

Results of verification test on effectiveness evaluation of alternative disinfection materials using influenza virus

April 30, 2020

National Institute of Technology and Evaluation

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Matters confirmed during the 1<sup>st</sup> Committee

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<Mission in this committee>

Due to the high demand of rubbing alcohol under the new coronavirus outbreak, therefore, this committee collected and analyzed literature information about the possible candidate substances that have a certain disinfecting effect among those that are available in ordinary households. Furthermore, by conducting a verification test, the effectiveness of the disinfection method will be evaluated urgently. At that time, consideration will be given to the balance of "securing scientific objectivity", "quick response", and "easy for the people to understand".

<Candidate substances that may have antiseptic effects>

No literature information is currently available on the evaluation of disinfection methods for new coronaviruses. Therefore, evaluation will be performed based on literature information on other coronaviruses, which are RNA envelope viruses similar to the new coronavirus.

Based on the document information about the antiviral effect of other is RNA type enveloped virus to coronavirus (SARS coronavirus, etc.) and influenza virus, the following are considered to be candidate substances that may have a certain disinfecting effect against the new coronavirus.

- 1) Surfactant (kitchen/dish detergent, etc.)
- 2) Hyaluronic acid (Generated by electrolysis)
- 3) Quaternary ammonium salt

Heating, alcohol disinfection, and sodium hypochlorite disinfection are already widely recognized and are not included in the above discussion. It should be noted that there are other materials that may have a disinfecting effect in addition to those listed above, but we have examined from the viewpoint that they can be continuously supplied even under the current conditions. As a rule, the candidate substances 1) to 3) above are for disinfecting goods.

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<Verification test of candidate substances>

It is ideal to immediately carry out a verification test with the new coronavirus, but it needs to be considered that it will take a reasonable period of time to complete the test. Therefore, it is urgent to carry out a verification test using another virus that can carry out verification in a short period of time as an option for the time being. In parallel, we will prepare for verification tests using the new coronavirus.

<Implementation of a verification test using a virus that can be used instead>

(Effectiveness of virus verification that can be used as an alternative)

Influenza A virus will be used for immediate verification tests. Since influenza A virus is classified as an RNA virus with an envelope like the new coronavirus, it is of some significance to infer the effect on the new coronavirus from the results of verification tests with the influenza virus.

(Selection of candidate substances for verification test)

- From the candidate materials, we start the verification test for the following.
  - Surfactant: Select 8 typical surfactants
  - Hypochlorous acid water: Four types are selected from electrolyzed water, strong acidic electrolyzed water, weakly acidic electrolyzed water and slightly acidic electrolyzed water that have a bactericidal effect and are designated as food additives (bactericidal agents).
  - Quaternary ammonium salt: Benzalkonium chloride is selected as a typical compound.

(Test protocol, validation, etc.)

The verification test protocol will be executed according to the method proposed by the committee secretariat.

Upon receipt of the results of the verification test, this committee will verify the validity of the results and the effectiveness of the alternative disinfection method, etc.

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### Results of Verification Test

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#### Substances subject to verification tests using influenza virus

Sample #	Name of the type of surfactant	Differentiation of surfactants
Surfactant 1	Pure soap content (potassium fatty acid)	Anionic surfactant
Surfactant 2	Linear alkylbenzene sodium sulfonate	
Surfactant 3	Alkyl glycoside	Nonionic surfactant
Surfactant 4	Fatty acid alkanolamide	
Surfactant 5	Polyoxyethylene alkyl ether	Zwitterionic surfactant
Surfactant 6	Alkyl betaine	
Surfactant 7	Alkylamine oxide	Cationic surfactant
Surfactant 8	Benzalkonium chloride	

Sample #	Name	Electrolytes	pH	Effective chlorine concentration (ppm)
Hypochlorous acid water 1	Strongly acidic electrolyzed water	Salt	~2.7	40
Hypochlorous acid water 2	Strongly acidic electrolyzed water	Salt	2.7~5.0	30
Hypochlorous acid water 3	Strongly acidic electrolyzed water	Chlorine	5.0~6.5	30
Hypochlorous acid water 4	Strongly acidic electrolyzed water	Salt + Chlorine	5.0~6.5	40

