fomites such as food, plastic egg tray, chicken cage and farm clothes [10–12]. Hence, effective disease prevention and control, particularly in the poultry industry [13], hinges on the critical application of disinfectants to mitigate pathogens transmission through various medium.

Potassium peroxymonosulfate (PPMS), also known as potassium monopersulfate (PMPS), acts as a broad-spectrum disinfectant by oxidizing bacterial and viral protein capsids. This process releases and inactivates the nucleic acids of viruses. The effectiveness of this compound in killing bacteria and viruses relies on various factors, including concentration, contact time, and the presence of organic materials [14]. Because of its safety and versatility, PPMS finds extensive application as a multipurpose virucidal disinfectants at specific concentrations [7,15]. For instance, it is employed in the livestock industry for disinfecting animal shelters, meat production facilities, and swimming pools [16]. Furthermore, products containing PPMS have demonstrated the ability to deactivate the severe acute respiratory syndrome coronavirus (SARS-CoV), much like NaClO [17,18]. While PPMS powder can cause severe skin and ocular burns upon direct contact due to its corrosive nature, the solution from does not cause irritation and is safe for both animals and humans [7,16]. Particularly, the efficacy of PPMS was assessed rifampicin-resistant *Salmonella* Infantis (rif-SI), *Escherichia coli* (*E. coli*), *Salmonella* Infantis (SI), and enveloped viruses like Newcastle disease virus (NDV) and avian influenza virus (AIV). This assessment covered diverse concentrations, organic material conditions, and exposure durations. Furthermore, its application on various carrier surfaces and clothes was tested to assess its bactericidal and virucidal efficacy, aiming to enhance biosecurity measures.

2. Materials and Methods

2.1. Sample preparation

To prepare PPMS solution for testing, PPMS powder (BIOX®, Biogénesis Bagó, Argentina) was freshly dissolved in distilled water (dW_2) at manufacturer's recommended concentrations of 1% (1X) and 0.5% (0.5X). To simulate organic material conditions at 5%, 500 µl of fetal bovine serum (FBS) was added to 10 ml of both concentrations before conducting the tests. The pH level of both concentrations was assessed before and after neutralization with a blocking solution, using a pH meter, to ensure accurate monitoring of the solution's acidity and alkalinity throughout the experiment.

Bacteria, viruses, medium, and cell preparation

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In this study, rif-SI, *E. coli,* and SI were subcultured on deoxycholate hydrogen sulphide lactose (DHL) agar and incubated overnight at 37 °C. The bacterial colonies were then picked and cultivated in Luria-Bertani (LB) medium (1% Bacto Tryptone, 0.5% Bacto Yeast Extract and 1%NaCl, pH 7.4) as described by Kunanusont et al. [10]. Cultured bacteria underwent two rounds of washing and centrifugation to eliminate organic residues, followed by suspension in phosphate buffer saline (PBS) before testing.

Virulent NDV, namely NDV/chicken/Asean Country/2013 [19,20] and a low pathogenic AIV, namely A/duck/Aomori/Japan/395/2004 H7N1, were propagated in 9-day-old chicken embryonic eggs following the method outlined by Jahangir et al. [21]. Three days post-inoculation, the allantoic fluid containing the propagated viruses was harvested and stored at -80°C, ready for subsequent testing purposes. Chicken embryo fibroblasts (CEF) and Madin-Darby canine kidney (MDCK) cells were primed for NDV and AIV titration in line with Jahangir et al.'s [21] methodology.

2.2. Blocking solution preparation and neutralization testing

Three blocking solutions, including FBS, 1M Tris-HCl (pH 7.4), and a mixture of 1M Tris-HCl (pH 7.4) and FBS in the ratio 7:3, were used for disinfectant neutralization. This testing was evaluated using rif-SI. Briefly, 400 µl of each disinfectant was mixed with 500 µl of each blocking solution. Subsequently, the solution mixture was immediately added to 100 µl of rif-SI and titrated onto DHL ager plates for bacterial titration. Each treatment was tested in triplicates, and titers were presented as the mean with standard error (SE).

