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Nestle Research validates Nanoshield with SARS-CoV-2 testing

Highlights:

- **A study conducted by Nestle Research over a combined period of five months has concluded Nanoveu's Nanoshield product to be highly effective against the SARS-CoV-2 virus that causes COVID-19**
- **The clinical testing has been published in the Applied and Environmental Microbiology journal**
- **>4.0 Log_{10} reduction of SARS-CoV-2 (>99.99% inactivation[1]) achieved at time-stamp '0' (15 minutes drying/contact time)**
- **Assessment was conducted by researchers from Nestlé Research, Institute of Food Safety and Analytical Science at a BSL-3 facility under high-security conditions**
- **Research assessed three key criteria simulating real-world conditions: immediate antiviral activity, long-term activity/durability after repeated cleaning and the effect of frequent touching**
- **Results confirm Nanoshield's status as a globally significant product with ability to make surfaces safer**

Nanoveu Limited ("Nanoveu" or the "Company") is pleased to announce its Nanoshield antiviral protection technology has received highly successful results in an assessment of its efficacy against SARS-CoV-2 (COVID-19).

The study, which was conducted by researchers from Nestlé Research, Institute of Food Safety and Analytical Science was published in the Applied and Environmental Microbiology journal (DOI: 10.1128/AEM.01098-21) and is available for public access:

https://journals.asm.org/doi/10.1128/AEM.01098-21

The report is appended to this announcement.

Assessment was conducted under a modified ISO 21702 protocol which has been designed to fill a gap in research knowledge regarding the efficacy of commercially available self-disinfecting surfaces under real-life conditions. The protocol was also required to be carried out in a biosafety

[1] The higher logarithmic reduction, the higher percentage of viral load is inactivated. For example; 1 Log_{10} = 90% reduction, 2 Log_{10} = 99% reduction, 3 Log_{10} = 99.9% reduction, 4 Log_{10} = 99.99% reduction. In Australia, the TGA requires product demonstrate at least a 4 Log_{10} against specific viruses to claim effectiveness.

level 3 (BSL-3) facility equipped with significant safety resources, sufficient to permit research on the novel coronavirus.

The results demonstrate Nanoshield was effective at reducing SARS-CoV-2 (COVID-19) immediately, in durability testing and also effective despite the presence of other organic matter.

Commenting on the ground-breaking result Nanoveu Executive Chairman and CEO Alfred Chong said:

"We have long believed the importance of scientific research in the efficacy of our products, and we welcome the validation of our antiviral claims as the leading antiviral coatings for high touch surfaces.

"Many businesses and government agencies have been looking to science to address the challenges of "living with COVID" as the pandemic rages through countries.

"Nanoveu's copper-based technology has demonstrated to be highly effective in a number of real-world scenarios, outperforming other products including quaternary ammonium compounds which lose their efficiency in the normal course of cleaning, and reactive oxygen species which are found not to be effective in everyday environments.

"Our robust network of suppliers, fabrication and manufacturing partners and testing agencies have ensured that we have a solid business-case for our clients looking for additional measure to mitigate the risk of SARS-CoV-2 transmission from high-touch surface.

"Now that the scientific research has been reviewed and published, Nanoshield becomes one of the most economical options to deliver protection and peace of mind. We are grateful to Nestlé Professional Group and their research laboratories for sharing this important finding. They are a fantastic example of a global brand which – once aware of the results – employed the Nanoshield product offering across their network. From a marketing perspective, the research also allows us and our distribution partners to directly address customer's main concern – that of protecting staff, customers and other partners from COVID-19.

"The testing of our products by Nestle's Class 3 laboratories and qualified scientists specifically against SARS-CoV-2 virus is a major achievement and without the assistance of our flagship customer Nestlé Professional, the exercise would have been prohibitively expensive and time consuming. I would like to take this opportunity to thank Nestlé Professional as well as Nanoveu shareholders for their support as we now prepare for the next phase of growth."

