[Results Report]

“NaOClean (Electrolyzed sodium hypochlorite water)”'s Disinfection Efficiency for the inactivity of Salmonella and Avian Influenza(AI) virus

Aug. 30. 2019

Jeonbuk National University
Bureau of University-Industry Cooperation & Research

1. General Information

1.1. General Information

○ Test Title: “NaOClean(Electrolyzed sodium hypochlorite water)”s disinfection capabilities for the inactivity of Salmonella and Avian Influenza(AI) virus

○ Test Institute and persons in charge

Name	Jeonbuk National University, Bureau of University-Industry Cooperation & Research			Name	Jo Jae Young	
Address	Bureau of University-Industry Cooperation & Research Jeonbuk National University, Baekjedaero 567, Deokjin-gu, Jeonju-si, Cheollabuk-do					
Person in charge	Lab	Jeonbuk National University Korea Zoonosis Research Institute	Title	Prof.	Name	TARK DONGSEOB
Contact	063-400-4084		E-mail		tarkds@jbnu.ac.kr	

○ Client

Name	D&D Electronics Co., Ltd	Title	Rep.	Name	Seo Soongi
Address	207 Anyang mega valley, Hagiro 268, Dongan-gu, Anyang City, Gyeonggi-do				
Contact	031-424-0100		E-mail	eunju@dndele.com	

1.2. Test Material

○ Product Name: ‘NaOClean’

○ Manufacturer: D&D Electronics Co., Ltd

○ License No.:

1.3. Test Schedule

○ Test Approved Date :

○ Test Start : Feb. 1. 2019

○ Test Finish : Aug. 29. 2019 (supplemented)

1. 일반사항

1.1. 일반사항

○ 시험제목: "나오크린(전해 차아염소산나트륨수)"의 살모넬라 및 조류인플루엔자 바이러스 불활화를 위한 소독효능 평가

○ 시험기관 및 책임자

기관명	전북대학교 산학협력단		기관장 성명		조 재 영	
주소	전라북도 전주시 덕진구 백제대로 567 전북대학교 산학협력단					
시험책임자	소속	전북대학교 인수공통전염병연구소	직위	교수	성명	탁동섭 (인)
연락처	063-400-4084		E-mail		tarkds@jbnu.ac.kr	

○ 의뢰자

업체명	㈜디엔디전자	직위	대표이사	성명	서순기
주소	경기도 안양시 동안구 학의로 268 안양메가밸리 207호				
연락처	031-424-0100		E-mail		eunju@dndele.com

1.2. 시험물질

○ 제품명: '나오크린'

○ 제조사: ㈜디엔디전자

○ 허가번호:

1.3. 시험일정

○ 시험설계서 승인일 :

○ 시험 개시일 : 2019년 2월 1일

○ 시험 종료일 : 2019년 8월 29일 (실험보완)

1.4. Pathogens for the Test

○ Virus : Avian Influenza

○ Bacteria : General bacteria(Salmonella)

1.5. Test Purpose and Method

The purpose of the test is to evaluate the disinfection capabilities of 'NaOClean' that may be used to stop the outbreak and spread of those pathogens(virus, bacteria) mentioned above 1.4. The test was carried out according to the Animal and Plant Quarantine Agency public notice No.2018-16 【guideline for disinfectant efficiency test】.

2. Material and Method

2.1. Test Material

Product Name	Ingredient Name	Content(ppm)	Remark
NaOClean	Electrolyzed sodium hypochlorite water	100, 200, 250, 700, 1000	

2.2. Notice Strain

Class.	Test Disease	Pathogen Name	Notice Standard Strain	Organization to parcel
Virus	Avian Influenza	Avian Influenza Virus	KVCC-VR1100013	Animal and Plant Quarantine Agency
Bacteria	General Bacteria	Salmonella typhimurium	KVCC-BA0400600	Animal and Plant Quarantine Agency

2.3. Preparation for Diluents

○ Hard Water : $CaCl_2$ 0.305g and $MgCl_2 \cdot 6H_2O$ 0.139g(w/v) were dissolved into distilled water(DW) 1L and then used under autoclave(121℃, 15minutes) at 4℃.

○ Organic Diluent : Hard water containing organic matters used for the dilution of the disinfectant

- For Bacteria : Dissolved in hard water to contain yeast extract 20%(w/v),

then stored under autoclave(121℃, 15minutes) at 4℃, and in case of its use, diluted it four times with hard water to make yeast extract 5% but to adjust for pH 7.0 by using 1N NaOH.

