Final Report on Efficacy Assessment of Disinfecting Substances
Alternative to Alcohol for Use Against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

The Committee on Efficacy Assessment of Disinfecting Substances
Alternative to Alcohol for Use Against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

June, 2020
Introduction

In March 2020, the Ministry of Economy, Trade and Industry (METI) requested that the National Institute of Technology and Evaluation (NITE) conduct an assessment of the virucidal activity of substances alternative to alcohol-based disinfectants in order to prevent the spread of SARS-CoV-2 infection.

More specifically, the request was to perform the urgent evaluation of virucidal activity of candidate substances against SARS-CoV-2, which were widely available as a daily commodity and expected to be alternatives to alcohol-based disinfectants. This was to be done through a literature survey, given the circumstances of the tight supply and increased demand of alcohol-based disinfectants due to the COVID-19 outbreak.

Toward above-mentioned purpose, NITE established the Committee on Efficacy Assessment of Disinfecting Substances Alternative to Alcohol for Use Against SARS-CoV-2 (Secretariat: METI and NITE). The Committee was responsible for the selection of disinfectant candidates for efficacy evaluation, approval of testing protocols, and other matters necessary for consideration. The Committee was also responsible for judging the validity of the evaluation results, as well as assessing and determining the virucidal activity of the candidate substances against SARS-CoV-2.

As a policy, the Committee tried to keep a good balance among ensuring scientific objectivity, facilitating prompt actions, and providing easy-to-understand information to citizens.

The intended application of the disinfecting substances evaluated by this Committee was the disinfection of environmental surfaces in households and community settings (hereinafter referred to as “surfaces”), which included but were not limited to the surfaces of furniture, sinks, bathtubs, toilets, remote controls for electric appliances, and other fixed items such as counter tops, stairway rails, floors and walls. The Committee assessed the virucidal activity of the candidate substances by using the liquid suspension method of the virus and samples for testing virucidal activity. Evaluations for safety and conditions for the actual use of the effective substances were outside of the scope of the Committee.
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1. Outline of the Committee
1-1. Members and Observers of the Committee (in Alphabetical Order)

**Members**

<table>
<thead>
<tr>
<th>Names</th>
<th>Titles and Affiliations as of June 25, 2020</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chairperson: MATSUMOTO Tetsuya, M.D., Ph.D.</td>
<td>Chief Professor, Department of Infectious Diseases, International University of Health and Welfare, and Vice President, Japanese Society for Infection Prevention and Control</td>
</tr>
<tr>
<td>FUKUZAKI Satoshi, Ph.D.</td>
<td>Professor, Graduate School of Bioresources, Mie University</td>
</tr>
<tr>
<td>HANAKI Ken-Ichi, D.V.M., Ph.D.</td>
<td>Director, Management Department of Biosafety and Laboratory Animal, National Institute of Infectious Diseases (NIID)</td>
</tr>
<tr>
<td>ISHIWATARI Yukinori</td>
<td>Representative Director, Japan Electrolyzed Water Association</td>
</tr>
<tr>
<td>KAGEYAMA Seiji, M.D., Ph.D.</td>
<td>Professor (Division of Virology) and Vice Dean, Faculty of Medicine, Tottori University</td>
</tr>
<tr>
<td>KUBOTA Hiroki, Ph.D.</td>
<td>Senior Researcher, Department of Food Additives, National Institute of Health Sciences (NIHS)</td>
</tr>
<tr>
<td>MATSUURA Yoshiharu, D.V.M., Ph.D.</td>
<td>Professor, Department of Molecular Virology, Research Institute for Microbial Diseases, Osaka University</td>
</tr>
<tr>
<td>SAIJO Hiroyuki</td>
<td>Senior Managing Director, Japan Soap and Detergent Association</td>
</tr>
<tr>
<td>UEMA Masashi, D.V.M., Ph.D.</td>
<td>Section Chief, 4th Section, Division of Biomedical Food Research, National Institute of Health Sciences (NIHS)</td>
</tr>
</tbody>
</table>

**Observers**

<table>
<thead>
<tr>
<th>Names</th>
<th>Titles and Affiliations as of June 25, 2020</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMOTO Yasuo, Ph.D.</td>
<td>Microbial Testing Laboratory Manager, Japan Textile Products Quality and Technology Center (QTEC), Kobe Testing Center</td>
</tr>
<tr>
<td>Names</td>
<td>Titles and Affiliations as of June 25, 2020</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-----------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>ISHIGURO Hitoshi, Ph.D.</td>
<td>Vice-Leader, Senior Researcher, Kanagawa Institute of Industrial Science and Technology (KISTEC)</td>
</tr>
<tr>
<td>KATAYAMA Kazuhiko, Ph.D.</td>
<td>Professor, Laboratory of Viral Infection I, Department of Infection Control and Immunology, Ōmura Satoshi Memorial Institute &amp; Graduate School of Infection Control Sciences, Kitasato University</td>
</tr>
<tr>
<td>NAGAI Takeshi, Ph.D.</td>
<td>Researcher, Kanagawa Institute of Industrial Science and Technology (KISTEC)</td>
</tr>
<tr>
<td>OGAWA Haruko, D.V.M., Ph.D.</td>
<td>Professor, Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine (OUAVM)</td>
</tr>
<tr>
<td>SEJIMA Shunsuke</td>
<td>President, Certified NPO Biomedical Science Association (BMSA)</td>
</tr>
<tr>
<td>TAKAGI Hirotaka</td>
<td>Senior Researcher, Management Department of Biosafety and Laboratory Animal, National Institute of Infectious Diseases (NIID)</td>
</tr>
<tr>
<td>TAKEDA Yohei, D.V.M., Ph.D.</td>
<td>Specially Appointed Assistant Professor, Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine (OUAVM)</td>
</tr>
</tbody>
</table>

Ministry Officials

<table>
<thead>
<tr>
<th>Names</th>
<th>Titles and Affiliations as of June 25, 2020</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESAKI Yoshihide</td>
<td>Deputy Director-General, Commerce and Service Industry Policy Group, METI</td>
</tr>
<tr>
<td>HINOSHITA Eiji, M.D., Ph.D.</td>
<td>Director, Tuberculosis and Infectious Diseases Control Division, Health Service Bureau, MHLW</td>
</tr>
<tr>
<td>KANAI Shinsuke</td>
<td>Director for Policy Planning, Crisis Management Unit, Minister's Secretariat, METI</td>
</tr>
<tr>
<td>TANAKA Makoto</td>
<td>Senior Investigator for Surcharge, Representation Division, Consumer Affairs Agency</td>
</tr>
<tr>
<td>TANAKA Tetsuya</td>
<td>Director, Bio-Industry Division, Commerce and Service Industry Policy Group, METI</td>
</tr>
<tr>
<td>YOSHIMURA Katsumoto</td>
<td>Director, Material Industries Division, Manufacturing Industries Bureau, METI</td>
</tr>
</tbody>
</table>
1-2. Summary of the Activities of the Committee

**The First Committee (April 15, 2020)**
- Several candidate substances possible to inactivate SARS-CoV-2 were selected based on the literature survey.

**The Second Committee (April 30, 2020)**
- The results of the evaluation studies of the selected candidates using the Influenza A virus as a surrogate for SARS-CoV-2 were reported.
- The initiation of collaborative studies with the National Institute of Infectious Diseases (NIID) and Kitasato University on the evaluation of the virucidal activity of the candidate substances against SARS-CoV-2 was reported.

**The Third Committee (May 21, 2020)**
- The first report on the evaluation studies using SARS-CoV-2 was presented. Five surfactants (including quaternary ammonium salts) were judged to be effective.

**The Fourth Committee (May 28, 2020)**
- The second report on the evaluation studies using SARS-CoV-2 was presented. Two additional surfactants (including quaternary ammonium salts) were judged to be effective.

**The Fifth Committee (June 25, 2020)**
- The third report of the evaluation studies using SARS-CoV-2 was presented. The results provided by the Obihiro University of Agriculture and Veterinary Medicine (OUAVM), Tottori University and the Japan Textile Products Quality Technology Center (QTEC), in addition to NIID and Kitasato University, revealed that two additional surfactants — hypochlorous acid water (HAW) of 35 ppm or higher of available chlorine concentration (ACC) and sodium dichloroisocyanurate (NaDCC) of 100 ppm or higher of ACC — were judged to be effective against SARS-CoV-2.
- In conclusion, the Committee judged that nine surfactants (including three quaternary ammonium salts) and HAW (including NaDCC) of certain concentration were effective against SARS-CoV-2.
1-3. Schedule of Activities of the Committee

Activities related to the Committee were carried out according to the following schedule.

<table>
<thead>
<tr>
<th>The Committee Meeting</th>
<th>April</th>
<th>May</th>
<th>June</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15th</td>
<td>21st</td>
<td>25th</td>
</tr>
<tr>
<td></td>
<td>1st</td>
<td>3rd</td>
<td>5th</td>
</tr>
<tr>
<td>nite Literature survey</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nite Evaluation study using Influenza A virus as a surrogate for SARS-CoV-2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>METI Disclosing the results to the public</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Figure 1. Schedule of Activities of the Committee**

**Table 1. List of Abbreviation and Acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC</td>
<td>Available chlorine concentration</td>
</tr>
<tr>
<td>COVID-19</td>
<td>Coronavirus Disease 2019</td>
</tr>
<tr>
<td>EW (≤pH 2.7)</td>
<td>Strongly acidic electrolyzed water (≤pH 2.7)</td>
</tr>
<tr>
<td>EW (pH 2.7-5.0)</td>
<td>Weakly acidic electrolyzed water (pH 2.7-5.0)</td>
</tr>
<tr>
<td>EW (pH 5.0-6.5)</td>
<td>Slightly acidic electrolyzed water (pH 5.0-6.5)</td>
</tr>
<tr>
<td>HAW</td>
<td>Hypochlorous acid water</td>
</tr>
<tr>
<td>METI</td>
<td>Ministry of Economy, Trade and Industry</td>
</tr>
<tr>
<td>NaDCC</td>
<td>Sodium dichloroisocyanurate</td>
</tr>
<tr>
<td>NIID</td>
<td>National Institute of Infectious Diseases</td>
</tr>
<tr>
<td>NITE</td>
<td>National Institute of Technology and Evaluation</td>
</tr>
<tr>
<td>OUAVM</td>
<td>Obihiro University of Agriculture and Veterinary Medicine</td>
</tr>
<tr>
<td>QTEC</td>
<td>Japan Textile Products Quality and Technology Center</td>
</tr>
<tr>
<td>SARS-CoV-2</td>
<td>Severe Acute Respiratory Syndrome Coronavirus 2</td>
</tr>
<tr>
<td>TCID&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Median tissue culture infectious dose</td>
</tr>
<tr>
<td>min</td>
<td>minutes</td>
</tr>
<tr>
<td>s</td>
<td>seconds</td>
</tr>
</tbody>
</table>
2. Candidate Substances for Assessment of their Disinfectant Activity Against SARS-CoV-2

2.1. Selection of Candidate Substances Alternative to Alcohol-Based Disinfectants for Efficacy Assessment

Candidate substance categories for efficacy assessment were selected based upon the following criteria:

- Enough supply of corresponding products was secured in the market.
- Virucidal activity against SARS-CoV-2 was anticipated through the literature survey.

Please note that no information was found indicating the virucidal activity of the candidate substances against SARS-CoV-2 as of May 2020. As such, the virucidal activity of the candidate substances against SARS-CoV-2 was presumed based on scientific evidence regarding their virucidal activities against relevant enveloped RNA viruses. These include other coronaviruses (e.g., SARS coronavirus) and influenza viruses in the literature information (Appendix 5: References 1 to 16).