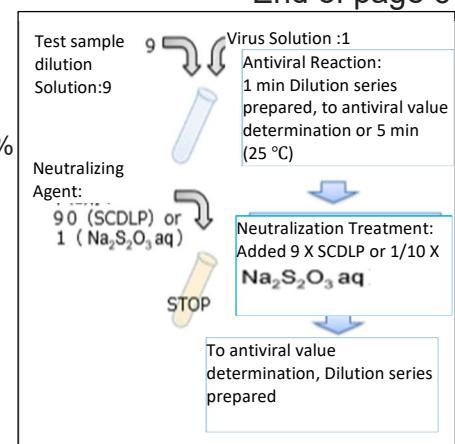
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### Outline of anti-virus evaluation method

- Host cell culture and virus culture (JIS standard)
- Dilution of test sample
  - Diluting the surfactant with sterile water to a concentration of 0.1-0.5%
  - No need to dilute hypochlorous acid water
- Antiviral reaction (ASTM standard)
  - Test sample dilution solution: virus solution = 9:1
  - Reaction time: 1 minute, 5 minutes (25 °C)
- Neutralization treatment (ASTM standard, JIS standard)
  - Surfactant: 9 times SCDLP (ASTM standard)
  - For hypochlorous acid water: 1/10 times 0.012N sodium thiosulfate solution (JIS standard)
- Infect host cells by diluting the reaction solution after neutralization (JIS standard)
- Evaluation method: Plaque method or TCID50 method (JIS standard)

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  - Surfactant: 9 times SCDLP (ASTM standard)
  - For hypochlorous acid water: 1/10 times 0.012N sodium thiosulfate solution (JIS standard)



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- Infect host cells by diluting the reaction solution after neutralization (JIS standard)
- Evaluation method: Plaque method or TCID50 method (JIS standard)



Plaque method:

Using 2 wells for each dilution stage, to calculate the number of viruses decrease from the number of plaques.



TCID50 method:

Using 4 wells or more for each dilution stage, to calculate the number of viruses decrease from the number of wells of virus-infected cells.

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### Verification Test

Surfactant classification	Name of surfactant type	Testing Institutions			
		A	B	C	C
		Plaque Method	Plaque Method	Plaque Method	Plaque Method
		H3N2 (A/HongKong/8/68) ATCC VR-1679	H3N2 (A/HongKong/8/68) ATCC VR-1679	H3N2 (A/HongKong/8/68) ATCC VR-1679	H1N1 (A/PR/8/34) ATCC VR-1469
Anionic surfactants	Pure soap (potassium fatty acid)				O
	Sodium chained alkylbenzene sulfonic acid	O	O	O	
Nonionic surfactants	Alkyl glycoside	O		O	
	Fatty acid alkanolamide	O	O		O
	Polyoxyethyleneoxy Alkyl Etail	O			O
Amphoteric ionic surfactants	Alkyl betaine	O	O		O
	Alkylamine oxides	O	O	O	
Cationic surfactants	Benzalkonium chloride		O		O
Classification of hypochlorite water	Electrolyte & Effective chlorine concentration				
Strongly acidic electrolyzed water	Electrolyte: Salt, 40ppm				O
Weakly acidic electrolyzed water	Electrolyte: Salt, 30ppm				O
Slightly acidic electrolyzed water 1	Electrolyte: Hydrochloric acid, 30ppm				O
Slightly acidic electrolyzed water 2	Electrolyte: Salt & Hydrochloric acid, 40ppm				O

O: Test conducted

Tests for eight surfactants and four types of hypochlorite water in four testing institutions.

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## Test results/ Testing institution A

		Sodium linear alkylbenzene sulfonic acid			Alkyl glycoside			Fatty acid alkanolamide			Positive Control	Negative Control
	Contact Time	0.5%	0.3%	0.1%	0.5%	0.3%	0.1%	0.5%	0.3%	0.1%	EtOH	PSB
1 <sup>st</sup> Time	1 min	< 2	< 2	< 2	< 2	< 2	< 2	4.29	4.52	4.85	< 2	7
	5 min	< 2	< 2	< 2	< 2	< 2	< 2	2.74	3.11	3.93	< 2	7
2 <sup>nd</sup> Time	1 min	< 2	< 2	< 2	< 2	< 2	< 2	4.37	4.76	5.04	< 2	7
	5 min	< 2	< 2	< 2	< 2	< 2	< 2	2.60	3.10	4.02	< 2	7

