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Statement of antimicrobial activity of copper-dispersed films from RuKaInnovation B.V.

RuKaInnovation B.V. (from hereon RuKaInnovation), Rudolf Wessels and Kamal Khandelwal being the Directors, requested TNO to evaluate their copper-dispersed films for antimicrobial activity (limited to antiviral and antibacterial activity) in an objective expert study. TNO has reviewed to the best of their ability, available test information regarding RuKaInnovation's copper-dispersed products as well as a limited set of peer-reviewed articles, resulting in this statement about the antimicrobial activity of RuKaInnovation's copper-dispersed products.

The products this statement deals with are Polyethylene (PE) films from RuKaInnovation containing copper as an antimicrobial component and differing in the presence of an acrylic adhesive with paper/ PET liner. Copper has long been recognized as an antimicrobial agent, reviewed in (1). In 2008, the EPA officially recognized copper and its alloys as the first effective metallic antimicrobial agent (<http://www.epa.gov/pesticides/factsheets/copper-alloy-products.htm>).

The antibacterial activity of RuKaInnovation PROMAXCOPPER (Manufacturing Code RF7000CU), from hereon also referred to as 'Film 1' has been tested by FITI, a South Korean company, against *Staphylococcus aureus* ATCC 6538P and *Escherichia coli* ATCC 8739 (2). Both bacteria were susceptible for the film, with a 4.6 log₁₀ reduction for *S. aureus* and a 6.2 log₁₀ reduction for *E. coli* after 24h exposure to the film.

RuKaInnovation has also had two of its copper-dispersed PE films tested for activity against bovine coronavirus (3). Film 1 (PROMAXCOPPER RF7000CU) is the same copper-dispersed PE film that has been tested for antibacterial activity (2), Film 2 is an improved version of Film 1 with a higher copper content (proprietary data - confidential). Both films showed a reduction in virus titres compared to the control, with the higher reduction observed for Film 2 with its higher copper content (3), summarized in Table 1 and represented in Fig. 1.

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Table 1. Reduction of bovine corona virus titres (log₁₀ TCID₅₀/ml) compared to the control after incubation of the virus on 2 copper-dispersed films of RuKaInnovation, compared to the negative control.

	Film 1	Film 2
0.5h	1.4	3.2
1h	2	3.6
4h	2	4.2
24h	2	4

For both PROMAXCOPPER films, virucidal activity was already observed after 30min of exposure. The virucidal activity against the tested bovine coronavirus further increased, leading to inhibitory efficiencies (log₁₀ TCID₅₀/ml) ranging from 2 for Film 1 to 4 for Film 2 after 24h exposure (Table 1). The improved Film 2, with its higher copper content therefore performed better than the original Film 1. According to the test facility, 'At 4.0 or higher, virus self-disinfectivity is recognized' (3).

The observed antiviral activity of the 2 films has been translated towards 'inhibitory ability%'. With the log₁₀ factor in mind, a 2 log₁₀ reduction as observed for Film 1 after 1h onwards, translates to 99% disinfection activity. For Film 2, with its 3.6 or higher log₁₀ reduction from 1h onwards, the disinfection activity is increased to >99.98%.

For the final decrease in bovine coronavirus titres, the natural decrease as observed in the negative control should also be taken into consideration. In this study, the decrease in the negative control equated to 1.6 log₁₀ reduction after 24h (Fig. 1 and (3)). Together, this leads to a final reduction of 5.6 (4 + 1.6) log₁₀ on Film 2 after 24h (also shown in Fig.1). In this study, starting with 10^{6.5} bovine coronaviruses, after 24h exposure to Film 2 only 7 (10^{0.9}) viruses could be detected (Fig.1 and (3)).