Assessment conditions and results

The testing analysed the effectiveness of Nanoveu's Nanoshield product and two other products under a number of conditions.

Nestle Research cite a lack of solid scientific evidence surrounding commercially available self-disinfecting surface coatings under conditions that mimic real-world use. They developed a novel, robust approach to evaluate the antiviral activity of such coatings, applying three criteria:

1. Immediate antiviral activity;
2. Effect after repeated cleaning of the coated surface; and
3. Antiviral activity in the presence of organic material (frequent touching).

Base-case test conditions saw the three commercially available products assessed against SARS-CoV-2 and human coronavirus HCoV-229E by placing the virus on coated and un-coated 25cm^2 surfaces.

The viral sample was dried upon the surfaces at room temperature for 15 minutes and subsequently assessed at the 0, 30 and 120 minute-mark. Short evaluation periods were chosen to reflect real-world use cases where antiviral activity needs to occur within a rapid timeframe.

In addition to base-case assessment, the test subjects were also analysed after repeated cleaning with the surfaces being wiped by a microfibre cloth 1, 7, 30 and 90 times over five days at room temperature.

The assumption of the repeated cleaning scenario is that one cleaning per day is a standard procedure for many high-touch surfaces. The various wiping frequencies can then be used to assess product effectiveness after simulating the amount of cleaning received at one day, one week, one month and three-month intervals.

Finally, the assessment also sought to understand the level of effectiveness while also hosting organic material introduced by finger-touching.

Prior to applying the viral load, the surfaces were touched by a finger 10 and 50 times, to simulate medium and high levels of daily touching.

In the assessment of antiviral activity after repeated cleaning the Nanoveu product performed exceptionally well, demonstrating antiviral activity following all rounds of cleaning. The QAC-based coating was removed after only one round of cleaning, limiting its effectiveness in this assessment.

The Nanoshield product was then assessed for effectiveness on a surface which has been subjected to human touching.

After 10 touches, the product returned strong results, with greater than 4.0 $\log_{10}$ reduction (99.99% inactivation) of HCoV-299E and 3.2 $\log_{10}$ reduction (>99.9% inactivation) of SARS-CoV-2 (COVID-19).

After 50 touches the product was still able to inactivate >90% of both coronaviruses at time stamp '0' (1.4 $\log_{10}$ reduction of HCoV-299E and 1.3 $\log_{10}$ reduction of SARS-CoV-2).

The results demonstrate that even under a high-use scenario the product retains its antiviral properties. However, for best performance a daily clean is recommended.

In addition to SARS-CoV-2 (COVID-19) and HCoV-299E, Nanoveu's antiviral technology has been independently demonstrated to be a highly effective agent for the inactivation of other viruses. The technology has been proven to eliminate 99.99%[2] of OC43, another coronavirus affecting humans in 30 minutes. The antiviral protection has also been validated against other bacteria and viruses including E. coli, Influenza A (subtype H3N2), and coronavirus MHV-A59[3]. Additionally, testing simulating 12 months outdoor weather exposure has found Nanoveu's products retain their effectiveness against MS2 Bacteriophage, a single-strand RNA virus[4].

- Ends –

This announcement has been authorised for release by Nanoveu's Executive Chairman and CEO.

[2] See announcements of 5 and 25 May 2020
[3] See announcement of 15 April 2020
[4] See ASX announcement of 18 February 2021

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About Nanoveu's Products:

Nanoshield is a film which uses a patented polymer of Cuprous embedded film to self-disinfect surfaces. Nanoshield antiviral protection which is available in a variety of shapes and forms, from mobile phone screen covers, to mobile phone cases and as a PVC commercial film, capable of being applied to a number of surfaces such as doorhandles and push panels. The perfectly clear plastic film contains a layer of charged copper nanoparticles which have antiviral and antimicrobial properties.

EyeFly3D is a film applied to digital displays that allowed users to experience 3D without the need for glasses on everyday mobile handheld devices.

