● Antiviral processing agent 「NOVARON® IV」

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1. Introduction

The COVID-19 pandemic has had a profound impact on human health and the economy. To protect ourselves from COVID-19, we are required to wear masks and take actions to avoid closed spaces, crowded places, and close-contact settings. However, it is said that an era of "living with COVID," wherein we coexist with the virus while protecting ourselves from infection, will arrive in the future. Against this backdrop, people are increasingly aware of the need for safe and secure environments, and interest in antiviral-processed products is growing. In fact, the adoption of antibacterial- and antiviral-processed articles in railway car interiors, public facilities, and medical institutions is becoming more widespread, and demand for antiviral processing agents has never been higher. In addition, the Japan Textile Evaluation Technology Council (SEK) and the Society of International sustaining growth for Antimicrobial Articles (SIAA) have established certification mark systems for antiviral processed products. These certification mark systems grant certification to products that exhibit a certain level of antiviral effect (reduction in virus count) and are safe, and the number of antiviral-processed products using these mark systems is rapidly increasing.

Many antiviral processing agents on the market are converted from conventional antibacterial agents, and there are few inorganic antimicrobial agents that are safe and have high antiviral efficacy. Inorganic antibacterial agents are mostly metal-based compounds, and their active ingredients, such as silver and copper, are said to exhibit antibacterial activity by inhibiting bacterial metabolism[1]. Although these antibacterial metals are also known to inactivate viruses[2], there is not necessarily a correlation between antibacterial and antiviral effects. An organism is generally defined as an individual specimen that reproduces, evolves, and metabolizes. Viruses are not classified as living organisms because they do not metabolize. If the action mechanism of antibacterial metals is to inhibit bacterial metabolism, it is not surprising that they have different effects on viruses that do not metabolize. Although existing grades of our silver-based antibacterial agent NOVARON also exhibit antiviral properties, their effects are low. In response, we have developed NOVARON IV, a new, unprecedented high-performance antiviral processing agent specializing in antiviral properties, as a next-generation amenity material following antibacterial agents. In addition to the NOVARON IV1000, which we have previously introduced[3], this article introduces the newly developed IV2000 and IV3000.

2. Types of viruses

A virus has a very simple structure and is basically made up of nucleic acids (DNA or RNA) and proteins. Structurally, viruses generally consist of a nucleic acid core surrounded by a capsid (composed of protein subunits called capsomeres). They are broadly classified into two types: (1) those wrapped in a membrane called an envelope (composed of an inner membrane protein and an outer lipoprotein complex), and (2) those without an envelope[4] (**Fig. 1**). A typical example of a virus with an envelope (1) is influenza virus, while a typical example of a virus without an envelope (2) is norovirus.

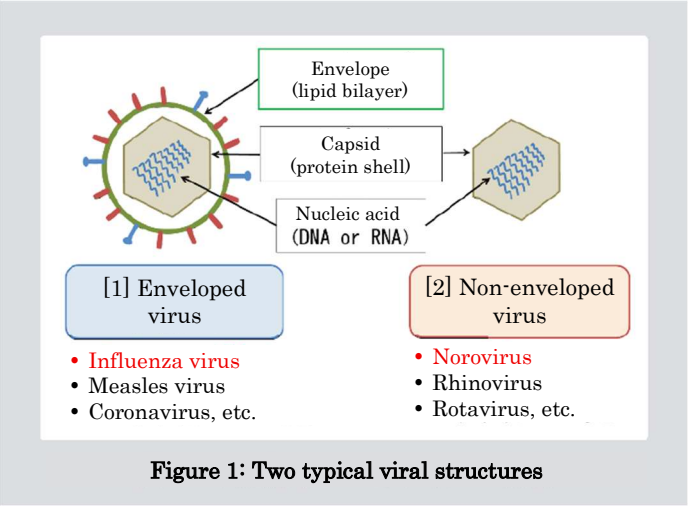

Figure 1: Two typical viral structures

3. Characteristics of NOVARON IV, a new anti-influenza virus processing agent

NOVARON IV is a new, highly safe antiviral processing agent composed entirely of inorganic components. In addition to its high antiviral effect, it has the advantages of heat resistance and resistance to discoloration.