- For Virus : Dissolved in the sterilized hard water to have 5%(v/v) fetal bovine serum(FBS).

○ Neutralization Solution for Embryonated Egg Cultivation : Added 10% FBS to sterilized Phosphate-buffered saline(PBS)

○ Solution to neutralize bacteria : Contained 5% heat inactivated horse serum(56℃, 30 minutes) in nutrient broth

2.4. Test Conditions and Design

The test conditions and methods followed annex 1 「test for disinfectant of bacteria」 and annex 2 「test for virus disinfectant efficiency」 of Animal and Plant Quarantine Agency notification No. 2018-16(2018.5.31.). Hard water-diluent containing the pathogen to be sterilized, or 5% organic diluent were mixed with hard water-diluted solution of disinfectant and 5% organic diluent and then at 4℃ for 30 minutes the efficiency of the disinfectant was evaluated as below Table1.

Table1. Treatment in the evaluation of the disinfectant efficiency

Treatment	Hard Water	Organic	Disinfectant	Pathogen	Remark
1 (Organic Low)	+	-	+	+	Hard Water Condition
2 (Organic High)	+	+	+	+	Organic/ Hard Water Condition
3 (Control for Pathogen)	+	-	-	+	Control of treatment 1, 2
4 (Control for Toxicity)	+	-	+	-	Control of treatment 3

「+」 and 「-」 mean compositions of each treatment, organic 「low·high」 organic content of materials to be sterilized. treatment 4(disinfectant's toxicity control) is limited to the evaluation of the disinfectant efficiency.

2.5. Test Evaluation Standard and Decision of Efficient Dilute Magnification

○ “Efficient dilute magnification” is the rate of diluent/minimized efficient ingredients of the disinfectant that is effective under the condition of

organic low or high, "dilute magnification recommended" means the disinfectant dilute magnification for quarantine.

○ For the disinfection of bacteria, effective concentration for disinfection dilution step was determined by more than 4 nutrient broths that have no proliferation among 5 nutrient broths having the same disinfectant dilute magnification.

○ For the disinfection of virus, effective concentration was recognized as a dilute magnification where more than pathogen $10^{4.0}$ EID_{50} 5/ml are dead or inactivated in comparison with pathogen control. The effective dilute magnification of the product was carried out by 3 tests repeatedly and the value showed higher than 4 when converted into common logarithm, and the magnification was set to be the median of the result value within the error range of arithmetic mean 20%(±10%).

3. 'NaOClean's disinfection efficiency test for virus

3.1. 'NaOClean's disinfection efficiency test for Avian Influenza Virus

□ Virus Culture

Avian Influenza Virus(KVCC-VR1100013) was cultivated in chicken embryos of 9~10 days 2~3 times consecutively for the use of activated virus. Virus was inoculated in allantoic cavity and cultivated for 72 hours and then kept more than 3 hours at 4℃. Allantoic fluid was taken and its solid ingredients were eliminated by centrifugation(3,000rpm, 4℃, 30 minutes), then the allantoic fluid containing virus was stored in iced water for short time. In addition, for some virus fluid obtained from the cultivation, their virus titer was confirmed through EID_{50}/ml standard calculation, while virus fluid showing higher than $10^{8.0}EID_{50}$/ml was stored at –80℃. In this test, virus fluid of titer higher than $10^{8.0}EID_{50}$/ml was diluted at the ratio of 1/10 by using PBS, which led to virus fluid of higher than $10^{7.0}EID_{50}$/ml for use.

□ Disinfectant Diluent

According to the Animal and Plant Quarantine Agency 2018-16(2018.5.31.) 【disinfectant efficiency test guideline】 article 2(definition of term), hard water and organic diluent were used. In principle, hard water element and organic

element were tested by hard water and organic diluent respectively, the regulation of article 3.1 was applied. For the disinfectant to be tested, its dilute magnification was the concentration before reaction.

□ To Dilute Disinfectant

For the dilution of the disinfectant, in case of organic (low treatment 1), 50, 100, 200, 250ppm, and in case of organic (high treatment 2), 50, 100, 200, 250ppm were used respectively. Besides, pathogen control(treatment 3) with no disinfectant and disinfectant toxicity control diluted by hard water(treatment 4) were also kept at 4℃ before their reaction.