The preliminary evaluation study of the candidates listed in Table 2 was performed based on the above-mentioned criteria, and the following four substance categories were selected as promising candidates:

- Surfactants (household detergents etc.)
- Hypochlorous acid water (electrolytically-generated and non-electrolytically-generated)
- Quaternary ammonium salts
- Sodium percarbonate (synonymous with sodium carbonate peroxyhydrate)
Table 2. Promising Substance Categories for Disinfection Against SARS-CoV-2

<table>
<thead>
<tr>
<th>Substance Category</th>
<th>Supply Capacity</th>
<th>Viricidal activity</th>
<th>Targets of use</th>
<th>Necessity for the efficacy assessment against SARS-CoV-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot Water (Heating)</td>
<td>Excellent</td>
<td>Effective</td>
<td>Food Surfaces</td>
<td>Already Recommended by the Government; No Evaluation Required</td>
</tr>
<tr>
<td>Alcohol (70% or higher)</td>
<td>Insufficient</td>
<td>Effective</td>
<td>Hand (Food) Surfaces</td>
<td>Already Recommended by the Government; No Evaluation Required</td>
</tr>
<tr>
<td>Chlorine Bleach (Sodium Hypochlorite)</td>
<td>Good (Demand Increasing)</td>
<td>Effective</td>
<td>Surfaces</td>
<td>Already Recommended by the Government; No Evaluation Required</td>
</tr>
<tr>
<td>Surfactants (Household Detergents etc.)</td>
<td>Excellent</td>
<td>Likely</td>
<td>(Food) Surfaces</td>
<td>Evaluation Study Needed</td>
</tr>
<tr>
<td>Hypochlorous Acid Water (HAW) (Electrolytically-Generated and Non-Electrolytically-Generated)</td>
<td>Good</td>
<td>Likely</td>
<td>(Hand) Food Surfaces</td>
<td>Evaluation Study Needed</td>
</tr>
<tr>
<td>Quaternary ammonium salts</td>
<td>Good (Demand Increasing)</td>
<td>Likely</td>
<td>(Hand) Food Surfaces</td>
<td>Evaluation Study Needed</td>
</tr>
<tr>
<td>Sodium Percarbonate salts (Synonymous with Sodium Carbonate Peroxyhydrate)</td>
<td>Good</td>
<td>Likely</td>
<td>Surfaces</td>
<td>Evaluation Study Needed</td>
</tr>
</tbody>
</table>

The above table was prepared by the secretariat of the Committee through a literature search. The status of supply capacity is reflected as of mid-April, 2020.

- While hydrogen peroxide, peracetic acid and iodine-based disinfectants were also listed as promising substance categories, they were not further evaluated because these substances were mainly used in a medical setting.
- Although there were many other promising candidate substances likely to be effective against SARS-CoV-2, they were not evaluated in the Committee mainly due to the limitation of their supply capacity for general use.
2-2. 1) Candidate Substances for Efficacy Assessment Against SARS-CoV-2 – Surfactants, Quaternary Ammonium Salts and Sodium Percarbonate

Twelve candidate substances were chosen for the evaluation study, as listed in Table 3. In addition to the eight surfactants (including one quaternary ammonium salt) that had been tested in the preliminary evaluation study using influenza A virus, three surfactants (including two quaternary ammonium salts) (i.e. sodium alkyl ether sulfates, benzethonium chloride and dialkyldimethylammonium chloride) and sodium percarbonate were added to the evaluation study based on a literature survey.

Table 3. Candidate Substances for the Evaluation Study (Surfactants, Quaternary Ammonium Salts and Sodium Percarbonate)

<table>
<thead>
<tr>
<th>Subcategory</th>
<th>Name of Substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anionic Surfactants</td>
<td>Potassium soap</td>
</tr>
<tr>
<td></td>
<td>Sodium soap</td>
</tr>
<tr>
<td></td>
<td>Sodium alkyl ether sulfates</td>
</tr>
<tr>
<td>Nonionic Surfactants</td>
<td>Alkyl glycosides</td>
</tr>
<tr>
<td></td>
<td>Fatty acid alkanol amides</td>
</tr>
<tr>
<td></td>
<td>Polyoxyethylene alkyl ether</td>
</tr>
<tr>
<td>Amphoteric Surfactants</td>
<td>Alkyl amidopropyl betaine</td>
</tr>
<tr>
<td></td>
<td>Alkyl dimethyl amine oxide</td>
</tr>
<tr>
<td>Cationic Surfactants (Quaternary Ammonium Salts)*</td>
<td>Benzalkonium chloride</td>
</tr>
<tr>
<td></td>
<td>Benzethonium chloride</td>
</tr>
<tr>
<td></td>
<td>Dialkyldimethylammonium chloride</td>
</tr>
<tr>
<td>Oxygen Bleach</td>
<td>Sodium percarbonate</td>
</tr>
</tbody>
</table>

* The three substances which were categorized into Cationic Surfactants were also known to be categorized into Quaternary Ammonium Salts.

2-2. 2) Candidates Substances for Efficacy Assessment Against SARS-CoV-2 – Hypochlorous Acid Water (HAW) (Electrolytically-Generated and Non-Electrolytically-Generated)

Several substances from both HAW (electrolytically-generated) and HAW (non-electrolytically-generated) categories were chosen for the evaluation study as listed in Tables 4-1 and 4-2. In this evaluation study, acidic solution containing hypochlorous acid as the main component were considered as HAW.
Please note: There are various types of products manufactured and sold under the name "hypochlorous acid water" in the market, and the majority of these products are categorized into HAW (non-electrolytically-generated); however, there are no widely-accepted product specifications or standards in this category. For instance, the following types of products are sold: a two-liquid premixed type, an ion-exchange type and a powder/tablet type.
Table 4-1. Candidate Substances for the Evaluation Study (Hypochlorous Acid Water (HAW): Electrolytically-Generated)

<table>
<thead>
<tr>
<th>Subcategory</th>
<th>Electrolytes</th>
<th>pH*</th>
<th>Available Chlorine Concentration* (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongly Acidic Electrolyzed Water (EW (≤pH 2.7))</td>
<td>Sodium chloride</td>
<td>≤2.7</td>
<td>20-60</td>
</tr>
<tr>
<td>Weakly Acidic Electrolyzed Water (EW (pH 2.7-5.0))</td>
<td>Sodium chloride, Hydrochloric Acid or</td>
<td>2.7-5.0</td>
<td>10-60</td>
</tr>
<tr>
<td>Slightly Acidic Electrolyzed Water (EW (pH 5.0-6.5))</td>
<td>Hydrochloric Acid + Sodium chloride</td>
<td>5.0-6.5</td>
<td>10-80</td>
</tr>
</tbody>
</table>

Table 4-2. Candidate Substances for the Evaluation Study (Hypochlorous Acid Water (HAW): Non-Electrolytically-Generated)

<table>
<thead>
<tr>
<th>Formulation Type</th>
<th>Ingredients</th>
<th>pH*</th>
<th>Available Chlorine Concentration* (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two-Liquid Premixed**</td>
<td>Sodium Hypochlorite+Hydrochloric Acid</td>
<td>5.0-6.5</td>
<td>100-300</td>
</tr>
<tr>
<td>Two-Liquid Premixed**</td>
<td>Sodium Hypochlorite+Carbonic Acid</td>
<td>5.0-6.5</td>
<td>100-300</td>
</tr>
<tr>
<td>Two-Liquid Premixed**</td>
<td>Sodium Hypochlorite+Acetic Acid</td>
<td>5.0-6.5</td>
<td>100-300</td>
</tr>
<tr>
<td>Ion-Exchange**</td>
<td>Sodium Hypochlorite</td>
<td>5.0-6.5</td>
<td>100-300</td>
</tr>
<tr>
<td>Powder/Tablet</td>
<td>Sodium Dichloroisocyanurate (NaDCC)</td>
<td>5.0-6.5</td>
<td>100-300</td>
</tr>
</tbody>
</table>

*The pH and available chlorine concentration (ACC) indicate the upper and lower limits of the sample. The actual pH and ACC of each tested sample was measured just before the evaluation study.

**Upon the evaluation of HAW of the non-electrolytically-generated type (except for the powder/tablet type), several products corresponding to each formulation type were purchased from the market and the actual evaluation tests were conducted in a blinded fashion. Please note that hydrochloric acid is the most-commonly used as a neutralizing acid among the two-liquid premixed types of HAW.
2-3. Definition of “Hypochlorous Acid Water” Used by This Committee

Various types of products are manufactured and sold under the name "hypochlorous acid water" in the market. Because there have been no widely-accepted product specifications or standards for these products, the following categorization is being tentatively used for this report.

Figure 2. Definition of “Hypochlorous Acid Water” Used by This Committee
3. Outline of Evaluation Studies with SARS-CoV-2 by Participating Organizations

3.1. The evaluation studies were performed by the following organizations in a collaboration manner:

- National Institute of Infectious Disease (NIID)
- Kitasato University
- Obihiro University of Agriculture and Veterinary Medicine (OUAVM)
- Tottori University
- Japan Textile Products Quality and Technology Center (QTEC)
Table 5. Materials and Test Conditions\(^1\) Used in the Evaluation Study of Each Participating Organization

<table>
<thead>
<tr>
<th>Organization</th>
<th>NIID</th>
<th>Kitasato University</th>
<th>OUAVM</th>
<th>Tottori University</th>
<th>QTEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium for Preparation of Virus Stock</td>
<td>DMEM</td>
<td>DMEM</td>
<td>DMEM</td>
<td>DMEM</td>
<td>DMEM</td>
</tr>
<tr>
<td>Concentration of FBS for Preparation of Virus Stock</td>
<td>5%</td>
<td>2%</td>
<td>1%</td>
<td>5%</td>
<td>1%</td>
</tr>
<tr>
<td>Detection Method of Virus</td>
<td>TCID(_{50})</td>
<td>CPE Observation and qRT-PCR</td>
<td>TCID(_{50})</td>
<td>TCID(_{50})</td>
<td>TCID(_{50})</td>
</tr>
<tr>
<td>Volume Ratio of Virus Stock to Test Substance</td>
<td>1:1(^a), 1:9(^b), 1:19(^c)</td>
<td>1:9(^b, c)</td>
<td>1:9(^b, c), 1:19(^c)</td>
<td>1:9(^b), 1:19(^b)</td>
<td>1:9(^b), 1:19(^b)</td>
</tr>
<tr>
<td>Initial Virus Concentration</td>
<td>&gt; 4 x 10(^6) TCID(_{50})/50 µl(^a)</td>
<td>&gt; 10(^6) pfu/µl</td>
<td>&gt; 1 x 10(^6) TCID(_{50})/ml</td>
<td>&gt; 1 x 10(^6) TCID(_{50})/ml</td>
<td>&gt; 1 x 10(^7) TCID(_{50})/ml</td>
</tr>
<tr>
<td>Substance Tested Surfactants: Non-Electrolytic HAW, Sodium Percarbonate, Electrolytic HAW</td>
<td>Surfactants</td>
<td>Non-Electrolytic HAW, Electrolytic HAW</td>
<td>Non-Electrolytic HAW</td>
<td>Non-Electrolytic</td>
<td>Non-Electrolytic</td>
</tr>
</tbody>
</table>

\(^1\) Test conditions are described in detail in Appendix 2.

FBS, fetal bovine serum; CPE, cytopathic effect; non-electrolytic HAW, HAW (non-electrolytically-generated); electrolytic HAW, HAW (electrolytically-generated)

\(^a\), for surfactants; \(^b\), for HAW (non-electrolytically-generated); \(^c\), for HAW (electrolytically-generated)
4. Results of the Evaluation Studies with SARS-CoV-2

4-1. Results of the Evaluation Studies with SARS-CoV-2 – Surfactants, Quaternary Ammonium Salts and Sodium Percarbonate

• Potassium soap 0.12% (5 min) and sodium soap 0.11% (5 min) showed a less than 99% reduction of viral titer in the evaluation study at NIID. Potassium soap 0.1% (5 min) and sodium soap 0.1% (10 min) did not show virucidal activity in the evaluation study at Kitasato University. On the other hand, potassium soap 0.24% (1 min) and sodium soap 0.22% (1 min) showed a more than 99.999% reduction of viral titer in the evaluation study at NIID.

• Sodium linear alkylbenzene sulfonates 0.1% (20 s) showed a more than 99.999% reduction of viral titer in the evaluation study at NIID. Virucidal activity was observed at 0.1% (5 min) in the evaluation study at Kitasato University.

• Sodium alkyl ether sulfates 0.5% (5 min) showed a less than 99.9% reduction of viral titer in the evaluation study at NIID. Virucidal activity was not observed at 0.1% (5 min) in the evaluation study at Kitasato University.

• Alkyl glycosides 0.05% (20 s) showed a more than 99.999% reduction of viral titer in the evaluation study at NIID. Virucidal activity was observed at 0.1% (1 min) in the evaluation study at Kitasato University.

• Fatty acid alkanol amides 0.2% (5 min) showed less than a 99.9% reduction of viral titer in the evaluation study at NIID. Virucidal activity was not observed at 0.1% (5 min) in the evaluation study at Kitasato University.

• Polyoxyethylene alkyl ether 0.1% (5 min) showed a more than 99.999% reduction of viral titer in the evaluation study at NIID. Virucidal activity was not observed at 0.1% (5 min) in the evaluation study at Kitasato University. Polyoxyethylene alkyl ether 0.2% (5 min) showed a more than 99.999% reduction of viral titer in the evaluation study at NIID.