Customskins are vending machines capable of precisely applying screen covers to mobile phones with an alignment accuracy of 150 microns.

Currently in research and development stage, EyeFyx is a vision correction solution using hardware and software to manipulate screen output addressing long-sightedness without the need to wear reading glasses.

Assessment of antiviral coatings for high-touch surfaces using human coronaviruses HCoV-229E and SARS-CoV-2

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Abstract

A novel and robust approach to evaluate the antiviral activity of coatings was developed, assessing three commercially available leave-on surface coating products for efficacy against human coronaviruses HCoV-229E and SARS-CoV-2. The assessment is based on three criteria that reflect real-life settings, namely (i) immediate antiviral effect, (ii) effect after repeated cleaning of the coated surface, and (iii) antiviral activity in the presence of organic material. The results showed that only a copper compound-based coating successfully met all three criteria. A quaternary ammonium compound-based coating did not meet the second criterion, and a coating based on reactive oxygen species showed no antiviral effect. Moreover, the study demonstrated that HCoV-229E is a relevant SARS-CoV-2 surrogate for such experiments. This new approach allows to benchmark currently available antiviral coatings and future coating developments to avoid unjustified claims. The deployment of efficient antiviral coatings can offer an additional

measure to mitigate the risk of transmission of respiratory viruses such as SARS-CoV-2 or influenza viruses from high-touch surfaces.

Importance

SARS-CoV-2, the virus responsible for the COVID-19 pandemic, is transmitted mainly by person-to-person through respiratory droplets whilst the contribution of fomite transmission is less important than suspected at the beginning of the pandemic. Nevertheless, antiviral coating solutions can offer an additional measure to mitigate the risk of SARS-CoV-2 transmission from high-touch surfaces. The deployment of antiviral coatings is not new, but what is currently lacking is solid scientific evidence of the efficacy of commercially available self-disinfecting surfaces under real-life conditions. Therefore, we developed a novel, robust approach to evaluate the antiviral activity of such coatings, applying strict quality criteria to three commercially available products to test their efficacy against SARS-CoV-2. We also showed that HCoV-229E is a relevant surrogate for such experiments. Our approach will bring significant benefit to evaluate the effect of coatings also on the survival of non-enveloped viruses, known to be more tolerant to desiccation and disinfectants and for which high-touch surfaces play an important role.

Introduction

The first reported cases of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pneumonia occurred in Wuhan, Hubei Province, China, in December 2019 and January 2020 (1, 2), and rapidly developed into the COVID-19 worldwide pandemic (3). The main transmission

route of SARS-CoV-2 is human-to-human by close contact through respiratory droplets and possibly aerosols (4). The persistence of infectious SARS-CoV-2 was shown to be high, up to 4 days, on various surfaces, such as stainless steel, plastic and glass, with infectivity better preserved in the presence of proteins (5, 6). Surfaces in hospital and community settings have been shown to be widely contaminated by SARS-CoV-2 RNA (7-9). SARS-CoV-2, other coronaviruses, such as human coronavirus HCoV-229E, or influenza viruses are efficiently and rapidly inactivated by alcohol solutions and disinfectants used for routine cleaning and sanitation (10-13), but chemical disinfectants are relatively short lived, for example in the case of alcohol due to evaporation. As an additional measure to the cleaning regime, antiviral coatings can contribute to the hygiene of high-touch surfaces. Modification and/or functionalization of surfaces (sometimes called "self-disinfecting surfaces" or coatings) to quickly inactivate microorganisms upon contact is a highly relevant research area (14-16).

A number of commercially available coatings advertise antiviral properties, however laboratory evidence demonstrating efficacy is mostly lacking. A robust methodology that mimics real-life conditions is urgently needed to evaluate antiviral claims of such products.

