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REPORT ON THE ANALYSIS OF VIRUSOCOCIDACY OF PAINTS SAMPLES REPORT No. 1/2021 (pages 6).

1. The Contractor:

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2. Client:

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3. Subject of research:

Paint samples entrusted by the client:

- NoEM paint (painted on polystyrene plate with a diameter of 6 cm).

- control (polystyrene plate with a diameter of 6 cm).

Virus tested:

Bovine Herpesvirus type 1 (BHV-1), virion diameter approximately 155-175 nm.
The BHV-1 virus was used to analyse the effectiveness of viral particle inactivation, which, like SARS CoV-2, is an enveloped virus, which determines its resistance to virucides.
The virus spreads through the air through the secretions of the lacrimal glands, saliva, nasal secretions and semen. BHV-1 is characterized by a very narrow range of hosts (it only infects cattle and sheep), which makes it safe for humans to work with.

4. Research Methodology.
The aim of the study was to assess the effectiveness of inactivation of viral particles by the analysed paint samples during: 10 minutes, 100 minutes and 24 hours.
The methodology was based on the guidelines contained in the ISO 21702 standard for the *"Measurement of antiviral activity on plastics and other non-porous surfaces"*.

5.1. Arrangement of trials in the experiment:

10 min incubation with viral lysate
- NoEM paint on polystyrene plate
- control polystyrene plate

100 min incubation with viral lysate
- NoEM paint on polystyrene plate
- control polystyrene plate

24h incubation with viral lysate
- NoEM paint on polystyrene plate -
control polystyrene plate

5.2. Methodology used:

5.2.1. Incubation of ink samples with viral lysate.

- 0.5 ml of viral lysate with a titer of 2x106 pfu / ml was applied to the painted surfaces of the plates (6 cm in diameter) and covered with a piece of polypropylene (PP) film 0.10 mm thick and an area of 4x4 cm.

- The plates were placed on wet blotting paper in Petri dishes (10 cm diameter) to minimize the effect of evaporation.

- The samples prepared in this way were incubated on the table at room temperature for 10 min, 100 min and 24h.

Fig-1. Paint samples and controls during incubation in Petri dishes.

5.2.2. Titration of the virus by the plaque size assay method.

- Immediately after the end of incubation, the viral lysate was carefully removed from the ink samples and titrated.

- The titration was performed in monolayer culture of MDBK cells in 12-well plates.

- Serial dilutions were made in a RPMI medium supplemented with 8% FBS (100 µl of viral lysate harvested after incubation was transferred to 900 µl of medium).

- After harvesting the medium from the cells, 500 µl of viral lysate at appropriate dilutions were applied to them and incubated for 1 h (37 ° C / 5% CO 2).

- After 1h incubation, the viral lysate was harvested from the cells. Subsequently, 1.5 ml of a 1% methylcellulose solution in the culture medium was applied to the cell culture and incubated for 6 days (37 ° C / 5% CO 2) to visualize the viral plaques.

Fig-2. MDBK cells in 12-well plates in a CO2 incubator.

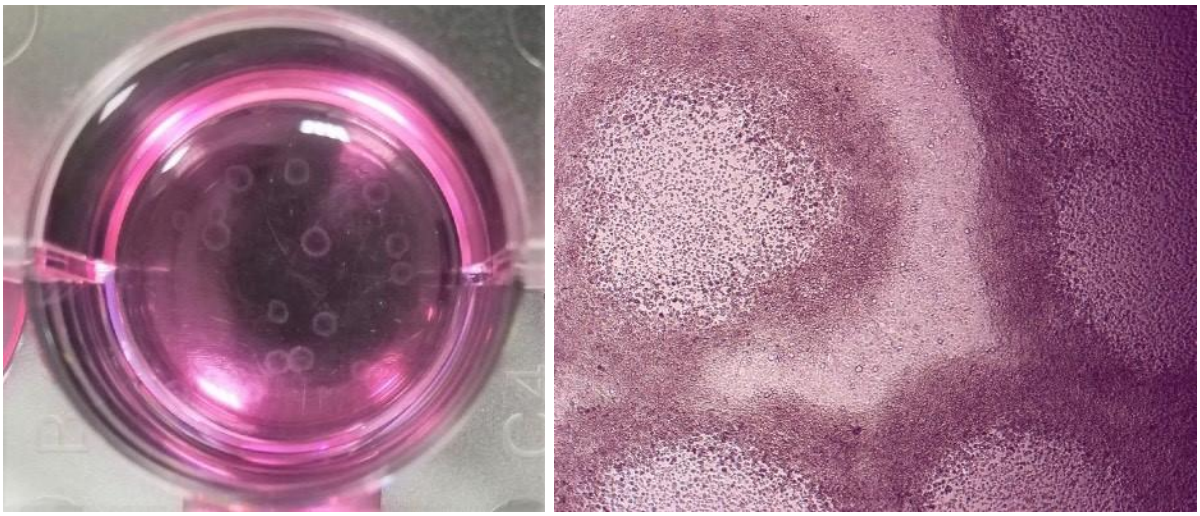


Fig-3. Viral plaques visible on 12-well plates and light microscopy.