		Polyoxyethylene Alkyl Etail			Alkyl betaine			Alkyl betaine			Positive Control	Negative Control
	Contact Time	0.5%	0.3%	0.1%	0.5%	0.3%	0.1%	0.5%	0.3%	0.1%	EtOH	PSB
1 <sup>st</sup> Time	1 min	2.74	2.40	< 2	6.88	6.81	7.19	< 3	< 2	< 2	< 2	7
	5 min	2.48	2.48	< 2	6.85	6.81	7.02	< 3	< 2	< 2	< 2	7
2 <sup>nd</sup> Time	1 min	2.85	2.65	< 2	6.88	6.85	6.85	< 3	< 2	< 2	< 2	7
	5 min	< 2	2.18	< 2	6.78	6.78	6.78	< 3	< 2	< 2	< 2	7

### Result by Plaque Method (N=2)

Antiviral reaction time: 1 minute and 5 minutes

Value: Common logarithm of infection values

Based on PBS



Decreased more than 4 digits

Decreased 3 digits

Decreased 2 digits

Decreased 1 digits

No Effective

- No cytotoxicity was confirmed.
- Sodium linear alkylbenzene sulfonic acid, alkyl glycoside, polyoxyethylene alkyl ether and alkyl amine oxide reduced the infection value by more than 4 digits.
- There was no decrease in infection value in alkyl betaine.
- The decrease in infection value in fatty acid alkanolamide varies depending on the concentration and reaction time.

### Test results/ Testing institution B

Assumed virus concentration Pfu/0.1ml		Sodium linear alkylbenzene sulfonic acid			Fatty acid alkanolamide			Alkylamine oxides		
		Dilution	0.5%	0.3%	0.1%	0.5%	0.3%	0.1%	0.5%	0.3%
10 <sup>4</sup>	Neutralization solution	0	0	0	2	8	Many	0	0	0
10 <sup>3</sup>	10 <sup>1</sup>	0	0	0	0	1	10	0	0	0
10 <sup>2</sup>	10 <sup>2</sup>	0	0	0	0	0	2	0	0	0
10	10 <sup>3</sup>	0	0	0	0	0	1	0	0	0
-	Medium only	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T

Assumed virus concentration Pfu/0.1ml		Alkyl betaine			Benzalkonium chloride			Positive Control EtOH	Negative Control PSB
		Dilution	0.5%	0.3%	0.1%	0.5%	0.3%	0.1%	
10 <sup>4</sup>	Neutralization solution	Many	Many	Many	Many	Many	Many	0	Many
10 <sup>3</sup>	10 <sup>1</sup>	Many	Many	Many	13	13	17	0	Many
10 <sup>2</sup>	10 <sup>2</sup>	Many	Many	Many	1	3	2	0	Many
10	10 <sup>3</sup>	18	20	19	0	0	0	0	27
-	Medium only	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T

Result by Plaque Method (N=2, this chart is an example data

Antiviral reaction time: only 5 minutes

Value: # of Plaque (Many: more than 60)

- : No virus inoculation

N/T: Not tested

Positive Control: Locally disinfecting ethanol (83% v/v)

Negative Control: Phosphate buffered saline

Based on PBSM



More than 4 digits in 5 minutes

3 digits in 5 minutes

2 digits in 5 minutes

No Effective

- No cytotoxicity was confirmed.
- Sodium linear alkylbenzene sulfonic acid and alkyl amine oxide reduced the infection value by more than 4 digits.
- The decrease in infection value in fatty acid alkanolamide varies depending on the concentration and reaction time.
- There was no decrease in infection value in alkyl betaine.
- The decrease in the infection value of benzalkonium chloride was 2 digits.

## Test results/ Testing institution C

Assumed virus concentration Pfu/0.1ml		Sodium linear alkylbenzene sulfonic acid			Alkylamine oxides			Alkyl glycoside			Positive Control EtOH	Negative Control PBS	
		Dilution	0.5%	0.3%	0.1%	0.5%	0.3%	0.1%	0.5%	0.3%	0.1%		
10 <sup>4</sup>	Neutralization solution	T	T	T	T	T	T	T	T	0	0	82% v/v	Many
10 <sup>3</sup>	10 <sup>1</sup>	T	T	0	T	T	0	0	0	0	0	0	Many
10 <sup>2</sup>	10 <sup>2</sup>	0	0	0	0	0	0	0	0	0	0	0	Many
10	10 <sup>3</sup>	0	0	0	0	0	0	0	0	0	0	0	14
-	Medium only	-	-	-	-	-	-	-	-	-	-		

Result by Plaque Method (N=1)

Antiviral reaction time: only 5 minutes

Value: # of Plaque (Many: more than 60)

T: Because of cytotoxicity, it is impossible to measure #f of plaques.