In this study, we provide a new approach comprised of three criteria to evaluate the antiviral potential of a surface coating, namely (i) immediate antiviral activity, (ii) antiviral activity of the coating after repeated cleaning, and (iii) the effect of organic material deposited by finger-contact on the antiviral activity of the coating. We tested the approach with SARS-CoV-2 and HCoV-229E as potential surrogate using three available commercial products claiming antiviral effects based on distinct effector mechanisms, i.e. reactive oxygen species (ROS), copper compounds, and quaternary ammonium compounds (QACs).

67 **Results and Discussion**

68 A systematic approach to evaluate the antimicrobial activity of coatings is currently lacking (17).
69 Standards such as the American Society of Testing and Materials (ASTM) Method E1153 (18)
70 and ISO 21702 (19) to assess the antiviral activity on nonporous surfaces only consider the
71 immediate antiviral activity which is insufficient, hence our objective to develop a testing
72 protocol with additional and meaningful hurdles that closely reflect real-life surface exposure.
73 We developed a comprehensive approach using three criteria to evaluate the antiviral potential of
74 different surface coatings. First a protocol was established to evaluate the immediate antiviral
75 activity, based on the experimental set-up of the ISO 21702, with modifications to better
76 represent real-life settings (19). The most important modification was to air-dry the inoculum 15
77 minutes instead of covering it with a cover film which keeps it wet. This allowed for
78 consideration of potential viral inactivation due to simple drying on the surface, as moisture of a
79 droplet will in most cases have evaporated when the next person touches the surface. The second
80 modification was that the contact times were shortened from 24 hours to 0, 30 or 120 minutes.
81 Indeed, an antiviral coating is only efficient if it reduces the viral load quickly, as there is
82 potentially only a very short interval between users of high-touch surfaces. In addition to the
83 immediate antiviral activity, two other key aspects were included in the evaluation, namely the
84 robustness towards cleaning, and the inherent capacity of the coating to work despite the
85 presence of organic material.

86 **Immediate antiviral activity.** The immediate antiviral activity of the three coatings was
87 evaluated by comparing the survival of the HCoV-229E, on non-coated versus coated surfaces
88 for each contact time (0, 30 or 120 minutes) at room temperature (Figure 1). At time 0
89 (corresponding to 15 minutes drying after spiking of the virus on the surfaces), no reduction of

HCoV-229E was obtained with the coating based on ROS (Figure 1A), whereas the coatings based on copper compounds and on QACs inactivated HCoV-229E by more than 3.5 and 2.0 $\log_{10}$, respectively (Figures 1B and 1C). At times 30 and 120 minutes, the ROS-based coating showed low (0.6 $\log_{10}$) and no antiviral activity, respectively (Figure 1A). The ROS-based coating, when activated by light, forms ROS with the moisture in the air. It is possible that we did not observe viral inactivation because no ROS were formed, or the ROS did not affect HCoV-229E within the time span of two hours. Another study using TiO_2-coated glass observed more than 3 $\log_{10}$ reduction of influenza virus, but only after 4 hours of UV-A exposure (20). Based on the results obtained with HCoV-229E, the ROS-based coating was not investigated any further.

The two coatings which showed immediate antiviral activity against HCoV-229E were evaluated for antiviral activity using SARS-CoV-2 after 0, 30 or 120 minutes of contact times at room temperature (Figures 1B and 1C). At time 0, a reduction of more than 4.0 $\log_{10}$ and more than 1.6 $\log_{10}$ of SARS-CoV-2 was observed on the copper compound-based and the QAC-based coatings, respectively (Figures 1B and 1C).

The antiviral effect of copper has previously been reported for HCoV-229E and SARS-CoV-2 (21, 22). The antiviral activity of the copper compound-based coating used in our study is thought to be caused by contact between the surfaces of the virus and the copper compound, causing denaturation of biomolecules (e.g. proteins) which results in viral inactivation. Comparable viral inactivation was described previously where a 30-minute exposure to Cu_2O, another copper compound, lead to a reduction of more than 5 $\log_{10}$ of bacteriophage Qβ, a small sized single stranded RNA virus (23). The mechanism of the QAC-based coating technology is based on positively charged quaternized nitrogen and carbon chain “spikes”. The negatively