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NOVARON IV1000 exhibits a high antiviral effect against enveloped viruses such as influenza virus, while NOVARON IV2000 exhibits a high antiviral effect against non-enveloped viruses such as norovirus. NOVARON IV3000 is highly effective against both enveloped and non-enveloped viruses (**Table 1**).

Table 1: Characteristics of NOVARON IV

Grade	Characteristic	Appearance	Average particle diameter (μm)
IV1000	For enveloped viruses	White	1
IV2000	For non-enveloped viruses	White	6
IV3000	For both enveloped and non-enveloped viruses	White	5

3.1 Physical property values of NOVARON IV1000

Since NOVARON IV1000 is white in appearance, it can be used for products where color tone is important without compromising the appearance (**Fig. 2**). It is a fine powder composed only of inorganic components, with an average particle diameter of approximately 1 μm. Owing to its high heat resistance, low moisture content (less than 1 wt%), and insolubility in water and solvents, it can be used to process products of various materials and shapes (**Table 2**).

Figure 2: NOVARON IV1000

Table 2: Typical physical properties of NOVARON IV1000

Item	Physical property
Appearance	White
Average particle diameter	1 μm
Heat resistance temperature	600°C
Moisture content	< 1 wt%

3.2 Antiviral effect

3.2.1 Antiviral effect of NOVARON IV1000

First, we evaluated the antiviral property against influenza A virus (ATCC VR-1679), which is an enveloped virus, by using the water dispersion of NOVARON IV1000. We carried out the test by the method shown in **Fig. 3**, with reference to ISO18184. We mixed 100 μL of influenza virus suspension with 900 μL of NOVARON IV1000 water dispersion, allowed the mixture to react at 25°C for 2 hours, and measured the infectious virus count (infection titer) by the plaque assay method. As a control for NOVARON IV1000 water dispersion, we carried out the same operation using phosphate buffer. The plaque assay is a method of measuring viral concentration based on the fact that virus-infected cells undergo degeneration. In this method, monolayer-cultured host cells are inoculated with serially diluted viral solutions, agar medium is added, and after incubation, the plaques formed are counted and multiplied by the dilution factor to calculate plaque-forming units (PFU).

Fig. 4 shows the results. The 0.1 wt% water dispersion of NOVARON IV1000 reduced the influenza virus by 99.99% compared to phosphate buffer solution alone without NOVARON IV1000, showing that NOVARON IV1000 has a high antiviral effect.