2.3. Bactericidal and virucidal efficacy in the aqueous phase

Four hundred microliters of each PPMS concentration were mixed with 100 µl of each pathogen and then incubated at room temperature for specified durations: 5 sec, 30 sec, 1 min, 3 min, 5 min, 10 min or 15 min. After the incubation period, the mixture underwent immediate neutralization using 500 µl of blocking solution. Subsequently, it was titrated on a DHL ager plate for bacterial titration or through CEF and MDCK cells for NDV and AIV, respectively. For verification of neutralizing efficacy, the blocking solution was preemptively added to each sample prior to the introduction of bacteria or virus, precisely timed at 0 sec. Every treatment within this study underwent testing in triplicate, and the resulting titers were expressed as mean with associated standard errors.

2.4. Bactericidal and virucidal efficacy on contaminated carriers

Sheets of commercial stainless steel, rubber, and plastic purchased from local markets were cut into small squares measuring 5.0×5.0 cm and used for bactericidal and virucidal testing in this study. After that, each sheet was cleaned by washing with detergent and tap water, and then rinsed with dW_2 to completely remove any detergent residue. Following cleaning, all sheets were sterilized in an autoclave at 121 °C for 15 min, dried, and stored in a 60 °C incubator until they were ready for testing.

During bactericidal and virucidal tests, individual sterile carrier sheets were placed in 90mm petri dishes under a level 2 biological safety cabinet. Subsequently, 100 µl of rif-SI or AIV containing 5% FBS was inoculated onto each carrier's surface. After inoculation, the bacteria or virus were evenly spread on the carriers' surfaces and allowed to remain within the biological safety cabinet for 3 min. The artificially contaminated carriers were tested for virus inactivation using both concentrations of PPMS. Additionally, they were compared to quaternary ammonium compounds (QAC) at the manufacturer-recommended concentration. Subsequently, 500 µl of each concentration was applied to the contaminated carrier surfaces. They were then incubated for 30 sec, 1, 3, and 5 min, respectively. After the incubation period, the bactericidal or virucidal activity of each tested sample was neutralized. This was achieved by placing the carrier into a stomacher bag containing 2 ml of blocking solution. Following neutralization, the recovery of bacteria or virus from each carrier surface involved vigorous hand rubbing over the bag and scraping with a sterile pipette tip to transfer the virus into the blocking solution. Subsequently, the resulting solution was transferred from the stomacher bag to a microtube. It was then diluted in a 10-fold serial dilution using PBS for bacterial

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titration. For virus titration, it was cultured in Dulbecco's modified eagle medium (DMEM, Life Technologies Corporation, NY, USA) containing 20% $NaHCO_3$, penicillin 100 units/ml, streptomycin 100 µg/ml, amphotericin B 0.5 µg/ml, in MDCK. For the negative control, 500 µl of dW_2 was added to each contaminated carrier and kept for 5 min. Subsequently, they were placed in the stomacher bag with blocking solution for the removal of bacteria or virus and subsequent titration.

2.5. Virucidal efficacy on artificial contaminated clothes

Double-fold rayon sheets (size 2×2 cm) underwent sterilization by autoclaving at 121 °C for 15 min, followed by drying in a 60 °C incubator before experimentation. The objective was to assess PPMS efficacy on the cloth's viral decontamination potential. For this evaluation, 100 µl of AIV contaminated with 33% organic material was mixed with 50 µl of FBS. The mixture was uniformly spotted on the rayon sheets and allowed to dry for 30 min at room temperature in a class II biosafety laboratory cabinet. After this drying period, the virus-contaminated sheets were transferred to microtubes containing 500 µl of each PPMS concentration or PBS for the negative control. This step aimed to assess the PPMS effect on the previously contaminated sheets. Subsequently, they underwent incubation for defined periods (5 sec, 30 sec, 1 min, 5min, 10 min and 15 min) to determine the optimal duration for effective decontamination. Post-incubation, each condition underwent immediate neutralization by the blocking solution and was then subjected to recovery in MDCK cells for virus titration. To varidate the neutralizing efficacy, the blocking solution was added to PPMS samples before replacing the contaminated rayon, ensuring an accurate assessment of neutralization. Futhermore, the experiment was repeated using sodium dichloroisocyanurate (NaDCC) and QAC at their recommended concentrations, providing a comparative analysis to further understand the efficiency of PPMS.