• Alkyl amidopropyl betaine 0.5% (5 min) showed less than a 99.9% reduction of viral titer in the evaluation study at NIID. Virucidal activity was not observed at 0.1% (5 min) in the evaluation study at Kitasato University.

• Alkyldimethylamine oxide 0.05% (20 s) showed a more than 99.999% reduction of viral titer in the evaluation study at NIID. Virucidal activity was observed at 0.05% (1 min) in the evaluation study at Kitasato University.

• Benzalkonium chloride 0.05% (2 min) showed a more than 99.999% reduction of viral titer in the evaluation study at NIID. Virucidal activity was observed at 0.05% (1 min) in the evaluation study at Kitasato University.
• Benzethonium chloride 0.05% (1 min) showed a more than 99.999% reduction of viral titer in the evaluation study at NIID. Virucidal activity was observed at 0.05% (5 min) in the evaluation study at Kitasato University.

• Dialkyldimethylammonium chloride 0.01% (40 s) showed a more than 99.999% reduction of viral titer in the evaluation study at NIID. Virucidal activity was observed at 0.01% (5 min) in the evaluation study at Kitasato University.

• Sodium percarbonate did not show virucidal activity at 1.0% (5 min) in the evaluation study at Kitasato University. This substance was evaluated only at Kitasato University.

• No cytotoxic effect was observed for each substance at the concentration described above.
Table 6. Summary of Results of the Evaluation Studies with SARS-CoV-2 (Surfactants, Quaternary Ammonium Salts and Sodium Percarbonate)

<table>
<thead>
<tr>
<th>Testing organization</th>
<th>Reduction Rate of Virus Titer</th>
<th>Concentration (%)</th>
<th>Virucidal Activity</th>
<th>Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NIID¹</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anionic Surfactants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium Soap</td>
<td>99.999% or more</td>
<td>0.24</td>
<td>-</td>
<td>0.10</td>
</tr>
<tr>
<td>Sodium Soap</td>
<td>99.999% or more</td>
<td>0.22</td>
<td>-</td>
<td>0.10</td>
</tr>
<tr>
<td>Sodium Linear Alkylbenzene Sulfonates</td>
<td>99.999% or more</td>
<td>0.10</td>
<td>+</td>
<td>0.10</td>
</tr>
<tr>
<td>Sodium Alkyl Ether Sulfates</td>
<td>Less than 99.9%</td>
<td>0.20</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Nonionic Surfactants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkyl Glycosides</td>
<td>99.999% or more</td>
<td>0.05</td>
<td>+</td>
<td>0.10</td>
</tr>
<tr>
<td>Fatty Acid Alkanol Amides</td>
<td>Less than 99.9%</td>
<td>0.20</td>
<td>-</td>
<td>0.10</td>
</tr>
<tr>
<td>Polyoxyethylene Alkyl Ether</td>
<td>99.999% or more</td>
<td>0.20</td>
<td>-</td>
<td>0.10</td>
</tr>
<tr>
<td>Amphoteric Surfactants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkyl Amidopropyl Betaine</td>
<td>Less than 99.9%</td>
<td>0.50</td>
<td>-</td>
<td>0.10</td>
</tr>
<tr>
<td>Alkyldimethylamine Oxide</td>
<td>99.999% or more</td>
<td>0.05</td>
<td>+</td>
<td>0.05</td>
</tr>
<tr>
<td>Cationic Surfactants (Quaternary Ammonium Salts)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzalkonium Chloride</td>
<td>99.999% or more</td>
<td>0.05</td>
<td>+</td>
<td>0.05</td>
</tr>
<tr>
<td>Benzethonium Chloride</td>
<td>99.999% or more</td>
<td>0.05</td>
<td>+</td>
<td>0.05</td>
</tr>
<tr>
<td>Dialkyldimethylammonium Chloride</td>
<td>99.99% or more</td>
<td>0.01</td>
<td>+</td>
<td>0.01</td>
</tr>
<tr>
<td>Sodium Percarbonate</td>
<td>NT</td>
<td>NT</td>
<td>-</td>
<td>1.00</td>
</tr>
</tbody>
</table>

¹ The results of the evaluation study at NIID show percentage reduction rates of the virus infectious titer by TCID₅₀ calculation method.

² In the evaluation study at Kitasato University, when approximately 10,000 virus particles were almost completely inactivated (i.e., less than the detection limit), it was judged as “virucidal activity observed.”

NT, Not tested; +, virucidal activity observed; -, virucidal activity not observed.
4.2. Results of the Evaluation Studies with SARS-CoV-2 – Hypochlorous Acid Water (Electrolytically-Generated and Non-Electrolytically-Generated) Except for Sodium Dichloroisocyanurate

- Hypochlorous acid water (HAW) (electrolytically-generated) from ACC 35 to 54 ppm (pH 2.4-5.9) showed a more than 99.9% reduction of viral titer in the reaction times from 20 s to 5 min in the evaluation study at NIID. On the other hand, the same type of HAW from ACC 19 to 26 ppm (pH 2.4-4.2) showed less than a 99.9% reduction of viral titer within the same reaction times.
- HAW (electrolytically-generated) of ACC 50 ppm (pH 5.0 and 6.0) did not show virucidal activity for the reaction times of 1 min and 5 min in the evaluation study at Kitasato University.
- HAW (electrolytically-generated) of ACC 32 ppm (pH 5.3) and 56 ppm (pH 2.5, pH 5.2) showed a more than 99.99% reduction of viral titer for the reaction times of 20 s, 1 min and 5 min in the evaluation study at OUAVM.
- HAW (non-electrolytically-generated) of ACC 100 ppm and 200 ppm (pH 6.0) showed a more than 99.9% reduction of viral titer for the reaction times of 20 s and 1 min in the evaluation study at Tottori University.
- HAW (non-electrolytically-generated) of ACC 51 ppm (pH 6.0) showed a more than 99.99% reduction of viral titer for the reaction times of 20 s and 1 min in the evaluation study at QTEC. On the other hand, the same type of HAW of ACC 27 ppm (pH 6.0) showed less than a 99.9% reduction of viral titer for the reaction times of 20 s and 1 min.
- No cytotoxic effect was observed for each substance at the concentration described above except for ACC 200 ppm of HAW (non-electrolytically-generated) in the evaluation study at OUAVM, at which slight cytotoxicity was observed in periphery of wells.
Table 7. Summary of Test Conditions of HAW in Participating Organizations.

<table>
<thead>
<tr>
<th>Organization</th>
<th>NIID(^1)</th>
<th>Kitasato University(^2)</th>
<th>OUAVM(^1)</th>
<th>Tottori University(^1)</th>
<th>QTEC(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of FBS for Preparation of Virus Stock</td>
<td>5%</td>
<td>2%</td>
<td>1%</td>
<td>5%</td>
<td>1%</td>
</tr>
<tr>
<td>Detection Method of Virus Titer</td>
<td>TCID(_{50})</td>
<td>CPE Observation and qRT-PCR</td>
<td>TCID(_{50})</td>
<td>TCID(_{50})</td>
<td>TCID(_{50})</td>
</tr>
<tr>
<td>Volume Ratio of Virus Stock to Test Substance</td>
<td>1:19</td>
<td>1:9</td>
<td>1:19</td>
<td>1:19</td>
<td>1:19</td>
</tr>
</tbody>
</table>

\(^1\) The results of the evaluation studies by these organizations show percentage reduction rates of the virus infectious titer by TCID\(_{50}\) calculation method.

\(^2\) In the evaluation study at Kitasato University, when approximately 10,000 virus particles were almost completely inactivated (i.e., less than the detection limit), it was judged as “virucidal activity observed.”
4-3. Summary of the Evaluation Studies with SARS-CoV-2 – Hypochlorous Acid Water (Electrolytically-Generated and Non-Electrolytically-Generated) Except for Sodium Dichloroisocyanurate

Figure 3. Virucidal Activities of HAW (Electrolytically-Generated and Non-Electrolytically-Generated) Except for Sodium Dichloroisocyanurate

All data for the reaction time of 20 s obtained by NIID, OUAVM, Tottori University and QTEC were plotted in the figure. Please note that the data for Kitasato University are for the reaction times of 1 min and 5 min.

- ○ Data of HAW (Electrolytically-Generated) at NIID
- □ Data of HAW (Electrolytically-Generated) at OUAVM
- △ Data of HAW (Non-Electrolytically-Generated) at OUAVM
- ◊ Data of HAW (Non-Electrolytically-Generated) at Tottori University
- ★★ Data of HAW (Non-Electrolytically-Generated) at QTEC

- 99.999% or more
- 99.99% or more
- 99.9% or more
- 99% or more
- Less than 99%
Table 8. Summary of Test Conditions of HAW by Participating Organizations (Figure 3)

<table>
<thead>
<tr>
<th>Testing Organization</th>
<th>NIID</th>
<th>Kitasato University</th>
<th>OUAVM</th>
<th>Tottori University</th>
<th>QTEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of FBS for Preparation of Virus Stock</td>
<td>5%</td>
<td>2%</td>
<td>1%</td>
<td>5%</td>
<td>1%</td>
</tr>
<tr>
<td>Ratio of Virus Stock to Test Substance</td>
<td>1:19</td>
<td>1:9</td>
<td>1:19</td>
<td>1:19</td>
<td>1:19</td>
</tr>
</tbody>
</table>

4-4. Summary of the Evaluation Studies with SARS-CoV-2 – Hypochlorous Acid Water (Electrolytically-Generated and Non-Electrolytically-Generated) Except for Sodium Dichloroisocyanurate at ACC of ACC 60 ppm or Less

HAW of 32 ppm or higher showed more than 99.99% reduction rate of viral titer in the studies of OUAVM and QTEC.

HAW of 35 ppm or higher showed a more than 99.9% reduction rate of viral titer in the studies by NIID.

Figure 4. Virucidal Activities of HAW (Electrolytically-Generated and Non-Electrolytically-Generated) Except for Sodium Dichloroisocyanurate at ACC of 60 ppm or less.

- : Data of HAW (Electrolytically-Generated) at NIID
  - : Data of HAW (Electrolytically-Generated) at OUAVM
  ▲ : Data of HAW (Non-Electrolytically-Generated) at OUAVM
  ✖ : Data of HAW (Non-Electrolytically-Generated) at QTEC
4-5. Summary of the Evaluation studies with SARS-CoV-2 – Sodium Dichloroisocyanurate (NaDCC)

• While the NaDCC of ACC 200 ppm or higher showed a more than 99.999% reduction of viral titer at the reaction times of 20 s to 5 min in the evaluation study with a 1:9 volume ratio of virus to sample at NIID, the NaDCC of AC 100 ppm showed a more than 99.9% reduction of viral titer at the reaction times of 40 s to 5 min.
• The NaDCC of ACC 50 ppm or higher showed a more than 99.99% reduction of viral titer for the reaction times of 20 s, 1 min and 5 min in the evaluation study with a 1:19 volume ratio of virus to sample at OUAVM.

Please note: The Committee treated NaDCC as a substance categorized into HAW (non-electrolytically-generated), but the results of the NaDCC solution was herein described separately from HAW for the following reason. While an aqueous solution of NaDCC contains a certain amount of hypochloric acid, free chlorine is released from dichloroisocyanurate by the dissociation equilibrium reaction. Thus, an aqueous solution of NaDCC has different properties from conventional HAW.

Table 9. Summary of the Test Conditions of Each Organization

<table>
<thead>
<tr>
<th>Organization</th>
<th>NIID</th>
<th>OUAVM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of FBS for Preparation of Virus Stock</td>
<td>5%</td>
<td>1%</td>
</tr>
<tr>
<td>Detection Method of Virus Titer</td>
<td>TCID₅₀</td>
<td>TCID₅₀</td>
</tr>
<tr>
<td>Volume Ratio of Virus Stock to Test Substance</td>
<td>1:9</td>
<td>1:19</td>
</tr>
</tbody>
</table>
5. Substances Judged to be Effective Against SARS-CoV-2
5-1. Substances Judged to be Effective Against SARS-CoV-2 – Surfactants, Quaternary Ammonium Salts and Sodium Percarbonate

◆ Criteria for Determining Efficacy
Virucidal efficacy of each surfactant, quaternary ammonium salt and sodium percarbonate substance was assessed based on the results of the evaluation studies. A substance was judged to be effective against SARS-CoV-2, when a more than 99.99% reduction of viral titer was shown by NIID and virucidal activity was also shown by Kitasato University. Even when positive results were shown only by either of these organizations, a conclusion was drawn after careful investigation of the results of the evaluation studies obtained by both of these organizations, as the potential for virucidal activity of such substances was still worth considering.