□ Disinfectant Reaction

The virus fluid of 1.0ml at 4℃ was mixed with hard water at 4℃ and 5% organic diluent 19.0ml for virus then 2.5ml was obtained at an interval of one minute and put into test tubes of disinfectant diluent of the same amount stored at 4℃(total 5.0ml) and made to react exactly for 30 minutes at 4℃, during that time well mixed every 10 minutes. In this case, for pathogen control(treatment 3), hard water instead of disinfectant was used to treat in the same way. For the disinfect toxicity control(treatment 4), not virus diluent but hard water was also used to treat with the same way.

□ Neutralization Reaction

When the reaction of disinfectant with pathogen was finished, in order to neutralize the efficiency of the disinfectant, 1.0ml was immediately picked out and put in a neutralization solution of the same amount at 37℃.

□ Measurement of the Degree of Loss of Virus Infectivity

The neutralized reaction fluid by using PBS was diluted with (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8}) and each 0.2ml was innoculated into allantoic cavity of 5 chicken embryos of 9~10 days per dilute magnification and they were cultivated for 5 days at 37℃. Candling was done everyday and embryonated eggs which died within 24 hours after inoculation were regarded as innoculation death and eliminated from the test. Inoculated eggs that died within 5 days after 24 hours of innoculation were all stored at 4℃. 5 days later, all the inoculated eggs were collected and their allantoic fluid was gathered and immediately their hemo-agglutination(HA) was identified on

96 well microplate for the final decision of infection.

□ To Calculate Virus Content and Evaluate Effective Magnification

To calculate virus content, Kaerber method was used, showing that the result of the pathogen control was more than $2\times10^{5.0}EID_{50}$/ml, while for disinfectant treatment, the dilute concentration contributed to the death or inactivity in virus content of higher than $10^{4.0}EID_{50}$/ml was determined as effective concentration. The final dilute magnification was calculated as median of the result derived from 3 repeated tests within error range of 20%. In the disinfectant toxicity control experiment, the toxicity was determined by whether or not embryonated eggs died of the disinfectant. The dilute magnification recommended was set equivalent to 80% of effective dilute magnification.

4. 'NaOClean"s Disinfection Efficiency Test on Bacteria

4.1. 'NaOClean"s Disinfection Efficiency Test on Salmonella typhimurium

□ Bacteria Culture

Salmonella typhimurium(KVCC-BA0400600) was cultivated on nutrient broth of autoclave 2~3 times consecutively and activated bacteria were used. The bacteria were cultured 22~26 hours at 37℃ and then kept under 37℃ just before their use. In this test, the concentration of bacteria was higher than 1×10^{8}CFU/ml.

□ Disinfectant Diluent

In compliance with the Animal and Plant Quarantine Agency article 2018-16(2018.5.31.)【disinfectant efficiency test guideline】2(definition of term), adequate hard water and organic diluent were used. In principle, hard water treatment was carried out with hard water, organic treatment was executed with organic diluent, respectively, the guideline of the article 3.1 was applied. The disinfectant diluent was prepared for the dilute magnification equivalent to dilution concentration of the pathogen fluid to be tested.

□ To Dilute Disinfectant

For the dilution of the disinfectant, hard water was added for 10, 20, 30, 40, 50, 100, 200, 250, 700ppm in case of organic low(treatment 1), while when it comes to organic high(treatment 2), the disinfectant was mixed for 10, 20, 30, 40, 50, 100, 200, 250, 700ppm for 5% organic diluent. The diluted test tubes were kept at 4℃ just before test. Hard water with no disinfectant(treatment 3) was also kept at 4℃ just before test.

□ Disinfectant Reaction

34ml of bacteria cultivated at 37℃ were mixed at hard water at 4℃, organic diluent 96ml for bacteria respectively, then at an interval of a minute, 2.5ml of mixed fluid was picked out and put into the test tube of disinfectant fluid having the same amount at 4℃ (total 5ml) and made to react for exactly 30 minutes at 4℃, during that time, well-mixed every 10 minutes. In case of the pathogen control(treatment 3), hard water instead of the disinfectant was used and treated in the same way.

□ Neutralization Reaction and Proliferation

After the reaction, in order to neutralize the efficiency of the disinfectant, 1.0ml was immediately picked out and put into a 9.0ml neutralization broth then 0.1ml was each mixed in 5 test tubes of nutrient broth and then cultivated for 48 hours at the constant temperature of 37℃.

□ Judgment of Bacteria Proliferation and Determination of Disinfectant's Effective Dilute Magnification

The effective dilute magnification was determined when more than 4 broths showed no proliferation among 5 broths of the same amount of dilute magnification at the final step of dilution. The effective dilute magnification was set as the median of the result derived from 3 tests within the error range of 20%. In this case, the standard concentration of bacteria of the pathogen control was made higher than $2\times10^{5.0}$CFU/ml. The dilute magnification recommended was decided at 80% of the effective dilute magnification.