2.6. Bacterial and virus titration

For bacterial titration, each sample treated with rif-SI, *E. coli*, and SI underwent a 10-fold serial dilution using PBS. Post-dilution, the samples were inoculated onto DHL agar plates and incubated at 37°C for overnight. After 24-hr, colony counting was performed to determine the colony-forming units (CFU)/ml [22].

Following a similar 10-fold serially dilution using DMEM, samples treated with NDV and AIV were inoculated onto CEF for NDA or MDCK cells for AIV titration. Before inoculation, trypsin (Trypsin, Sigma, St. Louis, MO, USA) was added to the DMEM to achieve a final concentration of 1.0 µg/ml. The inoculated tissue culture plates were placed in 37 °C, 5% CO_2 incubator and observed twice daily for 3 days to detect the cytopathic effect (CPE). After the 3-day incubation period, the hemagglutinin (HA) activity of the culture supernatant was assessed using 1% chicken red blood cells to observe any hemagglutinin activity. Finally, the 50% tissue culture infective dose ($TCID_{50}$/ml) was determined using the Behrens -Kärber method [22–24].

2.7. Inactivation and statistical analysis

The reduction factor (RF) was used to ascertain the inactivation of bacteria and viruses, determined by the formula: $RF = t_{pc} - t_a$. Here, t_{pc} denote the titer converted into a $\log_{10}$ index of the positive or PBS control, and t_a represents the titer converted into a $\log_{10}$ index of the recovered virus from the treated sample. Successful inactivation of bacteria and viruses was defined by RF values equal to or greater than 3 [23].

RF values were analyzed individually and represented as mean ± SE. Statistical significance was established using a one-way analysis of variance (ANOVA) *post hoc* test (SPSS, Armonk, NY, USA) comparing control and treatment groups when the p value was less than 0.05 [10].

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3. Results

3.1. pH

The pH values of PPMS at 1X and 0.5X in the absence and presence of organic material, both before and after neutralization using a blocking solution, are presented in Table 1. The pH values before neutralization without organic material were 2.23±0.01 and 2.28±0.20, respectively. In the presence of organic material, the values were 2.31±0.03 and 2.60±0.02, respectively. Additionally, the pH values after neutralization were 7.46±0.01 and 7.60±0.01, respectively, in the absence of organic material, and 7.49±0.01 and 7.62±0.01, respectively, in the presence of organic material.

Table 1. The pH (mean with standard error) of potassium peroxmonosulfate (PPMS) before and after neutralization with blocking solution.

Concentration	Before neutralization		After neutralization	
	Absence of organic material	Presence of organic material	Absence of organic material	Presence of organic material
1X[a]	2.23±0.01	2.31±0.03	7.46±0.01	7.49±0.01
0.5X[b]	2.28±0.20	2.60±0.02	7.60±0.01	7.62±0.01

[a]manufacturer's recommended concentration as 1% [b)] half of recommended concentration as 0.5%.

Table 2. Log_{10} CFU/ml (mean±standard error) of rifampicin-resistant *Salmonella* Infantis (rif-SI) after neutralization testing to each disinfectant using 3 blocking solutions.

Disinfectant	FBS[a)]	Tris HCl [b)]	Mix [c)]	Control
PPMS[d]	3.44±3.18	8.09±0.50 *	8.74±0.17 *	8.88±0.02
QAC[e]	8.43±0.11*	< 2.60±0.00	6.30±1.88	8.42±0.12
NaDCC[f]	8.63±0.12*	8.44±0.30 *	8.46±0.21 *	8.42±0.12

[a)]fetal bovine serum [b)]1M Tris hydrochloride [c)]30% fetal bovine serum and 70% Tris hydrochloride [d)]potassium peroxymonosulfate [e)]quaternary ammonium compounds [f)] sodium dichloroisocyanurate * Single asterisk indicates blocking agent could not inhibited the effective of disinfectant (reduced bacteria titer ≥1 log10 CFU/ml when compared with control).

3.2. Neutralization testing

The results of the neutralizing testing of the present disinfectants are shown in Table 2. PPMS was inhibited by Tris-HCl and mix solutions, while NaDCC was inhibited by all three blocking solutions. In contrast, QAC was solely inhibited by FBS.