- : No virus inoculation

Positive Control: Locally disinfecting ethanol (83% v/v)

Negative Control: Phosphate buffered saline



Cytotoxic



Based on PBSM

More than 4 digits in 5 minutes

3 digits in 5 minutes

2 digits in 5 minutes

No Effective

- Cytotoxicity was observed from the low dilution rate group and high concentration sample addition group of the reaction solution.
- As results of the tests with 0.1% concentration of Sodium linear alkylbenzene sulfonic acid, alkylamine oxide and alkyl glycoside, it was found that the decrease of the infection value by more than 4 digits.

**Test results/ Testing institution D**  
**Surfactants, Quaternary ammonium salts**

	Contact Time	Fatty acid potassium			Fatty acid alkanolamide			Polyoxyethylene alkyl ethers		
		1.0%	0.5%	0.1%	0.5%	0.3%	0.1%	0.5%	0.3%	0.1%
1 <sup>st</sup> Time	1 min	5.23	5.59	5.15	3.80	3.86	4.86	2.92	2.99	3.40
	5 min	4.59	3.40	3.15	2.40	2.92	4.80	2.30	2.54	2.80
2 <sup>nd</sup> Time	1 min	5.30	5.08	5.15	4.00	4.59	4.92	3.80	3.00	3.23
	5 min	4.86	3.88	2.88	2.40	3.15	4.73	2.41	2.65	2.80

	Contact Time	Alkyl betaine			Benzalkonium chloride			Positive Control EtOH	Negative Control PSB
		0.5%	0.3%	0.1%	0.5%	0.3%	0.1%		
1 <sup>st</sup> Time	1 min	6.92	6.87	6.8	4.72	3.99	4.67	< 1	7
	5 min	6.86	7.15	6.87	3.15	2.92	3.59	< 1	7
2 <sup>nd</sup> Time	1 min	7.04	7.00	6.95	4.08	4.46	4.15	< 1	7
	5 min	6.92	6.93	7.04	3.49	3.00	3.30	< 1	7

Based on PBS

Result by TCID50 method

Antiviral reaction time: only 5 minutes

Value: Common logarithm of infection value

Positive Control: Locally disinfecting ethanol (83% v/v)

Negative Control: Phosphate buffered saline



Decreased more than 4 digits

Decreased 3 digits

Decreased 2 digits

Decreased 1 digits

No Effective

- Cytotoxicity was confirmed with fatty acid potassium and benzalkonium chloride. (Neutralization stock solution group only)
- Polyoxyethylene alkyl ether (0.1%) showed a decrease in infection value by more than 4 digits in the reaction time of 5 minutes.
- Fatty acid alkanolamide (0.1%) showed a decreased in infection value by 2 digits in the reaction time of 5 minutes.
- Benzalkonium chloride (0.1%) showed a decreased in infection value by 3 digits in the reaction time of 5 minutes.
- There was no decrease in infection value in Alkyl betaine regardless of the concentration and reaction time.

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**Test results/ Testing institution D**  
**Hypochlorite water (electrolyzed)**

5. Inactivated test results

III-1-3, Confirmation of antiviral effect by test solution (virus inactivation test by various electrolyzed water)

Test Products	Working Time		
	0 (Initial)	1 min	5 min
Control (phosphate buffered saline)	1.4E + 07		8.2E + 06
Locally disinfecting ethanol		< 6.3	< 6.3
Strongly acidic electrolyzed water (salt) pH 2.7m 50ppm		< 6.3	< 6.3
Weakly acidic electrolyzed water (salt) pH 3.5, 42ppm		< 6.3	< 6.3
Slightly acidic electrolyzed water (hydrochloric acid) pH 5.83, 30ppm		< 6.3	< 6.3
Slightly acidic electrolyzed water (hydrochloric acid & Salt) pH 5.35, 30ppm		< 6.3	< 6.3

Detection limit: 6.3 TCID50/ mL

Decrease in infection value: Log 10 (Initial ÷ Infection value after each working time)

Notation of the decrease in infection value is truncated to two decimal places.