6. Results.

Table-1

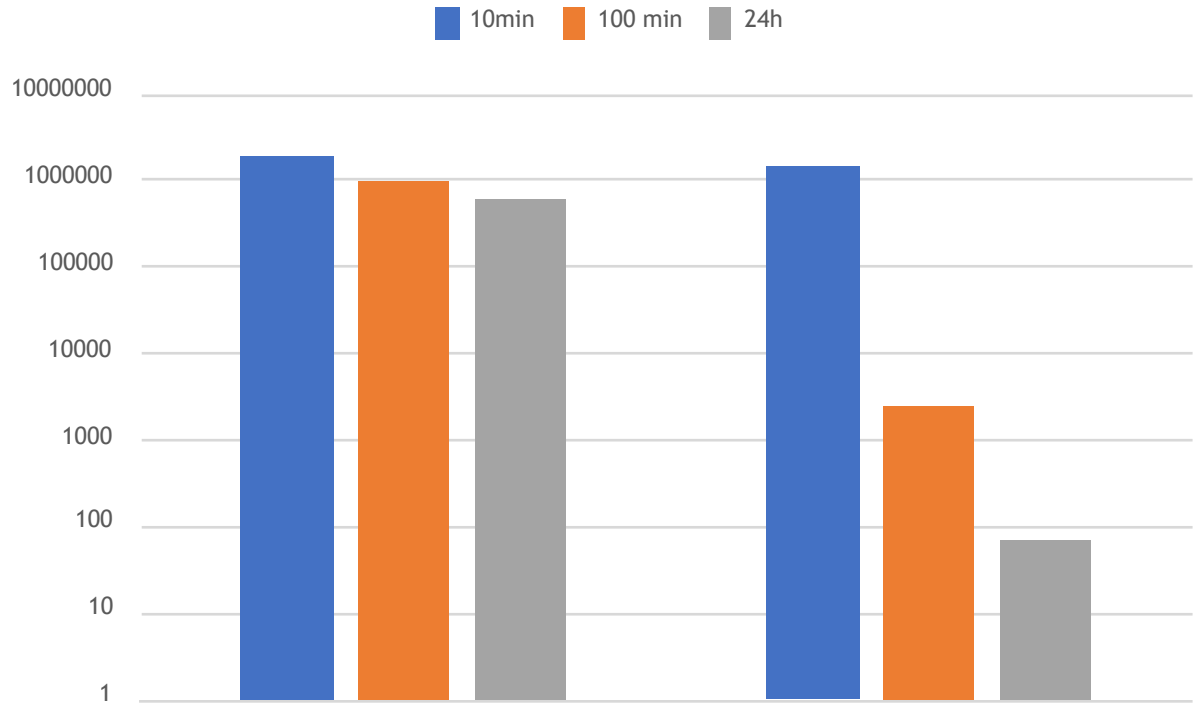
Amount of active viral particles remaining in the lysate after incubation with the test material (PFU / ml). Average of two independent biological replicates.

	10 min	100 min	24 h
Control	$2x10^6$	$1x10^6$	$6x10^5$
NoEM paint	$1,5x10^6$	$2,5x10^3$	7x10

Table-2

Bar graph - logarithmic scale.

The amount of active viral particles remaining in the lysate after incubation with the test material. Average of two independent biological replicates.



1 control polystyrene plate	2 NoEM paint

7. Conclusions:

The ability of the test product to inactivate the test virus (BHV-1) is determined on the basis of the decrease in its infectious titer, caused by contact with the test material. The criterion of virucidal activity of the tested product against a given virus is a decrease in the titer of infectious virus after 24 hours of incubation by at least 2 logs (difference in the logarithmic scale between the infectious titer of the virus in the control sample and the infectious titer of the virus after incubation with the test material).
The NoEM dye, after 100 min of incubation with the viral lysate, causes a decrease in its infectious titer compared to the control by over 2 logs.
After 24h of incubation, the decrease in infectious virus titer was 4 logs from the control.
The study showed the significant virucidal potential of NoEM paint with a recommendation for certification.

Chairman of the Board ProChimia Surfaces Sp. z o.o.
Piotr Barski