◆ Determination of Efficacy of the Tested Substances
- Potassium soap of 0.12% (5 min) and sodium soap of 0.11% (5 min) showed less than a 99% reduction of viral titer in the evaluation study at NIID. Potassium soap of 0.1% (5 min) and sodium soap of 0.1% (10 min) showed no virucidal activity in the evaluation study at Kitasato University. On the other hand, potassium soap of 0.24% (1 min) and sodium soap of 0.22% (1 min) showed a more than 99.999% reduction of viral titer in the evaluation study at NIID. Thus, potassium soap of 0.24% or higher and sodium soap of 0.22% or higher were judged to be effective.
- Sodium linear alkylbenzene sulfonates of 0.1% (20 s) showed a more than 99.999% reduction of viral titer in the evaluation study at NIID. Virucidal activity was also observed at 0.1% (5 min) in the evaluation study at Kitasato University. Thus, sodium linear alkylbenzene sulfonates of 0.1% or higher were judged to be effective.
- Sodium alkyl ether sulfates of 0.5% (5 min showed less than a 99.9% reduction of viral titer in the evaluation study at NIID. Virucidal activity was not observed at 0.1% (5 min) in the evaluation study at Kitasato University. Thus, sodium alkyl ether sulfates were not judged to be effective at a concentration of up to 0.5%.
- Alkyl glycosides of 0.05% (20 s) showed a more than 99.999% reduction of viral titer in the evaluation study at NIID. Virucidal activity was also observed at 0.1% (1 min) in the evaluation study at Kitasato University. Thus, alkyl glycosides of 0.1% or higher were judged to be effective.
- Fatty acid alkanol amides of 0.2% (5 min) showed less than a 99.9% reduction of
viral titer in the evaluation study at NIID. Virucidal activity was not observed at 0.1% (5 min) in the evaluation study at Kitasato University. Thus, fatty acid alkanol amides were not judged to be effective at a concentration of up to 0.2%.

- While polyoxyethylene alkyl ether of 0.1% (5 min) showed a 99.99% reduction of viral titer in the evaluation study at NIID, virucidal activity was not observed at 0.1% (5 min) in the evaluation study at Kitasato University. On the other hand, the substance of 0.2% (5 min) showed a more than 99.999% reduction of viral titer in the evaluation study at NIID. Thus, polyoxyethylene alkyl ether of 0.2% or higher was judged to be effective.

- Alkyl amidopropyl betaine of 0.5% (5 min) showed less than a 99.9% reduction of viral titer in the evaluation study at NIID. Virucidal activity was not observed at 0.1% (5 min) in the evaluation study at Kitasato University. Thus, alkyl amidopropyl betaine was not judged to be effective at a concentration of up to 0.5%.

- Alkyldimethylamine oxide of 0.05% (20 s) showed a more than 99.999% reduction of viral titer in the evaluation study at NIID. Virucidal activity was also observed at 0.05% (1 min) in the evaluation study at Kitasato University. Thus, alkyldimethylamine oxide of 0.05% or higher was judged to be effective.

- Benzalkonium chloride of 0.05% (2 min) showed a more than 99.999% reduction of viral titer in the evaluation study at NIID. Virucidal activity was also observed at 0.05% (1 min) in the evaluation study at Kitasato University. Thus, benzalkonium chloride of 0.05% or higher was judged to be effective.

- Dialkyldimethylammonium chloride of 0.01% (40 s) showed a more than 99.999% reduction of viral titer in the evaluation study at NIID. Virucidal activity was also observed at 0.01% (5 min) in the evaluation study at Kitasato University. Thus, dialkyldimethylammonium chloride of 0.01% or higher was judged to be effective.

- Sodium percarbonate was tested only at Kitasato University, and virucidal activity was not observed at 1.0% (5 min) in the evaluation study. Thus, sodium percarbonate was not judged to be effective at a concentration of up to 1.0%.
5.2. Summary of Substances Judged to be Effective Against SARS-CoV-2 and Precautions When Eliminating SARS-CoV-2 by Surfactants and Quaternary Ammonium Salts

**Substances Judged to be Effective by the Results of the Evaluation Studies**

- Sodium linear alkylbenzene sulfonates (0.1% or higher)
- Alkyl glycosides (0.1% or higher)
- Alkyldimethylamine oxide (0.05% or higher)
- Benzalkonium chloride (0.05% or higher)
- Benzethonium chloride (0.05% or higher)
- Dialkyldimethylammonium chloride (0.01% or higher)
- Polyoxyethylene alkyl ether (0.2% or higher)
- Potassium soap (0.24% or higher)
- Sodium soap (0.22% or higher)

Please note: These results were obtained using the surfactant components themselves. These were not the results of household detergent products that contain these surfactants.

**Precautions When Eliminating SARS-CoV-2 from Surfaces with Effective Surfactants**

- Effective elimination of SARS-CoV-2 can be expected by the appropriate use of various types of household detergents containing effective surfactants. It is important to adhere to the instructions for the use of each product.
- Please note that this assessment verified the efficacy of certain surfactants for disinfection of surfaces against SARS-CoV-2, but was not designed to verify the effectiveness of surfactants for sanitization of the hands or skin.
- It is also important to pay attention to the safety information on household detergent products. Regarding safety, it is necessary to appropriately use these products in consideration of the safety information and precautions provided by the manufacturer etc.
5-3. Substances Judged to be Effective Against SARS-CoV-2 – Hypochlorous Acid Water (Electrolytically-Generated and Non-Electrolytically-Generated) Except for Sodium Dichloroisocyanurate (NaDCC)

◆ Criteria for Determining Efficacy

Virucidal efficacy of each hypochlorous acid water (HAW) substance was assessed based on the results of the evaluation studies. A HAW substance was judged to be effective against SARS-CoV-2, when a more than 99.99% reduction of viral titer was shown by four of the institutes (NIID, OUAVM, Tottori Univ. and QTEC) and virucidal activity also was shown by Kitasato University. Even when positive results were not obtained by two of the organizations, a conclusion was drawn after careful investigation of the results of the evaluation studies of all of the organizations because the potential for virucidal activity of such samples was still worth considering.

The Committee assumed that the two substances of HAW had the same virucidal activity regardless of their production process, if the available chlorine concentration (ACC) and pH were the same for the substances. Based on this idea, the same criteria for determining efficacy against SARS-CoV-2 were applied to both electrolytically-generated and non-electrolytically-generated HAW.

On the other hand, the Committee judged the efficacy of an NaDCC solution separately from HAW using the following rationale, although it categorized this substance as HAW (non-electrolytically-generated). While an aqueous solution of NaDCC contains a certain amount of hypochloric acid, free chlorine is released from dichloroisocyanurate by the dissociation equilibrium reaction. Thus, the Committee assumes that an aqueous solution of NaDCC has different properties from conventional HAW.

◆ Determination of Efficacy of the Tested Substances

- HAW of an ACC of 32 ppm or higher demonstrated a more than 99.99% reduction of viral titer in the evaluation study at OUAVM, and HAW of an ACC 51 ppm or higher showed a more than 99.99% reduction of viral titer in the evaluation study at QTEC.
- HAW of an ACC of 35 ppm or higher showed a more than 99.9% reduction of viral titer in the evaluation study at NIID. The level of reduction of viral titer observed at NIID was somewhat different from the levels seen at OUAVM and QTEC. Although it was difficult to clearly identify a cause of this difference, it seemed likely that the composition of the virus stock solution and/or the content of the organic matter in the reaction solution had affected the level reduction of viral titer.
• HAW of an ACC of 50 ppm did not show any virucidal activity in the evaluation study at Kitasato University. The volume ratio of virus stock to the tested sample in the reaction solution was 1:9 in this study. Furthermore, the presence of virucidal activity could be judged in the study at Kitasato University only when approximately 10,000 virus particles were almost completely inactivated (i.e., less than the detection limit) by cytopathic effect and qRT-PCR. Lack of virucidal activity may have been due to these harsh conditions.

• In the study at OUAVM, virucidal activity of HAW of an ACC of 52 ppm was attenuated up to one-tenth, when the volume ratio of virus stock to tested sample in the reaction solution was changed from 1:19 to 1:9. Furthermore, virucidal activity of HAW of an ACC of 51 ppm was drastically attenuated up to one thousandth in the study at QTEC, when it was changed from 1:19 to 1:9. These results indicate that the virucidal effect was greatly influenced by the volume ratio of the virus solution and HAW in the reaction solution. These results offer an important suggestion as to the proper way to use HAW, particularly that at a low ACC concentration, for effective elimination of the virus.

• On the other hand, virucidal activity of HAW of an ACC of 200 ppm was maintained, even when the volume ratio was changed from 1:19 to 1:9 in the study at Tottori University (under the condition of 5% FBS in the virus stock solution). Virucidal activity of HAW of an ACC of 84 ppm was also maintained between these two volume ratios in the study at QTEC (under the condition of 1% FBS in the virus stock solution). From these results, together with the results of HAW at a lower ACC, it was suggested that HAW at a higher ACC would be able to maintain the virucidal effect even under more strict conditions (i.e., at a volume ratio of 1:9).

• It was reported that the activity of hypochlorous acid was attenuated after it bound to an organic matter such as a protein (Appendix 5, Ref. No. 17-21). The same tendency seemed to be observed in these evaluation studies. This observation also offered an important suggestion as to the proper way to use HAW for the effective elimination of the virus.

• The criterion for determining efficacy was basically set at a 99.99% or more reduction of viral titer in this series of evaluation studies. On the other hand, it was revealed though a literature search that the criteria for virucidal effects were set at 99.9% to 99.99% in various guidelines, depending on their purpose or use conditions. Considering these circumstances, it was still worthwhile to take a 99.9% reduction of viral titer into account as a feasible criterion for determining efficacy in the evaluation study at NIID.
Based on these results, the Committee judged that HAW of an ACC of 35 ppm or higher was effective, which showed a more than 99.9% reduction of viral titer in the evaluation study at NIID as well as a more than 99.99% reduction in the studies at OUAVM and QTEC.

5.4. Substances Judged to be Effective Against SARS-CoV-2 – Sodium Dichloroisocyanurate (NaDCC)

◆ Determination of Efficacy of the Tested Substance

• Basically, the same criteria were applied as those for HAW in determining the efficacy of NaDCC. NaDCC of an ACC of 50 ppm or higher, which showed a more than 99.99% reduction of viral titer in the evaluation study at OUAVM. On the other hand, NaDCC of an ACC of 200 ppm or higher showed a more than 99.999% reduction, but NaDCC of an ACC of 100 ppm showed a more than 99.9% reduction in the evaluation study at NIID. It should be taken into account that NaDCC of an ACC of 100 ppm showed a significant effect in the study conditions at NIID, which were harsher than those at OUAVM.

• Based on these results, the Committee judged that NaDCC of an ACC of 100 ppm or higher was effective, which showed a more than 99.9% reduction of viral titer in the evaluation study at NIID, as well as a more than 99.99% reduction in the study at OUAVM.
5-5. Summary of Substances Judged to be Effective Against SARS-CoV-2 and Precautions When Eliminating SARS-CoV-2 by Hypochlorous Acid Water

Substances Judged to be Effective by the Results of the Evaluation Studies

➢ The following ACC ranges of HAW and the NaDCC solution were judged to be effective among the samples of hypochlorous acid water (pH 6.5 or less) and the NaDCC solution tested in the evaluation studies.
   • For HAW (electrolytically-generated and non-electrolytically-generated), an ACC of 35 ppm or higher.
   • For an NaDCC solution, an ACC of 100 ppm or higher.

Please note that the Committee judged the efficacy of the NaDCC solution separately from HAW by the following rationale, although NaDCC was initially categorized as a HAW (non-electrolytically-generated). While an aqueous solution of NaDCC contains a certain amount of hypochloric acid, free chlorine is released from dichloroisocyanyrate by the dissociation equilibrium reaction. Thus, the Committee assumes that an aqueous solution of NaDCC has different properties from conventional HAW.

Precautions when Eliminating SARS-CoV-2 from Surfaces with Effective HAW, Including an NaDCC Solution

➢ Elimination of SARS-CoV-2 can be expected with the appropriate use of an effective HAW.

➢ Based on the characteristics of HAW, as well as the results of the series of evaluation studies, the recommended use of effective HAW for virus elimination are described as follows:
   • Remove dirt, such as organic matter (hand stains etc.), in advance.
   • Use an enough amount of HAW to eliminate the virus on the surface.