5. Test Results of ‘NaOClean’'s Disinfection Efficiency on Bacteria

5.1. Comprehensive Opinions for the Test Results

□ ‘NaOClean’'s Effective Concentration

Virus	Treatment 1/Hard water Condition (ppm)	Treatment 2/ Organic Condition (ppm)
(avian influenza virus)	100ppm	200ppm

□ ‘NaOClean’'s Recommended Dilute Concentration

<table>
<tr><td rowspan="2">Class.</td><td colspan="3">Object to disinfect</td><td>Low Organic Object</td><td>High Organic Object</td></tr>
<tr><td colspan="2">Object Name(pathogen name)</td><td>Test Result</td><td>pen space, its surface, equipment, vehicles</td><td>pen floor, feces, carcass, farm vehicles and mobile device</td></tr>
<tr><td rowspan="3">Dilute Concentration Recommended</td><td rowspan="2">Virus</td><td rowspan="2">avian influenza virus</td><td>Organic low (ppm)</td><td>Organic high (ppm)</td><td></td><td></td></tr>
<tr><td>100ppm</td><td>200ppm</td><td>125ppm</td><td>250ppm</td></tr>
</table>

※Indication of dilution concentration recommended : By comparison between hard water of specific virus and organic matter test results, 1.25 times(80%)higher disinfectant concentration are multiplied to determine dilution concentration recommended.

5.2. Test Results of ‘NaOClean’'s Disinfection Efficiency on Avian Influenza Virus

□ Final Result Decision

(unit: EID_{50}/mL)

Treatment	Test Condition	Disinfectant Concentration (ppm)	1st Test	2nd Test	3rd Test	Final Effective Concentration
1 (Organic low)	Organic low (Hard water Condition)	50ppm	$10^{4.2}$	$10^{4.4}$	$10^{4.8}$	100ppm
		100ppm	<10	<10	<10	
		200ppm	<10	<10	<10	
		250ppm	<10	<10	<10	
		Effective Concentration	100ppm	100ppm	100ppm	
2 (Organic high)	Organic high (Organic Condition)	100ppm	$10^{3.2}$	$10^{3.0}$	$10^{2.8}$	200ppm
		200ppm	<10	<10	<10	
		250ppm	<10	<10	<10	
		Effective Concentration	200ppm	200ppm	200ppm	
3 (Pathogen control)	Organic low (Hare water Condition)	-	$10^{6.0}$	$10^{6.4}$	$10^{6.4}$	-
4 (Toxicity control)	Organic low (Hard water Condition)	250ppm	<10	<10	<10	No Toxicity

* 0.2ml innoculation converted into 1ml

□ 1st Test Result : ‘NaOClean’s Disinfection Efficiency on Avian Influenza Virus by Its Concentration

Treatment	Disinfectant Concentration	Neutralization Fluid Dilute Magnification (virus growing number/fertilized egg innoculation number)							
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
1. Organic low	50ppm	4/5	5/5	3/5	3/5	0/5	0/5	0/5	0/5
	100ppm	0/5	0/5	0/4	0/4	0/5	0/5	0/5	0/5
	200ppm	0/5	0/5	0/5	0/5	0/4	0/4	0/5	0/5
	250ppm	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
2. Organic high	100ppm	5/5	4/5	1/5	0/5	0/5	0/5	0/5	0/5
	200ppm	0/5	0/4	0/5	0/5	0/5	0/5	0/5	0/5
	250ppm	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
3. Pathogen control		5/5	5/5	5/5	5/5	2/5	2/5	0/5	0/5
4. Toxicity control	250ppm	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

□ 2nd Test Result : ‘NaOClean’s Disinfection Efficiency on Avian Influenza Virus by Its Concentration

Treatment	Disinfectant Concentration	Neutralization Fluid Dilute Magnification (virus growing number/fertilized egg innoculation number)							
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
1. Organic low	50ppm	4/5	4/5	5/5	3/5	0/5	0/5	0/5	0/5
	100ppm	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	200ppm	1/5	0/5	0/5	0/5	0/4	0/5	0/5	0/5
	250ppm	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
2. Organic high	100ppm	5/5	3/5	1/5	0/5	0/5	0/5	0/5	0/5
	200ppm	0/5	0/5	0/2	0/3	0/5	0/5	0/5	0/5
	250ppm	0/5	0/5	0/5	0/4	0/5	0/5	0/5	0/5
3. Pathogen control		5/5	5/5	5/5	5/5	4/5	2/5	0/5	0/5
4. Toxicity control	250ppm	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