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Table 3. Reduction factor (mean±standard error of log10 CFU/ml or $TCID_{50}$/ml) of rifampicin-resistant *Salmonella* Infantis (Rif-SI), *Escherichia coli* (E. coli), *Salmonella* Infantis (SI), avian influenza virus (AIV) and Newcastle disease virus (NDV) subsequent to treated by potassium peroxymonosulfate at manufacturing-recommended concentration (1X) or 0.5X even in the absence or presence of organic materials.

	Exposure time	Recommendation dose (1X)				Half recommendation dose (0.5X)			
		Absence[a]		presence[b]		Absence		presence	
		control	RF [c]	control	RF	control	RF	control	RF
Rif-SI	0 sec	8.84±0.06[d]	0.02±0.09	8.84±0.06	0.03±0.21	8.84±0.06	0.14±0.10	8.84±0.06	0.01±0.07
	5 sec	8.84±0.06	4.58±1.91*	8.84±0.06	3.88±2.15*	8.84±0.06	2.71±0.78	8.84±0.06	2.12±1.52
	30 sec	8.84±0.06	≥6.24±0.06**	8.84±0.06	≥6.24±0.06**	8.84±0.06	6.14±0.12*	8.84±0.06	6.04±0.29*
	1 min	NT	NT	NT	NT	NT	NT	NT	NT
E. coli	0 sec	8.04±0.19	0.23±0.10	8.04±0.19	0.53±0.48	8.15±0.12	0.25±0.20	8.15±0.12	0.28±0.13
	5 sec	8.04±0.19	≥5.44±0.19**	8.04±0.19	≥5.44±0.19**	8.15±0.12	4.08±0.16*	8.15±0.12	2.58±1.28
	30 sec	8.04±0.19	≥5.44±0.19**	8.04±0.19	≥5.44±0.19**	8.15±0.12	≥5.55±0.12**	8.15±0.12	≥5.55±0.12**
	1 min	NT	NT	NT	NT	NT	NT	NT	NT
SI	0 sec	8.43±0.07	0.67±0.05	8.43±0.07	0.01±0.25	8.43±0.07	0.14±0.11	8.43±0.07	0.04±0.11
	5 sec	8.43±0.07	5.12±1.29*	8.43±0.07	4.81±0.97*	8.43±0.07	2.03±0.31	8.43±0.07	1.64±0.33
	30 sec	8.43±0.07	≥5.82±0.07**	8.43±0.07	≥5.82±0.07**	8.43±0.07	≥5.82±0.07**	8.43±0.07	≥5.82±0.07**
	1 min	NT	NT	NT	NT	NT	NT	NT	NT
AIV	0 sec	6.83±0.38	0.42±0.29	6.83±0.38	0.33±0.14	6.83±0.38	0.08±0.29	6.83±0.38	0.00±0.25
	5 sec	6.83±0.38	≥4.33±0.38**	6.83±0.38	≥4.33±0.38**	6.83±0.38	≥4.33±0.38**	6.83±0.38	≥4.33±0.38**
	30 sec	6.83±0.38	≥4.33±0.38**	6.83±0.38	≥4.33±0.38**	6.83±0.38	≥4.33±0.38**	6.83±0.38	≥4.33±0.38**
	1 min	6.83±0.38	NT	6.83±0.38	≥4.33±0.38**	6.83±0.38	NT	6.83±0.38	≥4.33±0.38**
NDV	0 sec	8.42±0.38	0.58±0.38	8.42±0.38	0.42±0.14	8.33±0.14	0.17±0.29	8.33±0.14	0.42±0.14
	5 sec	8.42±0.38	≥5.92±0.38**	8.42±0.38	2.08±0.14	8.33±0.14	3.33±0.29*	8.33±0.14	0.92±0.52
	30 sec	8.42±0.38	≥5.92±0.38**	8.42±0.38	≥5.92±0.38**	8.33±0.14	≥5.83±0.14**	8.33±0.14	2.50±0.25
	1 min	NT	NT	8.42±0.38	≥5.92±0.38**	8.33±0.14	≥5.83±0.14**	8.33±0.14	3.75±0.00*
	5 min	NT	NT	NT	NT	8.33±0.14	≥5.83±0.14**	8.33±0.14	5.50±0.43*
	10 min	NT	NT	NT	NT	8.33±0.14	≥5.83±0.14**	8.33±0.14	≥5.83±0.14**
	15 min	NT	NT	NT	NT	8.33±0.14	≥5.83±0.14**	8.33±0.14	≥5.83±0.14**