➢ Please note that this assessment verified the efficiency of HAW within a certain range of ACC for the disinfection of surfaces against SARS-CoV-2, but was not designed to verify the effectiveness of HAW for the sanitization of hands or skin.

➢ It is also important to pay attention to the safety information on HAW products. Regarding safety, it is necessary to appropriately use these products in consideration of the safety information and precautions provided by the manufacturer etc.
Figure 5. Tested Substances of Hypochlorous acid water (HAW) and Sodium Dichloroisocyanurate (NaDCC) Solution in the Evaluation Studies and their ACC Ranges Judged to be Effective

(See Table 4 for HAW Substances Tested in the Evaluation Studies.)

*Please note that the ranges of pH and ACC plotted for HAW (electrically-generated type) correspond to the ranges set forth by Japan's specifications and standards for food additives.
Important Reminder About the Efficacy Assessment Conducted by the Committee

The Committee judged efficacy of the candidate substances against SARS-CoV-2 based on the criterion set at a 99.99% (i.e., 4 log) or more reduction of viral titer. However, in general criteria for the evaluation of virucidal efficacy set by various guidelines are not necessarily limited to 99.99%, and such criteria seem to have been set based on their purpose or use conditions. Considering these circumstances, one can argue that the efficacy criterion (i.e., a 99.99% or more reduction) set by the Committee in the evaluation studies is somewhat strict. In short, this can be considered as the one with more certainty of virucidal effects. Since the actual virucidal effects are exerted not only by the virucidal potential of these substances but also by their proper use according to their properties, the Committee agrees that the use of the above-mentioned strict criterion leaves a certain margin.

Given the above background, even though a certain substance was not judged to be effective by the Committee against SARS-CoV-2 due to the lack of efficacy in a particular condition (e.g., in a certain concentration) of this series of evaluation studies, this does not necessarily imply denial of virucidal efficacy of the substance. Please note that the efficacy of substances was affected by the study conditions of the evaluation study.

In response to an emergency, the Committee conducted this efficacy assessment for several candidate substances as described above within a limited time frame and with limited resources. In other words, the Committee could not carry out the evaluation of many other substances that could be expected to have a virucidal effect against SARS-CoV-2. Although these substances fell outside of the scope of the evaluation, this does not imply that the Committee has made any determination on an efficacy of these substances against SARS-CoV-2.

The results of the efficacy assessment by the Committee are intended to be used for public relations activities to facilitate public understanding for the presence of alternative substances to alcohol to use for disinfection against SARS-CoV-2 and to encourage a better use of these substances for elimination of SARS-CoV-2 from surfaces in households and community settings.
This assessment was not conducted in accordance with the Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices (Pharmaceutical Affairs Law), the Food Sanitation Act or any other relevant Japanese laws and regulations.
Closing Remarks

In response to the request by METI under emergency conditions, NITE urgently established and has operated the Committee for the purpose of efficacy assessment of disinfectant substances alternative to alcohol for the use against SARS-CoV-2 as part of the government’s countermeasures against the spread of COVID-19. All evaluation studies have been made with the latest information obtained to the extent possible as of June 2020. We greatly expect that further research and evaluations will proceed and that new knowledge will be accumulated in this field.

There were many substances that could not be evaluated due to limited time and resources. We do hope that the results of these evaluation studies can be used for future studies of these substances by other research institutes and the private sector. We would be greatly pleased if the efforts made by the Committee helped prevent the spread of SARS-CoV-2 infection and continued to promote public health.

We would also like to express our deepest gratitude to all the Committee members, institutions and universities that participated in these studies, as well as everyone who contributed to these studies despite the limited time and resources.
Appendix
Testing Protocol of Each Organization
Outline of Testing protocol with SARS-CoV-2
- National Institute of Infectious Disease (NIID) -

- Preparation of virus stock
  - Medium: DMEM + 5% FBS
- Dilution of test substance
  - Surfactant: Diluted with DDW (final concentration: variable)
  - Sodium dichloroisocyanurate: Diluted with DDW (final concentration: 300, 200 and 100 ppm)
  - Hypochlorous acid water; electrolytically-generated type: No dilution required
- Antiviral reaction
  - Surfactant: 1 part of virus + 1 part of test substance solution
  - Sodium dichloroisocyanurate: 1 part of virus + 9 parts of test substance solution
  - Hypochlorous acid water; electrolytically-generated type: 1 part of virus + 19 parts of test substance
  - Reaction Time: 20 s, 40 s, 60 s, 2 min and 5 min.
  - Temperature: room temperature (RT)
- Neutralization of test substance
  - Surfactant: Diluted with equal volume of DMEM + 5% FBS and removed with resin
  - Sodium dichloroisocyanurate: Diluted with 7x volume of DMEM + 5% FBS + 0.01 M Na₂S₂O₃ and removed with resin
  - Hypochlorous acid water; electrolytically-generated type: Diluted with 7x volume of DMEM + 5% FBS + 0.01 M Na₂S₂O₃.
- Calculation of results
  - TCID₅₀ method
  - Positive Control: None
  - Negative Control: Culture medium
Outline of Testing protocol with SARS-CoV-2
- National Institute of Infectious Disease (NIID) -

(continued)

Case of surfactant

1 part of virus + 1 part of test substance
(e.g. 80 \(\mu l\) of virus stock (7 x 10^6 TCID\(_{50}\)/80 \(\mu l\)) +
80 \(\mu l\) of test substance solution in a test tube)

Stand at RT (20 s, 40 s, 60 s, 2 min and 5 min)

Mix with 6x volume of medium (DMEM + 5% FBS)
with resin to remove surfactant (= 7\(^{-1}\) dilution)
(e.g. Collect 20 \(\mu l\) of the reaction mixture and
transfer it to 120 \(\mu l\) of medium with 100 \(\mu l\) bed
volume resin (Bio-rad BioBeads SM-2))

Stand at RT for 5 min

Serial 7-fold dilutions with medium (ex, \(7^{-2} - 7^{-8}\)

TCID\(_{50}\) is calculated

Case of hypochlorous acid water; electrolytically-generated

1 part of virus + 19 parts of test substance
(e.g. 20 \(\mu l\) of virus stock (3 x 10^6 TCID\(_{50}\)/20 \(\mu l\)) +
380 \(\mu l\) of test substance in a test tube)

Stand at RT (20 s, 40 s, 60 s, 2 min and 5 min)

Mix with 6x volume of medium (DMEM + 5% FBS +
0.01 M Na\(_2\)S\(_2\)O\(_3\)) to neutralize/stop reaction (= 7\(^{-1}\) dilution)
(e.g. Add 20 \(\mu l\) of the reaction mixture to 120 \(\mu l\) of
medium in a well of titer plate.)

Serial 7-fold dilutions with DMEM + 5% FBS
(ex, \(7^{-2} - 7^{-8}\)

TCID\(_{50}\) is calculated
Confirmation of cytotoxicity of test substances and determination of quenching condition

- Prepare 10 times, 100 times, 1,000 times, and 10,000 times dilutions of test substances beforehand and confirm the cytotoxic effect on host cells.
- Determine the dilution ratio (quenching conditions) of the substance to be contacted with the virus stock solution. Avoid the use of dilution ratios indicating cytotoxicity.

Method

- On the day of the test, prepare 96 well plates so that Vero E6/TMPRSS2 cells are 90% confluent. Immediately before the test, wash the plates with serum-free DMEM three times and add 180 µl of DMEM + 2% FBS to each well.
- Add 27 µl of diluted test substance to each well of another empty 96 well plates.
- Put 3 µl of virus stock of SARS-CoV-2 (> 1 x 10^4 pfu/µl) into each well using a multi-pipette and leave for 1 min and 5 min at RT (antiviral reaction step).
- After the reaction, add 270 µl of DMEM + 2% FBS as a stopping solution and pipette 5 times immediately (quenching step).
- Take 20 µl aliquots from each well and transfer to wells (14 wells/one antiviral reaction) of host cell culture plates containing 180 µl medium in advance. Place 1 hour in CO_2 incubator at 37°C.
- Wash cells twice with DMEM + 2% FBS.
- Add 200 µl of DMEM + 2% FBS and place in CO_2 incubator at 37°C.
- Immediately after the addition of the medium (Day 0), and 1 day, 2 days and 3 days after the start of culture, observe the cytopathic effect (CPE).
- On the 3rd day, examine all wells with qRT-PCR (TOYOBO SARS-CoV-2 Detection Kit) and measure SARS-CoV-2 RNA titer.
Evaluation and judgment of disinfection effect

• If at least one of the 14 wells has CPE confirmed by the 3rd day and an increase in RNA titer in the well is confirmed, the disinfecting effect of the substance is judged to be null.
• If no increase in RNA titer is observed even if CPE is confirmed, the well is excluded from the evaluation target as this CPE is due to cytotoxicity.
• Only when CPE was not observed in all 14 wells and RNA titer was not increased, it is judged that the disinfection effect is present.
• This judgment means that about 30,000 infectious viruses can be almost completely disinfected (below the detection limit) by the tested substance.

• Please note that the number of infectious virus particles included in one cough droplet is assumed to be 10,000 according to the measured values of patient samples

• Positive control, 70v/v% ethanol
• Negative control, DDW
Outline of Testing protocol with SARS-CoV-2
- Obihiro University of Agriculture and Veterinary Medicine (OUAVM) -

- Preparation of virus stock
  - Medium: DMEM + 1% FBS

- Dilution of test substance (Hypochlorous acid water)
  - Electrolytically-generated type: No dilution required
  - Non-electrolytically-generated type: diluted with DDW (final concentration: 200, 100 and 50 ppm)

- Antiviral reaction
  - 1 part of virus stock + 9 parts of test substance or 1 part of virus stock + 19 parts of test substance
  - Reaction Time: 20 s, 60 s and 5 min
  - Temperature: Room temperature (RT)

- Neutralization of test substance
  - Diluted with dilution buffer (DMEM + 1% FBS + 0.01 M Na₂S₂O₃)

- Calculation of results
  - TCID₅₀ method
  - Positive Control: 70v/v% ethanol
  - Negative Control: DDW
Outline of Testing protocol with SARS-CoV-2
- Obihiro University of Agriculture and Veterinary Medicine (OUAVM) -

(continued)

Case of the ratio 1:9

1 part of virus stock + 9 parts of test substance
(e.g. 10 µl of virus stock (5 x 10^6 TCID$_{50}$/ml) + 90 µl of test substance in a test tube)

Incubate at RT (20 s, 60 s and 5 min)

Mix with 9x volume of dilution buffer (DMEM + 1% FBS + 0.01 M Na$_2$S$_2$O$_3$) to neutralize/stop reaction (= 10$^{-1}$ dilution)
(e.g. Add 20 µl of the reaction mixture to 180 µl of dilution buffer in cell-seeding 96 well plate)

Serial 10-fold dilutions with dilution buffer (ex, 10$^{-2} – 10^{-5}$)

TCID$_{50}$ is calculated

Case of the ratio 1:19

1 part of virus stock + 9 parts of test substance
(e.g. 10 µl of virus stock (5 x 10^6 TCID$_{50}$/ml) + 190 µl of test substance in a test tube)

Incubate at RT (20 s, 60 s and 5 min)

Mix with 9x volume of dilution buffer to neutralize/stop reaction (= 10$^{-1}$ dilution)
(e.g. Add 20 µl of the reaction mixture to 180 µl of dilution buffer in cell-seeding 96 well plate)

Serial 10-fold dilutions with dilution buffer (ex, 10$^{-2} – 10^{-5}$)

TCID$_{50}$ is calculated
Outline of Testing protocol with SARS-CoV-2
- Tottori University -

• Preparation of virus stock
  – Medium: DMEM + 5% FBS

• Dilution of test substance
  – Diluted with DDW (final concentration: 200 and 100 ppm)

• Antiviral reaction
  – 1 part of virus + 9 parts of test substance or 1 part of virus + 19 parts of test substance
  – Reaction Time: 20 s and 60 s
  – Temperature: Room temperature (RT)

• Neutralization of test substance
  – Add 0.02x volume of 0.3 M Na$_2$S$_2$O$_3$ solution

• Calculation of results
  – TCID$_{50}$ method
  – Positive Control: None
  – Negative Control: PBS buffer
Outline of Testing protocol with SARS-CoV-2
- Tottori University -
(continued)

**Case of the ratio 1:9**

1 part of virus + 9 parts of test substance
(e.g. 50 µl of virus stock (6 x 10⁶ TCID₅₀/ml) + 450 µl of test substance in a test tube)

- Incubate at RT (20 s and 60 s)
- Mix with 0.02x volume of 0.3 M Na₂S₂O₃ solution to neutralize/stop reaction (= 10⁰ dilution)
  (e.g. Add 10 µl of 0.3 M Na₂S₂O₃ solution to the reaction mixture in the tube)
- Serial 10-fold dilutions with dilution bufferᵃ (ex, 10⁻¹ – 10⁻⁶)
- TCID₅₀ is calculated

**Case of the ratio 1:19**

1 part of virus + 9 parts of test substance
(e.g. 25 µl of virus stock (6 x 10⁶ TCID₅₀/ml) + 475 µl of test substance in a test tube)

- Incubate at RT (20 s and 60 s)
- Mix with 0.02x volume of 0.3 M Na₂S₂O₃ solution to neutralize/stop reaction (= 10⁰ dilution)
  (e.g. Add 10 µl of 0.1 M Na₂S₂O₃ solution to the reaction mixture in the tube)
- Serial 10-fold dilutions with dilution bufferᵃ (ex, 10⁻¹ – 10⁻⁶)
- TCID₅₀ is calculated

ᵃ, 1 ml of 5% FBS + 19 ml of PBS + 400 µl of 0.3 M Na₂S₂O₃ solution
Outline of Testing protocol with SARS-CoV-2
- Japan Textile Products Quality and Technology Center (QTEC) -

• Preparation of virus stock
  – Medium: DMEM + 1% FBS

• Dilution of test substance
  – Diluted with DDW (final concentration: 80, 50, 25 ppm)

• Antiviral reaction
  – 1 part of virus + 9 parts of test substance or 1 part of virus + 19 parts of test substance
  – Reaction Time: 20 s and 60 s
  – Temperature: Room temperature (RT)

• Neutralization of test substance
  – add 0.1x volume of 0.1 M Na$_2$S$_2$O$_3$ solution, 0.1x volume of 10-fold EMEM medium and 0.05x volume of FBS (final concentration: 2% FBS).