charged microbial cell wall of bacteria is attracted to the "spikes" and consequently disrupted, leading to inactivation. The antiviral activity we observed may be the result of a similar mechanism, since SARS-CoV-2 virus particles are mostly negatively charged at neutral pH (24). QACs coated on glass were also shown to be effective against influenza virus (25). It is important to mention that we observed an immediate antiviral activity only if the QAC-based coating was applied by spraying without subsequent wiping. No immediate antiviral activity was observed when the coating was sprayed on the surface directly followed by wiping to evenly distribute the product on the surface (data not shown).

Antiviral activity after repeated cleaning. The antiviral activity of the coating based on copper compounds and QACs was evaluated by cleaning the surfaces 1, 7, 30 or 90 times using a microfiber cloth with a water-based detergent. This represents an accelerated protocol to simulate 1, 7, 30 or 90 rounds of cleaning. The antiviral activity was assessed by comparing the survival of HCoV-229E and SARS-CoV-2 on non-coated surfaces versus coated and cleaned surfaces (Figure 2). The antiviral activity of the copper compound-based coating remained intact for at least 90 rounds of cleaning (Figure 2A), whereas the antiviral activity of the QAC-based coating was removed after only one round of cleaning (Figure 2B). The coating sprayed on the surface was probably wiped off during cleaning. This is similar to the results from a controlled trial in a hospital setting and shows that the mode of application of a spray coating is pivotal and potentially less reliable compared to a ready-to-use adhesive film (26). Similar to the cleaning, disinfection with 70% ethanol did not affect the antiviral efficiency of the copper compound-based coating whereas the antiviral activity was lost for the QAC-based coating (Figure 3). These study results are necessary to define cleaning instructions (e.g. type of cloth and frequency) for the applied coating to ensure sustained antiviral activity.

Effect of organic material introduced by finger-touching. The copper compound-based coating successfully passed the two first criteria and was further evaluated for the third criterion. To assess this criterion, coated surfaces were finger-touched 10 or 50 times, prior to virus inoculation, to simulate the daily use of a high touch surface (e.g touch screens of vending machines). This experimental set-up allowed to evaluate the effect of organic material such as fingermark residues on antiviral activity. The antiviral activity was assessed by comparing the survival of HCoV-229E and SARS-CoV-2 on non-coated versus coated and touched surfaces (Figure 4). The antiviral activity of the copper compound-based coating was still high after 10 touches (> 4.0 $\log_{10}$ reduction of HCoV-229E and 3.2 $\log_{10}$ reduction of SARS-CoV-2), but lower after 50 touches (1.4 $\log_{10}$ reduction of HCoV-229E and 1.3 $\log_{10}$ reduction of SARS-CoV-2). Similar $\log_{10}$ reductions were obtained for HCoV-229E and SARS-CoV-2 after 50-times finger-touching (p-value of 0.83). Fifty touches correspond to a daily touching frequency of a highly used vending machine and shows that the copper compound-based coating may retain activity for roughly one day. Afterwards, cleaning is required to remove traces of organic material. Repeated cleaning with a microfiber cloth did not affect the antiviral activity as shown when the second criterion was evaluated (Figure 2A). The commercial copper compound-based coating fulfilled the three evaluation criteria and can be considered an efficient antiviral coating.

Comparison of HCoV-229E and SARS-CoV-2. This study assessed the antiviral activity of coatings using two viruses, HCoV-229E and SARS-CoV-2. Both viruses are human respiratory pathogens. They belong to the family *Coronaviridae* with a single-strand, positive-sense RNA genome approximately 26-32 kilobases in size and a similar structure with spike projections from the virus membrane (27, 28). Despite these similarities, HCoV-229E cannot serve as a legitimate surrogate for SARS-CoV-2 without comparison and calibration (29). Since the LOQ