[a)]absence of organic material. [b)] presence of organic materials. [c)]reduction factor [d)] the titer converted into an index in log_{10} of bacterial or virus control. NT: Not tested. *Single asterisk indicates bacterial or virus inactivation effective (≥3 log_{10} CFU/ml or $TCID_{50}$/ml) and RF are significantly different (P<0.05) from bacterial and virus control ** bacterial or virus titer undetectable level and RF are significantly different (P<0.05) from bacterial and virus control.

3.3. efficacy on contaminated carriers

As shown in Table 4, the bacterial quantity retrieved from the rif-SI contaminated carrier ranged between 7.62 to 7.85 log_{10} CFU/ml across all carriers within the dW_2 control range. Both concentrations of PPMS successfully inactivated rif-SI across all carriers within a 30 sec timeframe. While QAC managed inactivated within 1 min on stainless steel and within 30 sec on plastic, it failed to inactivate on rubber surfaces within 5 min.

As Table 5 indicates, the virus quantity retrieved from the AIV-contaminated carrier ranged between 5.50 to 6.00 log_{10} $TCID_{50}$/ml, aligning with the dW_2 control across all carriers. At both 1X and 0.5X concentrations, PPMS inactivated AIV across all carriers within 30 sec, except on stainless steel where inactivation occurred within 1 min. However, at 1X concentration, QAC inactivated within 5 min on stainless steel and plastic, yet failed to inactivate on rubber within the same timeframe.

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Table 4. Reduction factor (mean±standard error of log10 CFU/ml) of rifampicin-resistant *Salmonella* Infantis subsequent to treated on various surface carriers by potassium peroxymonosulfate (PPMS) at manufacturing-recommended concentration (1X) or 0.5X compared with recommended concentration of quaternary ammonium compound (QAC).

	Type of carriers	Concentration	Titer of control (log_{10} CFU/ml)	Reduction factor (log_{10} CFU/ml)			
				30 sec	1 min	3 min	5 min
PPMS	Stainless steel	1X	7.62±0.38	≥5.02±0.38**	≥5.02±0.38**	≥5.02±0.38**	≥5.02±0.38**
		0.5X	7.62±0.38	≥4.95±0.01**	≥4.95±0.01**	≥4.95±0.01**	≥4.95±0.01**
	Rubber	1X	7.66±0.22	≥5.06±0.22**	≥5.06±0.22**	≥5.06±0.22**	≥5.06±0.22**
		0.5X	7.66±0.22	≥5.02±0.03**	≥5.02±0.03**	≥5.02±0.03**	≥5.02±0.03**
	Plastic	1X	7.65±0.18	≥5.05±0.18**	≥5.05±0.18**	≥5.05±0.18**	≥5.05±0.18**
		0.5X	7.65±0.18	≥4.88±0.01**	≥4.88±0.01**	≥4.88±0.01**	≥4.88±0.01**
QAC	Stainless steel	1X	7.75±0.08	1.74±1.03	4.73±0.14*	4.61±0.65*	4.28±1.38*
	Rubber	1X	7.85±0.14	1.01±0.77	1.36±0.60	2.01±0.79	1.24±1.05
	Plastic	1X	7.75±0.11	4.96±0.28*	4.44±0.45*	4.95±0.28*	5.05±0.21*

*Single asterisk indicates bacterial inactivation effective (≥3 log_{10} CFU/ml) and RF are significantly different (P<0.05) from control ** bacterial titer undetectable level and RF are significantly different (P<0.05) from control.

Table 5. Reduction factor (mean±standard error of log10 $TCID_{50}$/ml) of avian influenza virus subsequent to treated on various surface carriers by potassium peroxymonosulfate at manufacturing-recommended concentration (1X) or 0.5X compared with recommended concentration of quaternary ammonium compound (QAC).