The 2 copper-dispersed films from RuKaInnovation clearly show antimicrobial activity in the performed tests against 2 bacteria and a virus. The original Film 1 that showed antibacterial activity against *S. aureus* and *E. coli*, was also assessed for antiviral activity against bovine coronavirus. Both films showed antiviral activity, with Film 2 being an improved version of Film 1 (a higher copper content), outperforming Film 1. Based on available literature, it is well accepted that the available copper content on the surface dictates the antibacterial activity. The higher the copper content, the higher the antibacterial activity and the faster the killing (inactivation) action (4). Therefore, it is safe to assume that Film 2 will have even better antibacterial activity than Film 1.

For highly virulent pathogens such as SARS-CoV-2, it is common practice to utilize a less virulent surrogate for the initial tests. In this case, bovine coronavirus has been tested and found to be susceptible for the PROMAXCOPPER films of RuKaInnovation. Copper has also been described in literature to have proven antiviral activities, e.g. against influenza A virus, HIV-1, and hepatitis C (4). In a

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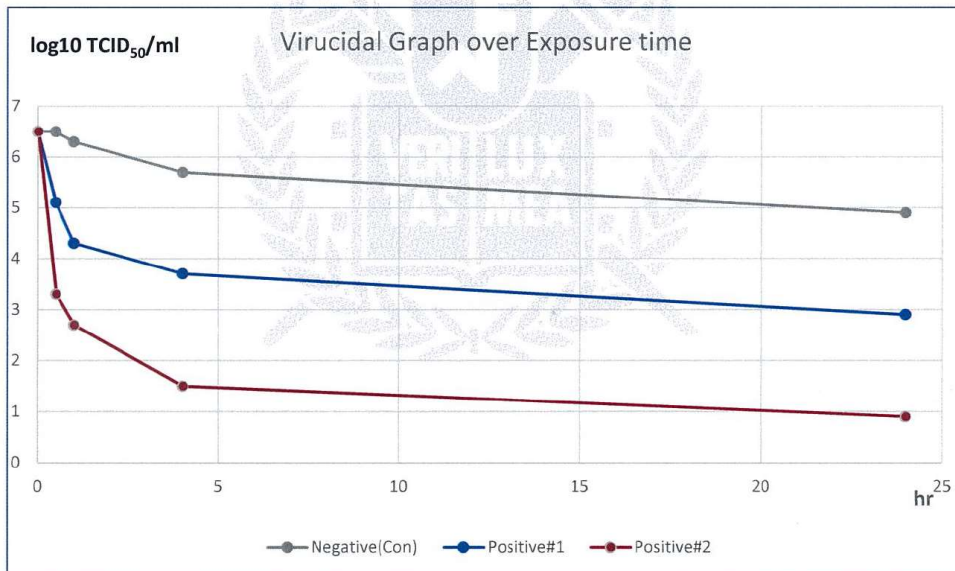


Figure 1. Graphic representation of the virucidal activity of the two tested copper-containing films in comparison to the negative control. Positive#1: Film 1; Positive#2: Film 2. Note: figure copied from (3).

recent study, aerosol and surface stability of SARS-CoV-2 was compared to SARS-CoV-1, including stability on copper (5). In this study, exposure to copper resulted in a reduction below the detection limit after 24h exposure for both viruses, with copper performing better than stainless steel or plastic, and comparable to cardboard. Slight differences were observed between SARS-CoV2 and SARS-CoV-1, with a slight trend of SARS-CoV-2 being less stable than SARS-CoV-1 (5).

Taken together, it is likely that the copper-dispersed PROMAXCOPPER films of RuKaInnovation will also have inhibitory activity against SARS-CoV-2. Whether this inhibitory activity is comparable to the inactivation as observed in (5), is at the moment impossible to say as the similarities/differences between the RuKaInnovation PROMAXCOPPER films and the copper surface tested in (5) are unavailable.

Conclusions about the antiviral activity of the improved PROMAXCOPPER film from RuKaInnovation:

The improved PROMAXCOPPER film (Film 2) of RuKaInnovation, with its increased copper content compared to the original PROMAXCOPPER Film 1, deactivated 99.9% of the tested bovine coronavirus after 30 minutes and 99.98% after 1h in comparison to the control. Similar disinfecting properties are expected on SARS-CoV-2.

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