□ 3rd Test Result : ‘NaOClean’'s Disinfection Efficiency on Avian Influenza Virus by Its Concentration

Treatment	Disinfectant Concentration	Neutralization Fluid Dilute Magnification (virus growing number/fertilized egg innoculation number)							
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
1. Organic low	50ppm	5/5	5/5	5/5	3/5	0/5	0/5	0/5	0/5
	100ppm	0/5	0/5	0/4	0/5	0/5	0/5	0/5	0/5
	200ppm	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	250ppm	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
2. Organic high	100ppm	5/5	2/5	1/5	0/5	0/5	0/5	0/5	0/5
	200ppm	0/4	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	250ppm	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
3. Pathogen control		5/5	5/5	5/5	5/5	4/5	2/5	0/5	0/5
4. Toxicity control	250ppm	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

□ Test Result Summary : ‘NaOClean’s Disinfection Efficiency on Avian Influenza Virus by Its Concentration

Treatment	Test Condition	Disinfectant Concentration	Virus Concentration(EID_{50}/0.2ml)			
			1st	2nd	3rd	Log reduction
1	Organic low	50ppm	$10^{3.5}$	$10^{3.7}$	$10^{4.1}$	< $10^{4.0}$
		100ppm	<10	<10	<10	>$10^{5.0}$
		200ppm	<10	<10	<10	>$10^{5.0}$
		250ppm	<10	<10	<10	>$10^{5.0}$
2	Organic high	100ppm	$10^{2.5}$	$10^{2.3}$	$10^{2.1}$	< $10^{4.0}$
		200ppm	<10	<10	<10	>$10^{5.0}$
		250ppm	<10	<10	<10	>$10^{5.0}$
4	3. Pathogen control		$10^{5.3}$	$10^{5.7}$	$10^{5.7}$	
5	4. Toxicity control	250ppm	<10	<10	<10	

6. Test Result of ‘NaOClean’s Disinfection Efficiency on Salmonella typhimurium

6.1. Comprehensive Opinions of the Test Results

□ ‘NaOClean’s Effective Concentration

Bacteria	Treatment 1/Hard water Condition (ppm)	Treatment 2/ Organic Condition (ppm)
general bacteria (Salmonella typhimurium)	30	1,000

□ ‘NaOClean’s Recommended Concentration

Class.	Object to disinfect				Low Organic Object	High Organic Object
	Object Name(pathogen name)		Test Result		pen space, its surface, equipment, vehicles	pen floor, feces, carcass, farm vehicles, mobile equipment
Recommended Concentration	general bacteria	Salmonella typhimurium	Organic low (ppm)	Organic high (ppm)		
			30	**1,000**	**37.5**	**1,250**

※Indication of recommended dilution concentration : By comparison between hard water of S. typhimurium and organic matter test results, 1.25 times(80%)higher disinfectant concentration are multiplied to determine dilution concentration recommended.

6.2. Test Results of ‘NaOClean’s Disinfection Efficiency on Salmonella typhimurium

□ Final Result Decision

Treatment	Test Condition	Disinfectant Concentration (ppm)	1st Test (Bacteria growing number/examination number)	2nd Test (Bacteria growing number/examination number)	3rd Test (Bacteria growing number/examination number)	Final Effective Concentration (ppm)
1 (Organic low)	Organic low (Hard water Condition)	10 20 30 40 50 100 200 250 700	5/5 2/5 0/5 0/5 0/5 0/5 0/5 0/5 0/5	5/5 2/5 0/5 0/5 0/5 0/5 0/5 0/5 0/5	5/5 2/5 0/5 0/5 0/5 0/5 0/5 0/5 0/5	30
		Effective Concentration	30	30	30	
2 (Organic high)	Organic high (Organic Condition)	100 200 250 700 800 900 1000	5/5 5/5 5/5 5/5 5/5 5/5 0/5	5/5 5/5 5/5 5/5 5/5 5/5 0/5	5/5 5/5 5/5 5/5 5/5 5/5 0/5	1000
		Effective Concentration	1000	1000	1000	
3. (Pathogen control)	Organic low (Hard water Condition)	-	$1x10^6$	$2x10^6$	$4x10^6$	-
4. (Toxicity control)	Organic low (Hard water Condition)	-				-