	Type of carriers	Concentration	Titer of control ($log_{10}TCID_{50}$/ml)	Reduction factor (log_{10} $TCID_{50}$/ml)			
				30 sec	1 min	3 min	5 min
PPMS	Stainless steel	1X	5.92±0.38	3.08±0.38*	3.17±0.29*	≥3.42±0.38**	NT
		0.5X	5.58±0.14	2.67±0.58	≥3.08±0.14**	≥3.08±0.14**	NT
	Rubber	1X	6.00±0.50	≥3.50±0.50**	≥3.50±0.50**	≥3.50±0.50**	NT
		0.5X	5.58±0.14	≥3.08±0.14**	≥3.08±0.14**	≥3.08±0.14**	NT
	Plastic	1X	6.00±0.43	≥3.50±0.43**	≥3.50±0.43**	≥3.50±0.43**	NT
		0.5X	6.00±0.25	≥3.50±0.25**	≥3.50±0.25**	≥3.50±0.25**	NT
QAC	Stainless steel	1X	5.75±0.50	1.33±0.52	1.17±0.29	1.67±0.63	≥3.25±0.50**
	Rubber	1X	5.50±0.25	1.00±0.66	1.17±0.38	1.67±0.38	2.42±0.38
	Plastic	1X	5.75±0.00	0.83±0.80	1.17±0.52	1.33±0.80	≥3.25±0.00**

NT: Not tested. *Single asterisk indicates virus inactivation effective (≥3 log_{10} $TCID_{50}$/ml) and RF are significantly different (P<0.05) from control ** virus titer undetectable and RF are significantly different (P<0.05) from control.

3.4. Virucidal efficacy on artificial contaminated clothes

The comparison of AIV inactivation on infected rayon sheets between PPMS, NaDCC and QAC at the manufacturer's recommended concentrations, against AIV artificially contaminated with 33% organic materials, is displayed in Table 6. PPMS at concentrations of 1X and 0.5X inactivated AIV on rayon sheet within 5 sec and 30 sec, respectively. In contrast, NaDCC at the recommended concentration exhibited AIV inactivation within 10 min, whereas QAC did not achieved inactivation within 30 min.

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4. Discussion

Initially, PPMS exhibits high acidic (pH 2.23 to 2.60). Nevertheless, after neutralization with the blocking solution, its pH shifted to a neutral range (pH 7.46 to 7.62). At o sec, none of the PPMS concentrations caused inactivation of rif-SI, *E. coli*, SI, NDV, or AIV. Consequently, there was no difference in titers compared to the bacteria or virus control. These finding suggest that the potent acidity of PPMS is its primary mechanism for elimination viruses or bacteria. Moreover, the efficacy of the blocking solution might impact the inhibitory activity of PPMS.

Neutralization tests revealed that Tris-HCl and FBS effectively halted the activity of PPMS and QAC, respectively. Conversely, NaDCC was impeded by all blocking solutions. Furthermore, these findings are pertinent to real farm scenatios, suggesting that a chosen disinfectant should maintain its inactivation efficacy even in the presence of organic materials such as feces. Thus, PPMS is recommended deu to its unaffected inactivation activity by organic material from FBS, whereas QAC and NaDCC were neutralized in their inactivation effectiveness.

Table 6. Reduction factor (mean±standard error of log10 $TCID_{50}$/ml) of avian influenza virus subsequent to treated on infected rayon sheet by potassium peroxymonosulfate (PPMS) at manufacturing-recommended concentration (1X) or 0.5X compared with recommended concentration of sodium dichloroisocyanurate (NaDCC) and quaternary ammonium compound (QAC) even virus contaminated with 33% of organic materials.

	PPMS		NaDCC	QAC
	1X	0.5X	1X	1X
t_{pc} [a)]	6.75±0.25	6.75±0.25	6.92±0.14	7.42±0.14
0 sec [b)]	0.33±0.14	0.08±0.72	0.25±0.25	0.58±0.38
5 sec [c)]	3.92±0.80*	2.92±2.53	083±0.14	NT
30 sec	≥4.25±0.25**	≥4.25±0.25**	1.08±0.38	NT
1 min	≥4.25±0.25**	≥4.25±0.25**	1.50±0.87	NT
5 min	NT	NT	2.08±0.72	1.00±0.00
10 min	NT	NT	3.08±0.72*	1.08±0.38
15 min	NT	NT	4.08±0.52*	1.83±1.28
30 min	NT	NT	≥4.42±0.14**	2.63±0.88

NT: Not tested. *Single asterisk indicates virus inactivation effective (≥3 $\log_{10}$ $TCID_{50}$/ml) and RF are significantly different (P<0.05) from control ** virus titer undetectable and RF are significantly different (P<0.05) from control.