• Calculation of results
  – TCID$_{50}$ method
  – Positive Control: 70 w/v% ethanol
  – Negative Control: PBS buffer
Outline of Testing protocol with SARS-CoV-2
- Japan Textile Products Quality and Technology Center (QTEC) -

(continued)

Case of the ratio 1:9

1 part of virus + 9 parts of test substance
(e.g. 100 µl of virus stock (1 x 10^7 TCID\textsubscript{50}/ml) + 900 µl of test substance in a test tube)

Contact at RT (20 s and 60 s)

Mix with 0.1x volume of 0.1 M Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3} solution to neutralize/stop reaction, and add 0.1x volume of 10-fold EMEM and 0.02x volume of FBS (final concentration: 2% FBS) (= 10\textsuperscript{9} dilution)
(e.g. Add 110 µl of 0.1 M Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3} solution to the reaction mixture in the tube, and add 123 ul of 10-fold EMEM and 25 µl of FBS)

Serial 10-fold dilutions with EMEM + 2% FBS (ex, 10\textsuperscript{-1} – 10\textsuperscript{-6})

TCID\textsubscript{50} is calculated

Case of the ratio 1:19

1 part of virus + 9 parts of test substance
(e.g. 100 µl of virus stock (1 x 10^7 TCID\textsubscript{50}/ml) + 1,900 µl of test substance in a test tube)

Contact at RT (20 s and 60 s)

Mix with 0.1x volume of 0.1 M Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3} solution to neutralize/stop reaction and add 0.1x volume of 10-fold EMEM and 0.02x volume of FBS (final concentration: 2% FBS) (= 10\textsuperscript{9} dilution)
(e.g. Add 220 µl of 0.1 M Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3} solution to the reaction mixture in the tube, and add 246 µl of 10-fold EMEM and 50 µl of FBS)

Serial 10-fold dilutions with EMEM + 2% FBS (ex, 10\textsuperscript{-1} – 10\textsuperscript{-6})

TCID\textsubscript{50} is calculated
The Results of Surfactants
Anionic Surfactant: Potassium Soap & Sodium Soap

Disinfection of SARS-CoV-2 by Potassium Soap (Potassium Laurate (Lau-K))

Disinfection of SARS-CoV-2 by Sodium Soap (Sodium Laurate (Lau-Na))

The concentrations of the component (Lau-K) calculated from the purity of the purchased reagent are as follows: 5 mM, 0.12%; 10 mM, 0.24%; and 20 mM, 0.48%.

The volume ratio of virus suspension to anionic surfactant solution is 1:1 (N=2).

The concentrations of the component (Lau-Na) calculated from the purity of the purchased reagent are as follows: 5 mM, 0.11%; 10 mM, 0.22%; and 20 mM, 0.44%.
Anionic Surfactant: Sodium Linear Alkylbenzenesulfonates (LAS)

Disinfection of SARS-CoV-2 by LAS

The volume ratio of virus suspension to anionic surfactant solution is 1:1 (N=2).

Testing Organization: NIID
Anionic Surfactant: Sodium Alkyl Ether Sulfates (AES)

The volume ratio of virus suspension to anionic surfactant solution is 1:1 (N=2).
Nonionic Surfactant: Alkyl Glycosides (AG)

Disinfection of SARS-CoV-2 by AG

Disinfection of SARS-CoV-2 by AAO & AG

AAO: Alkyldimethylamine Oxide (See page 20)

The volume ratio of virus suspension to nonionic surfactant solution is 1:1 (N=2).
Nonionic Surfactant: Fatty Acid Alkanol Amides (FAA)

Disinfection of SARS-CoV-2 by FAA

(ratio 1:1)

The test at 0.5% of FAA failed because of the adverse effect on the test system by the surfactant.

The volume ratio of virus suspension to nonionic surfactant solution is 1:1 (N=2).
Nonionic Surfactant: Polyoxyethylene Alkyl Ether (POE)

Disinfection of SARS-CoV-2 by POE

The volume ratio of virus suspension to nonionic surfactant solution is 1:1 (N=2).
Amphoteric Surfactant: Alkyl amidopropyl betaine (ALBT)

The volume ratio of virus suspension to amphoteric surfactant solution is 1:1 (N=2).
Amphoteric Surfactant: Alkyl dimethylamine Oxide (AAO)

Disinfection of SARS-CoV-2 by AAO

Disinfection of SARS-CoV-2 by AAO & AG

The volume ratio of virus suspension to amphoteric surfactant solution is 1:1 (N=2).
Cationic Surfactant: Benzalkonium Chloride (BZC)

The volume ratio of virus suspension to cationic surfactant solution is 1:1 (N=2).
Cationic Surfactant: Benzethonium Chloride (BZN)

The volume ratio of virus suspension to cationic surfactant solution is 1:1 (N=2).
Cationic Surfactant: Dialkyldimethyldimmonium Chloride (DDAC)

Disinfection of SARS-CoV-2 by DDAC

Since DDAC could not be fully removed by the resin at 0.05% or higher, the initial infectious titer was decreased, and the detection limit was increased due to the cytotoxicity.

The volume ratio of virus suspension to cationic surfactant solution is 1:1 (N=2).
Viral growth was confirmed by cytopathic effect and amplification of viral RNA by qRT-PCR.

<table>
<thead>
<tr>
<th>Classification of Disinfectants</th>
<th>Disinfectants</th>
<th>0.1</th>
<th>0.05</th>
<th>0.01</th>
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<td></td>
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<td>1 min</td>
<td>5 min</td>
<td>1 min</td>
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<tr>
<td>Anionic Surfactants</td>
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<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sodium Soap</td>
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<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High Purity Sodium Soap</td>
<td>-</td>
<td>*</td>
<td>-</td>
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<tr>
<td></td>
<td>Sodium Linear Alkylbenzene Sulfonates</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sodium Alkyl Ether Sulfates</td>
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<td>-</td>
<td></td>
</tr>
<tr>
<td>Nonionic Surfactants</td>
<td>Alkyl Glycosides</td>
<td>+</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fatty Acid Alkanol Amides</td>
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<td>-</td>
<td></td>
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<tr>
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<td>Polyoxyethylene Alkyl Ether</td>
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<td>-</td>
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</tr>
<tr>
<td></td>
<td>Alkyl Dimethyl Amine Oxide</td>
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<td>NT</td>
<td>+</td>
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<td>Cationic Surfactants</td>
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<td>Dialkyldimethylammonium Chloride</td>
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<tr>
<td>Oxygen Bleach</td>
<td>Sodium Percarbonate</td>
<td>**</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

CT, Cell toxicity; NT, Not tested; +, virucidal activity observed; -, virucidal activity not observed.
In this test, when approximately 10,000 viruses were almost completely inactivated (below the detection limit), it was judged as “virucidal activity observed”.
*, Exposure time 10 minutes; **, In only one well, RNA titer was not increased and cell death due to the substance was observed.
Viral growth was confirmed by cytopathic effect and amplification of viral RNA by qRT-PCR.
The Results of Hypochlorous Acid Water
Hypochlorous Acid Water; Non-electrolytically-generated Sodium dichloroisocyanurate (NaDCC)

Disinfection of SARS-CoV-2 by NaDCC (DiCLISC)

200 ppm of NaDCC showed more than 99.999% reduction of virus titer after 1 min.
100 ppm NaDCC showed more than 99.9% reduction of virus titer after 1 min.

Test condition
- Virus stock medium: DMEM+5% FBS
- The volume ratio of virus suspension to NaDCC solution solution is 1:9 (N=2).
Hypochlorous Acid Water; Electrolytically-generated (strongly acidic electrolyzed water)

Disinfection of SARS-CoV-2 by HAW; Electrolytically-generated (pH 2.4)

Test condition
- Virus stock medium: DMEM+5% FBS
- The volume ratio of virus suspension to HAW (ACC 35 ppm, pH 2.4) is 1:19 (N=2). (ACC: Available chlorine concentration)
Hypochlorous Acid Water; Electrolytically-generated (weakly and slightly acidic electrolyzed water)

Disinfection of SARS-CoV-2 by HAW; Electrolytically-generated (pH 3.5 and 3.8)

Disinfection of SARS-CoV-2 by HAW; Electrolytically-generated (pH 4.9 and 5.9)

Test condition
• Virus stock medium: DMEM+5% FBS
• The following four types of HAW were tested: ACC 44 ppm, pH 3.5; ACC 51 ppm, pH 3.8; ACC 54 ppm, pH 4.9; and ACC 50 ppm, pH 5.9. (ACC: Available chlorine concentration)
• The volume ratio of virus suspension to HAW is 1:19 (N=2).
Before conducting the test, the available chlorine concentration (ACC) was measured, and it was ensured that there was no discrepancy in the concentration at the time of generation. Viral growth was confirmed by cytopathic effect (CPE) and amplification of viral RNA by qRT-PCR. CPE was observed, and the elevated RNA titer reached the upper limit in all wells. In the 70% ethanol-treated group (as a positive control), cells were survived, and the qRT-PCR results were also negative in all wells.

### Table 2. Effect of Hypochlorous Acid Water; Electrolytically-generated on SARS-CoV-2

<table>
<thead>
<tr>
<th>Classification of Hypochlorous Acid Water</th>
<th>Electrolyte</th>
<th>pH</th>
<th>Available Chlorine Concentration (ppm)</th>
<th>Reaction Time</th>
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<tbody>
<tr>
<td>Slightly Acidic Electrolyzed Water</td>
<td>HCl</td>
<td>5.0</td>
<td>50</td>
<td>-</td>
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<tr>
<td></td>
<td></td>
<td>6.0</td>
<td>50</td>
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<td></td>
<td>HCl + NaCl</td>
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<td></td>
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<td>6.0</td>
<td>50</td>
<td>-</td>
</tr>
</tbody>
</table>

+, Virucidal activity observed; -, virucidal activity not observed
In this test, when approximately 10,000 viruses were almost completely inactivated (below the detection limit), it was judged as “virucidal activity observed”.

Before conducting the test, the available chlorine concentration (ACC) was measured, and it was ensured that there was no discrepancy in the concentration at the time of generation.

Viral growth was confirmed by cytopathic effect (CPE) and amplification of viral RNA by qRT-PCR. CPE was observed, and the elevated RNA titer reached the upper limit in all wells. In the 70% ethanol-treated group (as a positive control), cells were survived, and the qRT-PCR results were also negative in all wells.
Hypochlorous Acid Water; Electrolytically-generated (strongly acidic electrolyzed water)

DDW, double distilled water (negative control).
Strongly acidic electrolyzed water (AAC 56 ppm, pH 2.53).