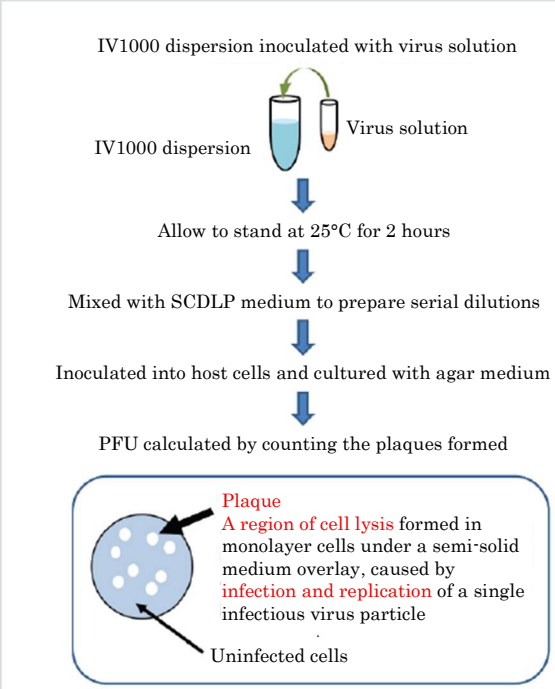

Figure 3: Antiviral property test method

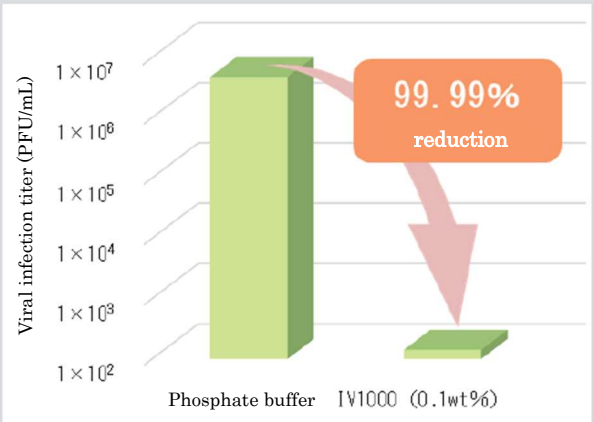

Figure 4: Antiviral effect of NOVARON IV1000

3.2.2 Antiviral property of NOVARON IV1000-treated fabric

We applied NOVARON IV1000 to fibers and evaluated changes in the influenza virus count on the treated fibers. We blended NOVARON IV1000 with a resin binder at a 1:1 solid content ratio, dip-coated polyester fabric with the mixture so that 1.0 g/m^2 of NOVARON IV1000 adhered to the fabric, and dried it at 120°C to produce the antiviral-treated fabric. We conducted the antiviral property test on this treated cloth according to JIS L 1922: Textiles-Determination of antiviral activity of textile products. This is a test method to quantitatively measure the inhibition effect on the activity of infectious viruses adhering to antiviral-treated products. We inoculated 0.4 g of NOVARON IV1000-treated fabric, cut into 2 cm × 2 cm pieces with 0.2 mL of influenza virus (H3N2) (ATCC VR-1679) suspension at 1 × 10^7 to 5 × 10^7 PFU/mL, and measured the viral infection titer at predetermined times by the plaque assay. To confirm the stability of the test virus on the fiber, we carried out a control test using the same procedure with a standard cotton cloth as the control sample. Although JIS L 1922 stipulates a standard contact time of 2 hours between the antiviral-treated product and the virus suspension, we also conducted a test with a contact time of 3 minutes to confirm the antiviral property of NOVARON IV1000-treated cloth in a short period of time.

Fig. 5 shows the results. While the viral infection titer of standard cotton cloth at 2 hours after contact with the virus suspension was 10^6, the titer was reduced to 10^2 with NOVARON IV1000-treated cloth, indicating that it reduced the viral infection titer on the treated cloth by 99.99%. In addition, the viral infection titer at 3 minutes after contact between the treated fabric and the virus suspension was already comparable to the level seen at 2 hours, indicating that the NOVARON IV1000-treated fabric exhibits antiviral activity within a very short time. Because NOVARON IV1000 exhibits rapid antiviral action, it shows promise for use in applications such as products handled by hand or other uses requiring quick effectiveness.