Generally, PPMS is a potassium salt of peroxymonosulfuric acid, widely used as an oxidizing agent. Several researchers, such as Kunanusont et al. [10] and Sonthipet et al. [7], have linked PPMS to potassium monopersulfate (PMPS). This compound function through oxidation, specifically targeting capsid proteins to disrupt viral nucleic acids. The current study conducted aqueous phase testing using both the manufacturer's recommended concentration and half of that concentration. This testing revealed that the bactericidal and virucidal efficacy did not vary concerning the absence or presence of organic material. These findings confirmed PPMS's consistent ability to inactivate bacteria and viruses, even when contaminated with organic material that affected their inactivation. However, various researchers [7,10,25] have described how organic material contamination in QAC might diminish the efficacy of bacteria and virus inactivation. Moreover, PPMS required an extended exposure time to 30 sec to achieve undetectable level of inactivation for both bacteria and viruses. The comparison of virucidal efficacy between NDV and AIV revealed that AIV was more susceptible to inactivation by PPMS than NDV. These results concurred with Ruenphet et al. [19,20] who reported that fresh charcoal ash, slaked lime and food additive grade calcium hydroxide inactivated AIV more easily than NDV.

The present study evaluated the disinfectant efficacy in an aqueous phase and applied it to artificial contaminated carrier surfaces and clothes, comparing PPMS's effectiveness with NaDCC and QAC. Generally, pathogens such as bacteria and viruses are excreted with organic materials or cell debris from infected animals and adhere firmly to surface equipment around animals and

environments [26,27]. The results showed that PPMS effectively inactivated rif-SI on all surface carriers at the recommended concentrations and half of the recommended concentrations within 30 sec. However, at half of recommended concentration, AIV inactivation on stainless steel took 1 min. The bactericidal and virucidal efficacy of QAC was significantly lower than PPMS on all carriers using manufacturer's recommended concentration. These carrier models are commonly used in vehicle tires, boots, tracks, and various animal farm equipment. This study reaffirms the efficacy and application of PPMS in inactivating bacteria and viruses on all carrier surfaces in the vicinity of animal farms.

Our model of AIV artificially contaminated with organic materials on rayon sheets was selected to simulate carpets, bedding, towels, or clothes. Both NaDCC and QAC are marketed as being able to destroy most bacterial and viral pathogens. In this study, we tested NaDcc and QAC on rayon, considering and comparing their efficacy with that of PPMS. QAC is a cationic detergent, whereas NaDCC is a slow-release chlorine source and a common disinfectant for animal farms and food processing industries such as car gate sprays, foot baths, and slaughterhouses. The advantage of QAC is its low toxicity and broad antimicrobial spectrum [28]. However, despite its advantages, inactivation efficacies of QAC are usually reduced, even with organic material contamination [29–32]. When used at recommended concentration, PPMS destroyed AIV within 5 sec., while half of the manufacturer's recommended concentrations could inactivate the virus inside rayon sheets within 30 sec. However, the manufacturer's recommended concentration of NaDCC and QAC required extended exposure time to 10 and more than 15 min, respectively. In summary, these results indicated a higher efficacy of PPMS than of NaDCC and QAC. Therefore, PPMS can be applied as an alternative disinfectant or a virucidal agent to inactivate virus-contaminated carpets, clothing, towels, and beddings, especially in animal farms or hospitals.

5. Conclusions

The present study demonstrated PPMS's ability to inactivate food-borne bacteria such as rif-SI, *E. coli* and SI, along with enveloped viruses like NDV and AIV. Moreover, PPMS displayed superior effectiveness on diverse carrier surfaces and artificially contaminated clothes in comparison to NaDCC and QAC. Nevertheless, the effectiveness in killing bacteria and viruses relies on optimal concentration, organic material presence, and exposure/contact duration. Hence, PPMS represents a viable alternative disinfectant, especially for bolstering biosecurity in managing bacteria and viruses in animal farms, slaughterhouses, or hospitals.

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