Virucidal effect of SAEW (56 ppm, pH 2.53) was observed in a short period (20-seconds exposure time) under the condition of test mixture at 1:19 (virus suspension (1% FBS):test agent).
Hypochlorous Acid Water; Electrolytically-generated (slightly acidic electrolyzed water)

Virus suspension (1% FBS):test agent = 1:19

<table>
<thead>
<tr>
<th></th>
<th>20 sec</th>
<th>1 min</th>
<th>5 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDW</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WAEW</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

DDW, double distilled water (negative control).
Slightly acidic electrolyzed water (AAC 54.5 ppm, pH 5.16).

Testing Organization: OUAVM
N=4 and two sets of experiment.

<table>
<thead>
<tr>
<th>Exposure time</th>
<th>20 sec</th>
<th>1 min</th>
<th>5 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test agents</td>
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<td>WAEW</td>
<td>DDW</td>
</tr>
<tr>
<td>Tube 1</td>
<td>6.25</td>
<td>≤1.25</td>
<td>6.25</td>
</tr>
<tr>
<td>Tube 2</td>
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</tr>
<tr>
<td>Tube 4</td>
<td>5.25</td>
<td>≤1.25</td>
<td>5.25</td>
</tr>
</tbody>
</table>

Infectivity reduction (log_{10} TCID_{50}/ml)

Mean± SD

|          | 6.0  | ±0.5 | 6.25 | ±0.48 | 5.625 | ±0.25 |

Difference value from DDW treatment

|        | ≥4.75 | -    | ≥4.875 | -    | ≥4.375 |

Virucidal efficacy (%)


Virucidal effect of WAEW (54.5 ppm, pH 5.16) was observed in a short period (20-seconds reaction time) under the condition of test mixture at 1:19 (virus suspension (1% FBS):test agent).
Hypochlorous Acid Water; Electrolytically-generated (slightly acidic electrolyzed water)

DDW, double distilled water (negative control).
Slightly acidic electrolyzed water (AAC 32 ppm, pH 5.3).

32 ppm WAEW (pH 5.3) showed virucidal effect in a short period (20-seconds exposure time) under the condition of test mixture at 1:19 (virus suspension (1% FBS):test agent).
Hypochlorous Acid Water; Non-electrolytically-generated Anonymous sample A

 Virucidal activity at 20 s reaction time

![Graph showing infectivity reduction](image)

<table>
<thead>
<tr>
<th>Test agents</th>
<th>DDW</th>
<th>A (original)</th>
<th>A (200 ppm)</th>
<th>A (100 ppm)</th>
<th>A (53 ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tube 1</td>
<td>6.25</td>
<td>≤1.25</td>
<td>≤1.25</td>
<td>≤1.25</td>
<td>≤1.25</td>
</tr>
<tr>
<td>Tube 2</td>
<td>5.75</td>
<td>≤1.25</td>
<td>≤1.25</td>
<td>≤1.25</td>
<td>≤1.25</td>
</tr>
<tr>
<td>Tube 3</td>
<td>6.25</td>
<td>≤1.25</td>
<td>≤1.25</td>
<td>≤1.25</td>
<td>≤1.25</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>6.0833 ± 0.29</td>
<td>≤1.25 ± 0</td>
<td>≤1.25 ± 0</td>
<td>≤1.25 ± 0</td>
<td>≤1.25 ± 0</td>
</tr>
<tr>
<td>Difference value from DDW treatment</td>
<td>-</td>
<td>≥4.8333</td>
<td>≥4.8333</td>
<td>≥4.8333</td>
<td>≥4.8333</td>
</tr>
<tr>
<td>Virucidal efficacy (%)</td>
<td>-</td>
<td>≥99.9985</td>
<td>≥99.9985</td>
<td>≥99.9985</td>
<td>≥99.9985</td>
</tr>
</tbody>
</table>

N=3 and two sets of experiment.  \(\leq 1.25\): below the detection limit

DDW, Double distilled water (negative control).
O, Original concentration (not disclosed) of anonymous sample A (pH 6.0).
The numbers (200, 100 and 53) are available chlorine concentrations (ppm) of diluted anonymous sample A.

Anonymous sample A (200, 100 and 53 ppm, pH 6.0) showed virucidal effect against SARS-CoV-2 in a short period (20-seconds exposure time) under the condition of test mixture at 1:19 (virus suspension (1% FBS):test agent).

Slight cytotoxicity was observed in periphery of wells for the specimen highlighted in light blue.
Hypochlorous Acid Water; Non-electrolytically-generated
Anonymous sample B

**Virucidal activity at 20 s reaction time**

<table>
<thead>
<tr>
<th>Test agents</th>
<th>DDW</th>
<th>B (215 ppm)</th>
<th>B (100 ppm)</th>
<th>B (52 ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tube 1</td>
<td>6.25</td>
<td>≤1.25</td>
<td>≤1.25</td>
<td>≤1.25</td>
</tr>
<tr>
<td>Tube 2</td>
<td>6.25</td>
<td>≤1.25</td>
<td>≤1.25</td>
<td>≤1.25</td>
</tr>
<tr>
<td>Tube 3</td>
<td>5.75</td>
<td>≤1.25</td>
<td>≤1.25</td>
<td>≤1.25</td>
</tr>
<tr>
<td>Mean± SD</td>
<td>6.08</td>
<td>± 0.29</td>
<td>± 0</td>
<td>± 0</td>
</tr>
<tr>
<td>Difference value from DDW treatment</td>
<td>-</td>
<td>≥4.83</td>
<td>≥4.83</td>
<td>≥4.83</td>
</tr>
</tbody>
</table>

**Residual infectivity titer**

- **DDW**, Double distilled water (negative control).
- The numbers (215, 100 and 52) are available chlorine concentrations (ppm) of diluted anonymous sample B (pH 6.2).
- Slight cytotoxicity was observed in periphery of wells for the specimen of 215 ppm at 1 and 5 minutes.

Anonymous sample B (215, 100 and 52 ppm, pH 6.2) showed virucidal effect against SARS-CoV-2 in a short period (20-seconds reaction time) under the condition of test mixture at 1:19 (virus suspension (1% FBS):test agent).
Hypochlorous Acid Water; Non-electrolytically-generated Anonymous sample C

**Virucidal activity at 20 s reaction time**

<table>
<thead>
<tr>
<th>Test agents</th>
<th>DDW</th>
<th>C (Original)</th>
<th>C (200 ppm)</th>
<th>C (103 ppm)</th>
<th>C (52 ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tube 1</td>
<td></td>
<td>6.25</td>
<td>6.25</td>
<td>6.25</td>
<td>6.25</td>
</tr>
<tr>
<td>Tube 2</td>
<td></td>
<td>5.75</td>
<td>5.75</td>
<td>5.75</td>
<td>5.75</td>
</tr>
<tr>
<td>Tube 3</td>
<td></td>
<td>5.75</td>
<td>5.75</td>
<td>5.75</td>
<td>5.75</td>
</tr>
<tr>
<td>Mean± SD</td>
<td>5.92 ± 0.29</td>
<td>6.25 ± 0</td>
<td>6.25 ± 0</td>
<td>6.25 ± 0</td>
<td>6.25 ± 0</td>
</tr>
<tr>
<td>Difference value from DDW treatment</td>
<td>-</td>
<td>3.67</td>
<td>4.67</td>
<td>4.67</td>
<td>4.67</td>
</tr>
<tr>
<td>Virucidal efficacy (%)</td>
<td>-</td>
<td>≥99.9785</td>
<td>≥99.979</td>
<td>≥99.979</td>
<td>≥99.979</td>
</tr>
</tbody>
</table>

N=3 and two sets of experiment.

Global cytotoxicity was observed for the specimens highlighted in gray, and slight cytotoxicity was observed in periphery of wells for the specimen highlighted in light blue.

Anonymous sample C (200, 103 and 52 ppm, pH 5.2) showed virucidal effect against SARS-CoV-2 in a short period (20-seconds reaction time) under the condition of test mixture at 1:19 (virus suspension (1% FBS):test agent).

DDW, Double distilled water (negative control).
O, Original concentration of anonymous sample C (pH 5.2).
The numbers (200, 103 and 52) are available chlorine concentrations of diluted anonymous sample C.
Anonymous sample D (156, 98 and 50 ppm, pH 5.9) showed virucidal effect against SARS-CoV-2 in a short period (20-seconds reaction time) under the condition of test mixture at 1:19 (virus suspension (1% FBS):test agent).
**Hypochlorous Acid Water; Non-electrolytically-generated Sodium dichloroisocyanurate (NaDCC)**

<table>
<thead>
<tr>
<th>Tube</th>
<th>DDW</th>
<th>308 ppm</th>
<th>205 ppm</th>
<th>99 ppm</th>
<th>52 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.4</td>
<td>≤1.4</td>
<td>≤1.4</td>
<td>≤1.4</td>
<td>2.4</td>
</tr>
<tr>
<td>2</td>
<td>5.9</td>
<td>≤1.4</td>
<td>≤1.4</td>
<td>≤1.4</td>
<td>≤1.9</td>
</tr>
<tr>
<td>3</td>
<td>5.9</td>
<td>≤1.4</td>
<td>≤1.4</td>
<td>≤1.4</td>
<td>≤1.9</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>6.07 ± 0.29</td>
<td>≤1.4 ± 0</td>
<td>≤1.4 ± 0</td>
<td>≤1.4 ± 0</td>
<td>≤2.07 ± 0.29</td>
</tr>
<tr>
<td>Difference value from DDW treatment</td>
<td>-</td>
<td>≥4.667</td>
<td>≥4.667</td>
<td>≥4.667</td>
<td>≥4</td>
</tr>
</tbody>
</table>


N=3 and two sets of experiment. ≤1.4: below the detection limit

Slight cytotoxicity was observed in periphery of wells for the specimen highlighted in light blue.

DDW, Double distilled water (negative control). The numbers (308, 205, 99 and 52) are available chlorine concentrations (ppm) of NaDCC solution (pH 6.2).

**NaDCC (308, 205, 99 and 52 ppm, pH 6.2) showed virucidal effect against SARS-CoV-2 under the condition of test mixture at 1:19 (virus suspension (1% FBS): test agent). 200 ppm NaDCC showed more than 99.999% reduction of virus titer after 1 minute.**
Hypochlorous Acid Water; Non-electrolytically-generated Anonymous sample A

Testing condition:
- virus culture suspension: DMEM medium with 5% FBS
- virus titer: $10^{6.8}\text{TCID}_{50}/\text{mL}$
- virus suspension: test agent: 1:19 or 1:9
- number of repeats: $N=4$
- positive control: None
- negative control: PBS (Phosphate Buffered Saline)

At 60-seconds reaction time, 200 ppm of anonymous sample A (pH 6.0) showed reduction of infectious virus titer of 99.9% or more in both test suspension ratios of 1:9 and 1:19 as compared with the negative control group (PBS). On the other hand, at 100 ppm, it was 99.9% or more at 1:19, but under the condition of 1:9, no significant virus titer reduction was observed.