was reached, the maximum log_{10} reduction was obtained for both viruses when testing the immediate antiviral activity, the first criterion, of the copper compound and QAC-based coatings. The maximum log_{10} reduction was also observed for both viruses on the copper compound-based coating after repeated cleaning (second criterion). No log_{10} reduction was observed for both viruses on the QAC-based coating after repeated cleaning. The evaluation of the effect of organic material introduced by the finger-touching in the third criterion also showed a similar behavior of SARS-CoV-2 and HCoV-229E. After 50 finger touches, the LOQ was not reached allowing for calculation of the p-value (p-value = 0.83) which indicated that the log_{10} reductions obtained for both viruses were not significantly different. Together these results show that both viruses behaved similarly in all experiments representing the three evaluation criteria (immediate antiviral activity, antiviral activity after repeated cleaning and the effect of organic material introduced by finger-touching), demonstrating that HCoV-229E is a relevant SARS-CoV-2 surrogate for the evaluation of these surface coating products. Generating data with human coronavirus surrogates, that can be handled in biosafety level (BSL)-2 laboratories is important, as studies with SARS-CoV-2 must be conducted in BSL-3 facilities, limiting the number of laboratories available.

In conclusion, a harmonized protocol will allow regulators and users to evaluate claims related to antiviral surfaces. It would be of interest to further elucidate the mode of action of these surfaces, especially when in contact with organic material and when exposed to extreme temperatures and pH conditions. It will also be useful to benchmark currently available coatings against novel technical solutions. Microbial tolerance to biocidal compounds present in coatings is unlikely due to the multitarget nature or non-specific action of the chemicals used, as described for QACs

(30). The coated surfaces will be regularly cleaned, thus avoiding long-term exposure and potential microbial tolerance to these biocidal compounds.

In the future our approach to evaluate and verify the antiviral activity of coatings could be expanded to also encompass the effect on non-enveloped viruses, known to be more tolerant to desiccation and disinfectants, such as Noroviruses, which are transmitted by the fecal-oral route and for which high-touch surfaces play an important role.

Materials and Methods

Virus and preparation of suspension. HCoV-229E (ATCC VR-740) and SARS-CoV-2, kindly provided by Prof. Isabella Eckerle (Geneva University Hospitals, Center for Emerging Viral Diseases), were propagated, assayed and titrated on human lung fibroblast MRC-5 cells (ATCC CCL-171) and on kidney African Green Monkey Vero C1008 [Vero 76, clone E6, Vero E6] (ECACC 85020206) cells, respectively, as described previously for enteric viruses (31). Briefly, the cells were passaged in Eagle's Minimum Essential Medium (EMEM) (ATCC, 30-2003) supplemented with 10% Fetal Bovine Serum (FBS) (ATCC, 30-2020) and 1% Penicillin/Streptomycin 100 X (Sigma, P0781) followed by incubation at 37°C with 5% CO_2. Viruses were propagated on their respective host cells followed by incubation at 35°C with 5% CO_2 for 1 to 2 h to allow the adsorption of the viruses to the cells. The adsorption was stopped by adding 25 ml of EMEM supplemented with 2% FBS and 1% Penicillin/Streptomycin 100 X followed by incubation at 35°C with 5% CO_2. Viral stocks were purified and concentrated by a polyethylene glycol precipitation (0.25 volume of 5x polyethylene glycol/NaCl solution) as described in ISO-15216 (32). The pellets were resuspended in phosphate-buffered saline (PBS)

(Sigma, D8662). Viral titers determined as the 50% tissue culture infective dose ($TCID_{50}$) per milliliter as described previously (31), were 7.0 ± 0.3 $\log_{10}$ $TCID_{50}$/ml for SARS-CoV-2 and ranged from 6.5 ± 0.1 $\log_{10}$ $TCID_{50}$/ml to 7.0 ± 0.1 $\log_{10}$ $TCID_{50}$/ml for HCoV-229E.