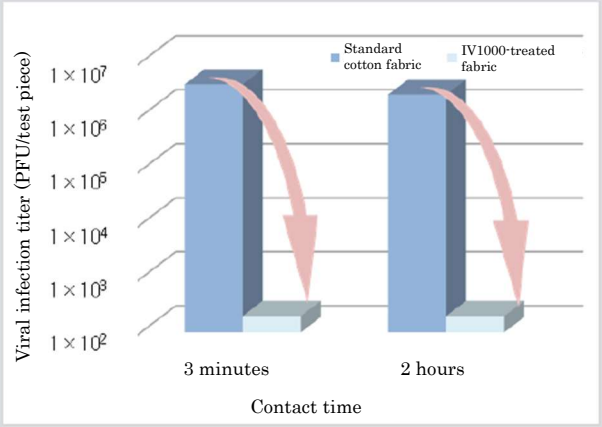

Figure 5: Antiviral property and rapid action of NOVARON IV1000-treated fabric

3.2.3 Antiviral property of NOVARON IV2000-treated fabric

Next, we treated polyester fabrics with NOVARON IV2000 so that 3.0 g/m^2 adhered to the fabric in the same manner as described in **3.2.2**, and evaluated the antiviral property of the processed fabric according to JIS L 1922. Since JIS L 1922 states that feline calicivirus is to be used as a substitute for norovirus, a representative example of a non-enveloped virus, we used feline calicivirus (ATCC VR-782) in this test. Other than the virus strains, host cells, and media used, the test method we used was nearly the same as the test method shown in **3.2.2**.

Fig. 6 shows the results. While the viral infection titer of standard cotton cloth at 2 hours after contact with the virus suspension was 10^7, the titer was reduced to 10^2 with NOVARON IV2000-treated cloth, indicating that it reduced the viral infection titer on the treated cloth by 99.99% or more. We found that NOVARON IV2000-treated fabric has high antiviral efficacy against feline calicivirus, a non-enveloped virus.

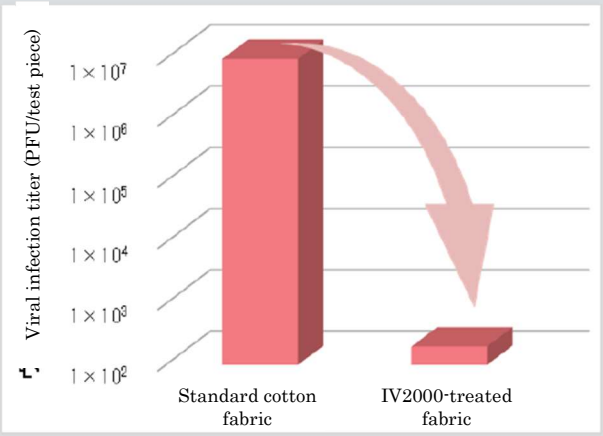

Figure 6: Antiviral property of NOVARON IV2000-treated fabric

3.2.4 Antiviral property of NOVARON IV3000-treated fabric

Next, we used NOVARON IV3000-treated fabric to carry out an antiviral property test against both influenza virus and feline calicivirus in accordance with JIS L 1922. We produced the treated fabric in the same manner as described in **3.2.2**, so that 6.0 g/m^2 of NOVARON IV3000 adhered to the fabric.

Fig. 7 shows the results. While the viral infection titer of standard cotton fabric at 2 hours after contact with the virus suspension was 10^6, the titer was reduced to 10^2 for influenza virus and 10^3 for feline calicivirus with NOVARON IV3000-treated fabric, indicating that it reduced the viral infection titer on the treated fabric by 99.99% or more and 99.97%, respectively. The above results show that NOVARON IV3000-treated fabric has a high antiviral effect against both influenza virus and feline calicivirus.