Numbers (200 and 100 ppm) are available chlorine concentrations of anonymous sample A (pH 6.0)
Hypochlorous Acid Water; Non-electrolytically-generated type
Anonymous sample A

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>Available Chlorine Concentration (ppm)</th>
<th>Set Value</th>
<th>Measured Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anonymous sample A</td>
<td>6.0</td>
<td></td>
<td>80</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25</td>
<td>27</td>
</tr>
</tbody>
</table>

Testing condition:
- virus culture suspension: DMEM with 1% FBS
- virus titer: $1.8 \times 10^7$ TCID$_{50}$/ml
- virus suspension: test agent: 1:19
- number of repeats: N=3
- positive control: 70 w% EtOH
- negative control: MilliQ water (sterile)

84 ppm and 51 ppm of sample A (pH 6.0) at 60-seconds reaction time showed more than 99.99% reduction of viral titer compared with control treatment (DDW). 27 ppm of sample A showed reduction of viral titer with only 99% or more.
The Result of Preliminary Evaluation Tests Using Influenza A Virus as a Surrogate for SARS-CoV-2
Evaluation test using *Influenza A Virus* Test Agents

Table 1a. List of the Surfactants

<table>
<thead>
<tr>
<th>Classification of Surfactant</th>
<th>Name of Surfactant</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anionic Surfactants</td>
<td>Potassium Soap (Potassium Laurate)</td>
<td>Lau-K</td>
</tr>
<tr>
<td></td>
<td>Sodium Linear Alkylbenzene Sulfonates</td>
<td>LAS</td>
</tr>
<tr>
<td>Nonionic Surfactants</td>
<td>Alkyl Glycosides</td>
<td>AG</td>
</tr>
<tr>
<td></td>
<td>Fatty Acid Alkanol Amides</td>
<td>FAA</td>
</tr>
<tr>
<td></td>
<td>Polyoxyethylene Alkyl Ather</td>
<td>POE</td>
</tr>
<tr>
<td>Amphoteric Surfactants</td>
<td>Alkyl Amidopropyl Betaine</td>
<td>ALBT</td>
</tr>
<tr>
<td></td>
<td>Alkyl Dimethyl Amine Oxide</td>
<td>AAO</td>
</tr>
<tr>
<td>Cationic Surfactants</td>
<td>Benzalkonium Chloride&lt;sup&gt;a&lt;/sup&gt;</td>
<td>BZC</td>
</tr>
</tbody>
</table>

<sup>a</sup> Also categorized into quaternary ammonium salts

Table 1b. List of Hypochlorous Acid Water; Electrolytically-Generated

<table>
<thead>
<tr>
<th>Classification of Hypochlorous Acid Water</th>
<th>Electrolyte</th>
<th>pH</th>
<th>Set Value of Available Chlorine Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongly Acidic Electrolyzed Water</td>
<td>NaCl</td>
<td>≤2.7</td>
<td>40</td>
</tr>
<tr>
<td>Weakly Acidic Electrolyzed Water</td>
<td>NaCl</td>
<td>2.7-5.0</td>
<td>30</td>
</tr>
<tr>
<td>Slightly Acidic Electrolyzed Water</td>
<td>HCl</td>
<td>5.0-6.5</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>HCl+NaCl</td>
<td>5.0-6.5</td>
<td>40</td>
</tr>
</tbody>
</table>
Outline of Testing Protocol with *Influenza A Virus*

- **Cell culture and virus stock** (refer to JIS L 1922/ISO 18184)
- **Dilution of test substances**
  - Surfactants: Diluted with DDW (final concentration 0.1-0.5%)
  - Hypochlorous acid water: No dilution required
- **Antiviral reaction** (refer to ASTM E1052-20)
  - 1 part of virus + 9 parts of test substance
  - Reaction Time: 1 min and 5 min
  - Temperature: 25 degrees Celsius
- **Neutralization of test substances** (refer to JIS L 1922/ISO 18184 and ASTM E1052-20)
  - Surfactants: Diluted with 9x volumes of SCDLP medium
  - Hypochlorous acid water: Add one-tenth volume of 0.012 M Na$_2$S$_2$O$_3$ solution
- **Calculation of results** (refer to JIS L 1922/ISO 18184)
  - Plaque assay or TCID$_{50}$ method
Outline of Testing protocol with *Influenza A Virus* (continued)

**Case of surfactants**

1 part of virus + 9 parts of test substance (e.g. 100 µl of virus stock (1 x 10⁸ TCID₅₀/ml) + 900 µl of test substance in a test tube)

Contact at 25°C (1 min and 5 min)

Mix with 9x volumes of SCDLP medium to neutralize/stop reaction (= 10⁻¹ dilution) (e.g. Add 100 µl of the reaction mixture to 900 µl of SCDLP medium in another test tube)

Serial 10-fold dilutions with cell culture medium (ex, 10⁻² – 10⁻⁶)

Plaque assay or TCID₅₀ method

**Case of hypochlorous acid water**

1 part of virus + 9 parts of test substance (e.g. 100 µl of virus stock (1 x 10⁸ TCID₅₀/ml) + 900 µl of test substance in a test tube)

Contact at 25°C (1 min and 5 min)

Mix with 0.1x volume of 0.012 M Na₂S₂O₃ solution to neutralize/stop reaction (= 10⁰) (e.g. Add 100 µl of 0.012 M Na₂S₂O₃ solution to the reaction mixture in the tube)

Serial 10-fold dilutions with cell culture medium (ex, 10⁻¹ – 10⁻⁶)

Plaque assay or TCID₅₀ method
Evaluation test using *Influenza A Virus*

**Summary of Results**

○ The results obtained from four participating organizations to this study are as follows:
  (concentration: 0.1 %, reaction time: 5 min, reduction of virus titer was expressed as $\log_{10}$)

  ➢ **Surfactants**
    - ≥ 4 (99.99%) : Sodium linear alkylbenzene sulfonates, Alkyl glycosides, Polyoxyethylene alkyl ether, Alkyl dimethyl amine oxide
    - ≥ 3 (99.9%) : Potassium soap
    - ≥ 2 (99%) : Fatty acid alkanol amides, Benzalkonium chloride
    - Ineffective : Alkyl amidopropyl betaine

  ➢ **Hypochlorous acid water; electrolytically-generated**
    - ≥ 4 (99.99%) : 4 types of hypochlorous acid water tested in this study (see page 76)

○ The results obtained from participating organizations were closely related each other, so it was judged that the study was successfully performed.

○ These results using influenza A virus indicated that the following substances may have the virucidal activity against SARS-CoV-2 with high probability, and we decided to proceed with the evaluation study of these substances using SARS-CoV-2.

  ● 5 types of surfactants:
    Sodium linear alkylbenzene sulfonates, Alkyl glycosides, Polyoxyethylene alkyl ether, Alkyl dimethyl amine oxide, Potassium soap

  ● 4-types of hypochlorous acid water; electrolytically-generated (see page 76)
Virucidal efficacy of tested substances against *Influenza A Virus*

Summary of Results

Table 2a. Virucidal Efficacy of Surfactants against Influenza A Virus

<table>
<thead>
<tr>
<th>Classification of Surfactant</th>
<th>Surfactant</th>
<th>Participating Organization</th>
<th>Detection Method of Virus Titer</th>
<th>Strain of Influenzae Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Anionic Surfactants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lau-K</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>LAS</td>
<td>&gt; 10⁴</td>
<td>&gt; 10⁴</td>
<td>&gt; 10⁴</td>
<td></td>
</tr>
<tr>
<td>Nonionic Surfactants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>&gt; 10⁴</td>
<td>NT</td>
<td>&gt; 10⁴</td>
<td>NT</td>
</tr>
<tr>
<td>FAA</td>
<td>&gt; 10²</td>
<td>&gt; 10²</td>
<td>NT</td>
<td>&gt; 10²</td>
</tr>
<tr>
<td>POE</td>
<td>&gt; 10⁴</td>
<td>NT</td>
<td>NT</td>
<td>&gt; 10⁴</td>
</tr>
<tr>
<td>Amphoteric Surfactants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALBT</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>-</td>
</tr>
<tr>
<td>AAO</td>
<td>&gt; 10⁴</td>
<td>&gt; 10⁴</td>
<td>NT</td>
<td>&gt; 10⁴</td>
</tr>
<tr>
<td>Cationic Surfactants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BZC</td>
<td>NT</td>
<td>&gt; 10²</td>
<td>NT</td>
<td>&gt; 10³</td>
</tr>
</tbody>
</table>

Values show decrease in infectious titer after the reaction time of 5 minutes with each surfactant of 0.1 %.
NT, Not tested; -, ineffective (reduction rate was less than 10²)
Table 2b. Effect of Hypochlorous Acid Water; Electrolytically-Generated on Influenza A Virus

<table>
<thead>
<tr>
<th>Classification of Hypochlorous Acid Water</th>
<th>Electrolyte</th>
<th>Participating Organization</th>
<th>Strain of Influenzae Virus</th>
<th>Detection Method of Virus Titer</th>
<th>TCID&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongly Acidic Electrolyzed Water</td>
<td>NaCl</td>
<td>A</td>
<td>H3N2 (A/HongKong/8/68)</td>
<td>Plaque</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>ATCC VR-1679</td>
<td></td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>H1N1 (A/PR/8/34)</td>
<td></td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td></td>
<td></td>
<td>&gt; 10&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weakly Acidic Electrolyzed Water</td>
<td>NaCl</td>
<td>A</td>
<td>H3N2 (A/HongKong/8/68)</td>
<td>Plaque</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>ATCC VR-1679</td>
<td></td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>H1N1 (A/PR/8/34)</td>
<td></td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td></td>
<td></td>
<td>&gt; 10&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Slightly Acidic Electrolyzed Water</td>
<td>HCl</td>
<td>A</td>
<td>H3N2 (A/HongKong/8/68)</td>
<td>Plaque</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>ATCC VR-1679</td>
<td></td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>H1N1 (A/PR/8/34)</td>
<td></td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td></td>
<td></td>
<td>&gt; 10&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>HCl + NaCl</td>
<td>NT</td>
<td>A</td>
<td>H3N2 (A/HongKong/8/68)</td>
<td>Plaque</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>ATCC VR-1679</td>
<td></td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>H1N1 (A/PR/8/34)</td>
<td></td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td></td>
<td></td>
<td>&gt; 10&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values show decrease in infectious titer after both 1 and 5 min reaction times. Influenzae A virus used in this study was prepared with ultra-centrifuge. NT, Not tested
Acknowledgment

We would like to thank the organizations below for their participation in the evaluation study using Influenza A virus

BOKEN Quality Evaluation Institute (BOKEN)
Kanagawa Institute of Industrial Science and Technology (KISTEC)
Kitasato Research Center for Environmental Science
Japan Textile Products Quality and Technology Center (QTEC)

(Alphabetical order)
References
Candidate disinfecting substances for evaluation studies

1. 【written in Japanese】日本医師会
「消毒・滅菌の概要」 https://www.med.or.jp/kansen/guide/steri_sum.pdf
「消毒薬一覧」 https://www.med.or.jp/kansen/guide/steri_list.pdf
2. 【written in Japanese】国立感染症研究所「SARSに関する消毒（三訂版）」
http://idsc.nih.go.jp/disease/sars/sars03w/index.html
3. CDC, Coronavirus Disease 2019 (COVID-19)／How to Prepare
4. EPA, List N: Disinfectants for Use Against SARS-CoV-2
https://www.epa.gov/pesticide-registration/list-n-disinfectants-use-against-sars-cov-2
5. 【written in Chinese】中国国家衛生健康委員会「消毒剤使用指南」（2020年2月18日）
http://www.nhc.gov.cn/zhjcj/s9141/202002/b9891e8c86d141a08ec45c6a18e21dc2

Surfactants

6. The Inactivation of the virus of epidemic influenza by soaps
7. 【written in Japanese】SARSに関する消毒（三訂版）（感染症情報センター）2003/12/18
http://idsc.nih.go.jp/disease/sars/sars03w/index.html
Quaternary ammonium salts

8. Effects of Chlorine, Iodine, and Quaternary Ammonium Compound Disinfectants on Several Exotic Disease Viruses

9. Inactivation of Viruses by Benzalkonium Chloride

http://koreascience.or.kr/article/JAKO201421154209242.page

11. 【written in Japanese】塩化ベンゼトニウムのエンベロープウイルスに対する効果
井上潔, 池谷文夫, 山崎隆義, 原武史, Gyobyo Kenkyu, 26: 195-200, 1991

12. Effects of chlorine, iodine, and quaternary ammonium compound disinfectants on several exotic disease viruses
https://www.jstage.jst.go.jp/article/jvms/62/1/62_1_85/_article
Sodium Percarbonate

13.【written in Japanese】ノロウイルスの不活化条件に関する調査（国立医薬品食品衛生研究所食品衛生管理部 平成19－21年度報告書）
平成21年度  https://www.mhlw.go.jp/topics/syokuchu/kanren/yobou/pdf/houkokusyo_110613_03.pdf

Hypochlorous Acid Water

14. Effect of weak acid hypochlorous solution on selected viruses and bacteria of laboratory rodents.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4160982/pdf/expanim-63-141.pdf
15. Evaluation of sprayed hypochlorous acid solutions for their virucidal activity against avian influenza virus through in vitro experiments
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4363024/
16.【written in Japanese】インフルエンザウイルスA, アデノウイルス, ヘルペスウイルス等に対する次亜塩素酸水の効果
https://www.jstage.jst.go.jp/article/jsei1986/14/4/14_4_255/_pdf/-char/ja
Hypochlorous Acid Water (continued)

17. 【written in Japanese】強酸性電解水中の残留塩素に対する有機物の影響
https://www.jstage.jst.go.jp/article/nskkk1995/47/4/47_4_287/_article/-char/ja/

18. The efficiency of disinfection of acidic electrolyzed water in the presence of organic materials
https://www.jstage.jst.go.jp/article/analsci/16/4/16_4_365/_article/-char/ja

19. Effect of weak acid hypochlorous solution on selected viruses and bacteria of laboratory rodents
https://www.jstage.jst.go.jp/article/expanim/63/2/63_13-0021/_article

20. Comparative antibacterial activities of neutral electrolyzed oxidizing water and other chlorine-based sanitizers
https://www.nature.com/articles/s41598-019-56248-7

21. Reduction of microbial contamination on the surfaces of layer houses using slightly acidic electrolyzed water