Antiviral coating solutions. ROS-based coated (the type of ROS is not described by the supplier) and non-coated 25 cm^2 glass surfaces, copper compound-based coated and non-coated 25 cm^2 polyethylene terephthalate (PET) films and QAC-based spray were kindly provided by the suppliers and are commercially available as Kastus glass cover commercialized by Kastus (Dublin, Ireland), Nanoshield commercialized by Nanoveu Limited (Subiaco, Australia) and Zoono Microbe Shield (Z-71) spray commercialized by Zoono group (Auckland, New Zealand), respectively. The ROS-based glass and the copper compound-based PET films are ready to employ coatings to be applied like a phone screen protector. The ROS-based coating forms ROS with the moisture in the air when activated by light. The QAC-based coating needs to be sprayed on the surface of interest by the customer. In our study, this coating was sprayed on 25 cm^2 poly-methyl methacrylate surfaces and distributed on the whole surface using the side of a micropipette tip followed by drying in a biosafety cabinet for at least 10 min.

Evaluation of the antiviral activity. The experimental set up was based on the ISO 21702 method (19) with slight modifications. The inoculum was dried for 15 minutes instead of covering it with a cover film which keeps it wet and the contact times were shortened from 24 hours to 0, 30 and 120 minutes, as the antiviral activity needs to act fast for high-touch surfaces to ensure inactivation between users.

Immediate antiviral activity. The immediate antiviral activity against HCoV-229E and SARS-CoV-2 was evaluated by comparing the survival of the viruses on non-coated surfaces and coated surfaces after 0, 30 or 120 minutes of contact times at room temperature.

Antiviral activity after repeated cleaning. The antiviral activity against HCoV-229E and SARS-CoV-2 was evaluated by comparing the survival of the viruses on non-coated surfaces versus coated surfaces previously cleaned 1, 7, 30 or 90 times using a microfiber cloth over 5 days at room temperature. As one cleaning per day is a standard procedure for many high-touch surfaces, this protocol simulates one day, one week, one month and three months of cleaning, respectively. Cleaning was carried out with Suma Star D1 detergent (10-20% sodium dodecylbenzene sulfonate, 5-10% sodium lauryl ether sulfate, 1-<3% ethyl alcohol) according to the supplier recommendations (Diversey Europe, Münchwilen, Switzerland) or 70% ethanol for disinfection.

Organic material effect introduced by finger-touching. The antiviral activity against HCoV-229E and SARS-CoV-2 was determined by comparing the survival of the viruses on non-coated surfaces versus coated surfaces finger-touched 0, 10 or 50 times by 10 volunteers, meaning 0, 1 or 5 finger-touching per person per 25 cm^2, respectively. This corresponds to a medium (10) and high (50) daily touching frequency of a high-touch surface. The volunteers were asked to not wash or disinfect their hands prior to the finger-touching. Each finger-touching was performed using 3 fingers applied several times on the surface in order to cover the 25 cm^2.

Virus inoculation on coated and non-coated surfaces. One hundred µl of HCoV-229E (5.5 or 6.0 log_{10} $TCID_{50}$) or SARS-CoV-2 (6.0 log_{10} $TCID_{50}$) which corresponds to viral loads in saliva of infected patients (33, 34) was spread on a 25 cm^2 non-coated or coated surface and dried for 15 minutes in a biosafety cabinet at room temperature. According to visual inspection, 15 minutes was the minimum time required to have a dry inoculum on the different surfaces employed in this study.

Virus recovery from non-coated and coated surfaces. Viruses were recovered by intensively swabbing the surface using a cotton-tipped swab (VWR, 115-1881) pre-dipped in Dey-Engley Neutralizing broth (Sigma, D3435) 5-fold diluted in PBS (Sigma, D8537). The swab was transferred to a 1.5-ml tube containing 0.5 ml of Dey-Engley Neutralizing broth 5-fold diluted in PBS. The plastic part of the swab was cut in order to close the tube and the tube was vortexed vigorously for 1 minute to release the viruses. The recovered viruses were 5-fold serially diluted and enumerated by determining the $TCID_{50}$ (31). Preliminary experiments demonstrated that Dey-Engley Neutralizing both 5-fold diluted did not affect the enumeration of the viruses.