Decrease in infection value	
After 1 min	After 5 min
	0.2
> 6.3	> 6.3
> 6.3	> 6.3
> 6.3	> 6.3
> 6.3	> 6.3
> 6.3	> 6.3
> 6.3	> 6.3
> 6.3	> 6.3
> 6.3	> 6.3
> 6.3	> 6.3
> 6.3	> 6.3

Result by TCID50 method

Antiviral reaction time: 1 minute and 5 minutes

Each Sample Value: Infection value (TCID50/ml)

Decrease in infection value: See the annotations below the table

Positive Control: Locally disinfecting ethanol (83% v/v)

Negative Control: Phosphate buffered saline

4 types of Hypochlorite water that is used in this testing showed a decrease in infection value by more than 4 digits in the reaction time of 1 minute and 5 minutes

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## Summary of Test Results 1

Degree of decrease in infection value	Surfactants	Electrolyzed water
	0.1% concentration, Decreased by more than 4 digits in 5 minutes	Decreased by more than 4 digits in 1 minute and 5 minutes
	0.1% concentration, Decreased by 3 digits in 5 minutes	
	0.1% concentration, Decreased by 2 digits in 5 minutes	
	0.1% concentration, Decreased by less than 2 digits in 5 minutes	

Surfactant classification	Name of surfactant type	Testing Institutions			
		A	B	C	C
		Plaque Method	Plaque Method	Plaque Method	Plaque Method
Anionic surfactants	Pure soap (potassium fatty acid)				
	Sodium chained alkylbenzene sulfonic acid				
Nonionic surfactants	Alkyl glycoside				
	Fatty acid alkanolamide				
	Polyoxyethyleneoxy Alkyl Etail				
Amphoteric ionic surfactants	Alkyl betaine				
	Alkylamine oxides				
Cationic surfactants	Benzalkonium chloride				
Classification of hypochlorite water	Electrolyte & Effective chlorine concentration				
Strongly acidic electrolyzed water	Electrolyte: Salt, 40ppm				
Weakly acidic electrolyzed water	Electrolyte: Salt, 30ppm				
Slightly acidic electrolyzed water 1	Electrolyte: Hydrochloric acid, 30ppm				
Slightly acidic electrolyzed water 2	Electrolyte: Salt & Hydrochloric acid, 40ppm				

## Summary of Test Results 2

- **Surfactants (8 surfactants, including 1 Quaternary ammonium salt)**

- None of testing institution could confirm that alkyl betaine could decrease the virus infection value in this verification study.
- The use of fatty acid alkanolamide. in 0.1% concentration and 5-minute reaction conditions confirmed a decrease in viral infection value by more than 2 digits
- The use of Benzalkonium chloride (quaternary ammonium salt) in 0.1% concentration and 5-minute reaction conditions confirmed a decrease in viral infection value by more than 2 digits.
- The use of fatty acid potassium in 0.1% concentration and 5-minute reaction conditions confirmed a decrease in viral infection value by more than 3 digits.
- The use of 4 surfactants other than the above in 0.1% concentration and 5-minute reaction conditions confirmed a decrease in viral infection value by more than 4 digits.

- **4 types of Hypochlorite water (generated by electrolysis)**

- Viral infection value was decreased by all tested hypochlorite water in 1 minute and 5 minutes by more than 4 digits in this test.

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## Summary of Test Results 3

### Decrease in virus infection value

- More than 4 digits

- Sodium linear alkylbenzene sulfonic acid, alkyl glycoside, polyoxyethylene alkyl ether, alkyl amine oxide
- Hypochlorite water

- More than 3 digits

- Fatty acid potassium

How much of the decreasing infection value should effective antiviral products have?

- More than 2 digits

- Fatty acid alkanolamide, benzalkonium chloride

- No Effective

- Alkyl betaine

<Reference> Examples of virus inactivation effects and criteria for determining antiviral effects

- EN 14476: 2013+A1 2015 (European Standard Test Terms for Disinfectants)
  - If decrease more than 4log10: Virus inactivated
- JIS L 1922, ISO 18184:2014 (Antiviral test method of textile products)

- If decrease more than  $3\log_{10}$ : Effective enough
- If decrease more than  $2\log_{10}$  but less than  $3\log_{10}$ : Effective

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### **The Institutions that helped with this testing**

(Japanese Alphabetical order)

Kanagawa Institute of Industrial Science and Technology

Kitasato Research Center for Environmental Science

Japan Textile Products Quality and Technology Center

Boken Quality Evaluation Institute

Thank you very much for your cooperation in this testing.

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