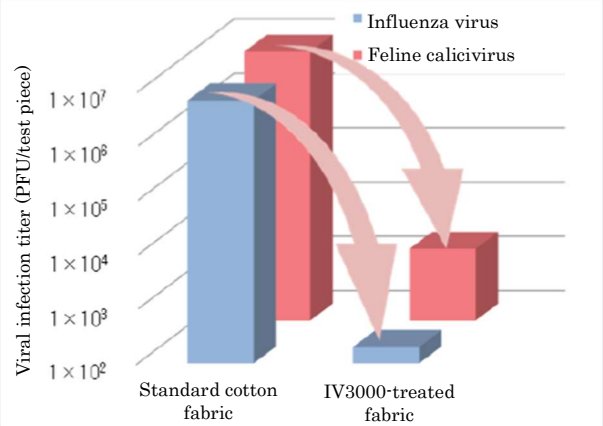

Figure 7: Antiviral property of NOVARON IV3000-treated fabric

3.3 Water resistance (washing durability) of NOVARON IV1000-treated fabric

The certification standard for antiviral properties established by the Japan Textile Evaluation Technology Council requires an antiviral activity value of 3.0 or higher, calculated as shown in **Equation 1**. The antiviral activity value is calculated as the difference between the average common logarithm of infection titers for 3 specimens immediately after inoculation of the test virus suspension on the standard fabric and the average common logarithm of infection titers for 3 specimens after 2 hours of action by the antiviral-treated fabric, when an antiviral property test is carried out in accordance with JIS L 1922. An activity value of 3.0 means a virus reduction rate of 99.9%, and a larger activity value means that the agent is more effective. In addition, it is necessary to confirm the washing durability by carrying out the antiviral property test after subjecting the specimen to washing treatment once to 10 times depending on the product application.

Antiviral activity value:

$Mv = Log(Va) - Log(Vb)$ ... (**Equation 1**)

Log(Va): Average common logarithm of the infection titer of 3 specimens immediately after inoculation of the test virus suspension on the standard fabric

Log(Vb): Average common logarithm of the infection titer of 3 specimens after 2 hours of action by the antiviral-treated fabric

We prepared treated fabric so that 1.0 g/m^2 of NOVARON IV1000 adhered to the fabric, and carried out washing treatment 10 times according to the "Washing Methods for SEK Mark Textile Products" to evaluate the anti-influenza virus property of the treated fabric before and after washing. **Fig. 8** shows the results. The viral infection titer of the standard cotton fabric immediately after inoculation with the virus suspension was 10^6 both before and after washing, whereas the viral infection titer of the NOVARON IV1000-treated fabric after 2 hours of action was 10^2 before washing and decreased to 10^3 after 10 washings (99.99% and 99.96% reduction, respectively). We calculated the antiviral activity value based on these viral infection titers, and obtained an activity value of 4.4 before washing and 3.4 even after 10 washings, meeting the SEK antiviral mark certification standard of 3.0 or higher. **Fig. 9** shows the activity values calculated from the infection titers in **Fig. 8**. In addition to its high antiviral effect, NOVARON IV1000-treated fabric has been confirmed to have good washing durability, making it suitable for items that come into contact with water or require laundering.

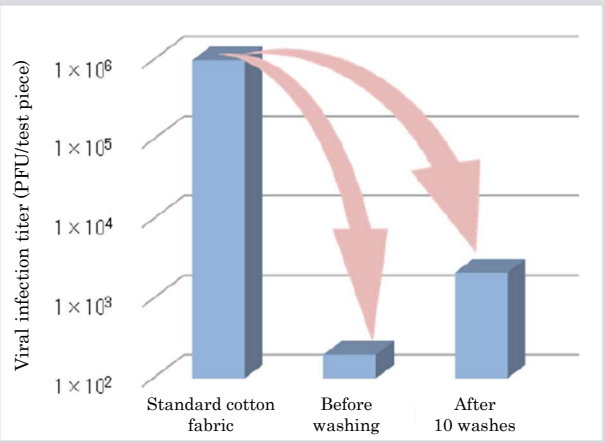

Figure 8: Washing durability (infection titer) of NOVARON IV1000-treated fabric

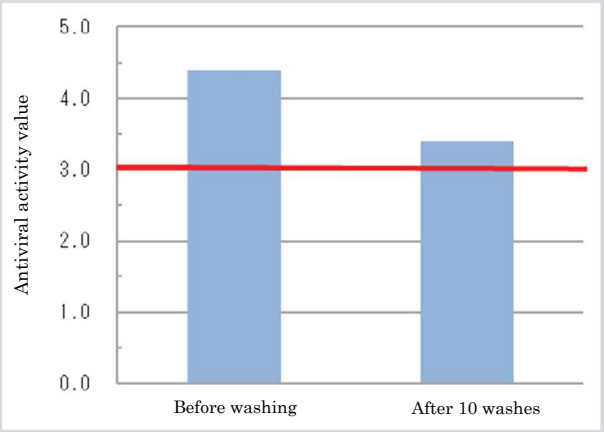

Figure 9: Washing durability (activity value) of NOVARON IV1000-treated fabric

3.4 Safety

Table 3 shows the safety data for NOVARON IV1000. It shows high safety in all test items of acute oral toxicity, primary skin irritation, skin sensitization, and mutagenicity (Ames test).