Data analysis. Viral counts (N_x and N_0) were expressed in $\log_{10}$ $TCID_{50}$/25 cm^2 where N_x is the viral titer recovered from the coated surface and N_0 the titer recovered from the non-coated surface (mean of 3 replicates). Plotted values are mean viral count ± standard deviation. The limit of quantification (LOQ) of the method was 1.05 $\log_{10}$ $TCID_{50}$/25 cm^2. Nevertheless, in some cases the LOQ was coating dependent since cytotoxicity on the cells was observed. The cytotoxicity induced by the coating solutions was evaluated by swabbing 25 cm^2 coated surface (not inoculated with viruses) and analyzed as described above (virus recovery from non-coated and coated surfaces) and inoculated on MRC-5 and Vero C-1008 cell lines. Each condition was tested in triplicate. The copper compound-based coating did not induce cytotoxicity and the LOQ for both viruses was 1.05 $\log_{10}$ $TCID_{50}$/25 cm^2, whereas the QAC-based coating inducted cytotoxicity on the two cell lines increasing the LOQ for both viruses to 3.15 $\log_{10}$ $TCID_{50}$/25 cm^2. Values below the LOQ were entered as LOQ with an asterisk in the graphs. Reduction in infectious virus count (inactivation) was calculated as N_x/N_0 and expressed in $\log_{10}$. The statistical significance of $\log_{10}$ reductions of HCoV-229E and SARS-CoV-2 obtained with the copper compound-based coating after 50 finger touches (Figure 4) was performed by a two-

sampled *t*-test (unequal variance) using Microsoft Excel® for Microsoft 365 MSO. P-values below 0.05 were considered as significantly different.

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Competing interests

The authors declare no competing interests.

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388

389 **Figure 1.** Survival of HCoV-229E (blue) and SARS-CoV-2 (grey) on non-coated surfaces (solid
390 bars) and on coated surfaces (hatched bars) after 0, 30 or 120 minutes of contact times at room
391 temperature (A: ROS-based, B: copper compound-based, C: QAC-based). Time "0" corresponds
392 to 15 minutes drying after spiking of the virus on the surfaces. Bars with asterisks highlight the
393 $\log_{10}$ values below the LOQ. The LOQ for both viruses was 1.05 $\log_{10}$ $TCID_{50}$/25 cm^2 for the
394 copper compound-based coating, whereas the QAC-based coating inducted cytotoxicity on the
395 two cell lines increasing the LOQ for both viruses to 3.15 $\log_{10}$ $TCID_{50}$/25 cm^2. Error bars
396 represent the standard deviation; n=3.

397 **Figure 2.** Survival of HCoV-229E (blue) and SARS-CoV-2 (grey) on non-coated surfaces (solid
398 bars) and on coated surfaces (hatched bars) after 1, 7, 30 or 90 rounds of cleaning with a water-
399 based detergent using a microfiber cloth (A: copper compound-based, B: QAC-based). Bars with
400 asterisks highlight the $\log_{10}$ values below the LOQ. Error bars represent the standard deviation;
401 n=3.

402 **Figure 3.** Survival of SARS-CoV-2 (grey) on non-coated surfaces (solid bars) and on coated
403 surfaces (hatched bars) after 1, 7, 30 or 90 rounds of disinfection with 70% ethanol using a

microfiber cloth (A: copper compound-based, B: QAC-based). Bars with asterisks highlight the $\log_{10}$ values below the LOQ. Error bars represent the standard deviation; n=3.

Figure 4. Survival of HCoV-229E (blue) and SARS-CoV-2 (grey) on non-coated surfaces (solid bars) and on copper compound-based coated surfaces (hatched bars) previously finger-touched 0, 10 or 50 times. Bars with asterisks highlight the $\log_{10}$ values below the LOQ. Error bars represent the standard deviation; n=3.

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