Table 3: Safety of NOVARON IV1000

Test item	Result
Acute oral toxicity	LD50 > 2000 mg/kg
Primary skin irritation	P.I.I. = 0 (no irritation)
Skin sensitization	Negative
Mutagenicity (Ames test)	Negative

4. Mechanism of the antiviral action

In general, infection of a human cell by influenza virus is triggered by hemagglutinin (HA) proteins present in the outer membrane of the virus, which recognize sialo-sugar chains on the cell surface (**Fig. 10**)[5]. Inhibiting the function of this HA on the virus would result in expression of an antiviral effect. To elucidate the mechanism of antiviral action of NOVARON

IV1000, we investigated whether NOVARON IV1000 inhibited the HA activity of influenza virus. Specifically, we used influenza virus A/Hong Kong/1/1968 (H3N2) as the virus, and confirmed the presence of HA activity inhibition by the hemagglutination test. The test was conducted at the Department of Biochemistry, Graduate School of Pharmaceutical Sciences, University of Shizuoka. As a result, we found that NOVARON IV1000 was highly effective in inhibiting the HA activity. This indicates that the anti-influenza viral mechanism of NOVARON IV1000 is inhibition of viral infection of cells through inhibition of HA activity. Although we have not experimentally verified this for NOVARON IV2000 and NOVARON IV3000, we presume they inhibit infection of cells by inactivating proteins on the virus.

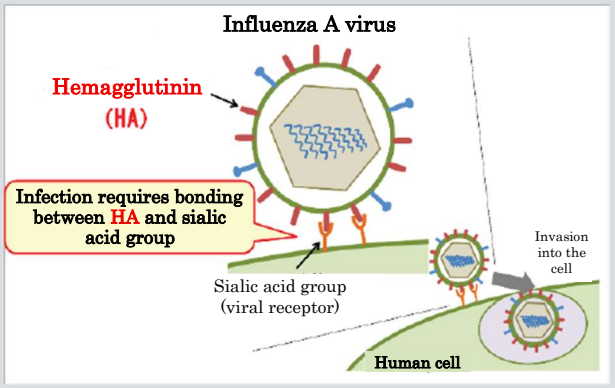

Figure 10: Conceptual diagram of infection of a cell by influenza virus

5. Antiviral effect against other viruses

We evaluated the antiviral property against coronaviruses by using some of the active ingredients of NOVARON IV1000. However, we used feline enteric coronavirus (WSU 79-1683) as the coronavirus (testing institution: Kitasato Research Center for Environmental Sciences[6]). In this test, we added some of the active ingredients of NOVARON IV1000 to 5 mL of 1.8×10^6 $TCID_{50}$/mL virus suspension to make it 1 w/v%, allowed it to act for 2 hours at 25°C, and measured the viral infection titer after the action by the $TCID_{50}$ method. We operated the control test in a similar procedure using only the virus suspension. As shown in **Fig. 11**, the infection titer of the virus suspension alone was 1.3×10^6 $TCID_{50}$/mL after 2 hours, whereas the infection titer was 2.8×10^2 $TCID_{50}$/mL when some of the active ingredients of NOVARON IV1000 were allowed to act, reducing the infection titer by 99.97% compared to the viral suspension alone.

We also evaluated the antiviral property against measles virus (ATCC VR-24) using NOVARON IV1000 in a similar fashion (testing organization: Kitasato Research Center for Environmental Sciences[7]). The results showed that the infection titer of the virus suspension alone decreased from 9.8×10^5 $TCID_{50}$/mL to 1.3×10^5 $TCID_{50}$/mL after 2 hours, whereas the addition of NOVARON IV1000 reduced it to less than 1.3×10^1 $TCID_{50}$/mL (below the detection limit) after 2 hours. Compared to the virus suspension alone, the addition of NOVARON IV1000 reduced the viral infection titer by 99.99% or more, indicating a high antiviral effect (**Fig. 12**).

The above results confirm that NOVARON IV1000 is effective not only against influenza viruses but also against other enveloped viruses.

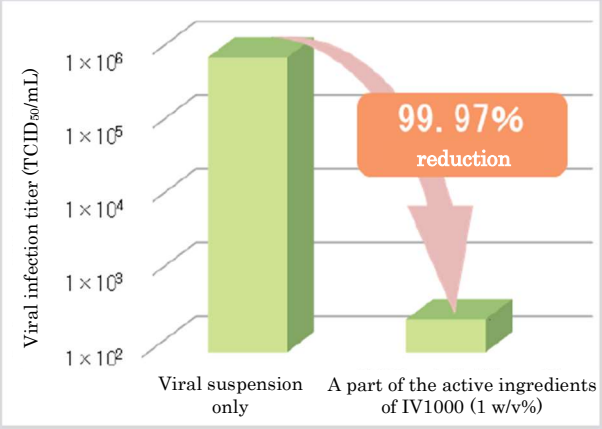

Figure 11: Antiviral property against feline enteric coronavirus

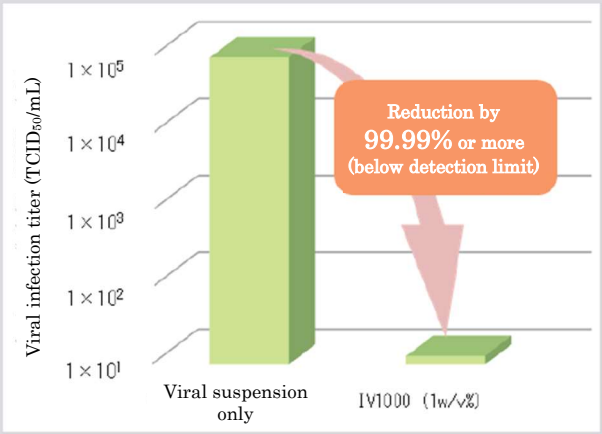

Figure 12: Antiviral property against measles virus

6. Application examples

There are various applications for NOVARON IV1000, including filters for air purifiers, house interior products such as carpets and mats, automobile interior products such as car seats and car mats, bedding products such as sheets, pillows, and bedding cotton, textile products such as masks, hats, and clothing, housing construction materials such as wall and floor coverings, and household goods such as plastic bags, plastic gloves, LCD protective films, and remote control covers. Because NOVARON IV2000 is effective against non-enveloped viruses, it is suitable for use in sheets that cover the vomit of virus-infected persons, personal protective equipment such as gloves and gowns to be used during waste disposal, garbage bags, and cleaning tools, in addition to the examples of applications for NOVARON IV1000. Due to the antiviral effects against both enveloped and non-enveloped viruses, NOVARON IV3000 can be applied to a wider range of target viruses and can be used for both of the above applications.

7. Conclusion

In addition to NOVARON IV1000, an antiviral processing agent highly effective against enveloped viruses with excellent heat resistance, processability, and safety, as well as fast-acting and water-resistant properties, we have newly developed NOVARON IV2000, which is highly effective against non-enveloped viruses, and NOVARON IV3000, which is effective against both types of viruses. At present, we have received many inquiries from various industries mentioned in the application examples above. We will continue further development to bring our products to more end-users and help provide comfortable living spaces for as many people as possible.

Reference

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