

## **Report on the Antiviral Activity of SD90+/SD-Pro against SARS-CoV-2**

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This report summarizes findings of antiviral activity of the surface coating product SD90+ (also marketed as SD-Pro) against the SARS-CoV-2 virus. These experiments were performed under laboratory conditions where no contact was made with the coated surface. Surfaces were sprayed with product, allowed to dry and then challenged with live virus immediately and from 1 to 8 days later.

### **Test Facility**

Experiments supporting these findings were conducted at the University of Nebraska Medical Center's (UNMC) biosafety level-3 (BSL-3) laboratory suite. The UNMC BSL-3 laboratory suite has been certified by the Federal Select Agent Program as a Tier 1 Select Agent facility.

### **Test Methods**

The University of Nebraska Medical Center received one lot of the surface coating product SD90+/SD-Pro to evaluate for initial activity and residual activity after 1 to 8 days. Following initial validation tests to determine the likely contact time required for viral inactivation, two rounds of experiments were performed. For the first experiment, glass cover slips were prepared by coating each with SD90+/SD-Pro, sprayed from a distance of 4-6 inches over a time of 5-10 seconds, and allowed to dry. Two contact times (20 and 60 minutes) were evaluated in triplicate for each residual activity point. In the second experiment, 15 x 100 mm petri dishes were prepared by coating each with SD90+/SD-Pro, sprayed from a distance of 4-6 inches over a time of 5-10 seconds, and allowed to dry. A single contact time of 20 minutes was evaluated in triplicate for each residual activity point.

Both experiments were completed with a stock of SARS-CoV-2 virus that was prepared by infecting Vero-E6 cells, growing in Minimum Essential Medium (MEM) enriched with 5% fetal bovine serum (FBS), and, after cytopathic effect (CPE) was observed, harvesting the supernatant. This stock was then concentrated using a Vivaflow concentrator (100 kD membrane) to reach a final titer of  $2.33 \times 10^6$  TCID50/mL ( $1.6 \times 10^6$  pfu/mL). Each coupon was exposed to 500  $\mu$ L of viral stock ( $\sim 1.17 \times 10^6$  TCID50/coupon). Each glass coupon was recovered in 10 mL of MEM to recover virus from the surface. Each petri dish was recovered by washing the surface with 500  $\mu$ L of MEM repeatedly. MicroSpin S-400 HR columns (GE Healthcare) were used to neutralize any activity from the SD90+, following Zhang et al., 2018 using 100  $\mu$ L of the recovered sample. Following neutralization, all recovered samples were assayed for residual viral activity, in triplicate, by TCID50.

### **Test Dates:**

February-March 2021

## Test Agent

The antimicrobial active ingredient in SD 90+/SD-Pro is 3-(trimethoxysilyl) propyldimethyl-octadecyl ammonium chloride and is EPA registered under Global Shield Solutions as DTN-100 (Reg. No. 92415-1). The composition and approximate concentration of DTN-100 in SD90+/SD-Pro is  $\leq 5\%$  with inert ingredients (as determined by the US EPA) present at  $\geq 95\%$ .

## **Test Results**

The results of the first experiment with glass cover slips are summarized in Table 1. In general, three log (3.0) inactivation was consistently achieved after either 20 or 60 minutes of contact time, across all residual times. However, two inconsistent data points were observed: one coupon (Day 0, 60 min contact time, replicate C) and one coupon (Day 8, 20 min contact time, replicate A) showed no virus inactivation. To address these outliers and whether they were caused by difficulty in manipulating the small-sized cover slips used, the second experiment was performed with petri dishes. The results of this second experiment are shown in Table 2. At all residual times, 3 log reduction of the virus was observed after the 20 minutes contact time.

SD90+/SD-Pro Coating Validation - Glass Slides					
Coating	Infection Time	Replicate	Titer (TCID <sub>50</sub> /mL)	Log Reduction	% Reduction
D-0	20 minutes	A	1.58E+00	3.00	99.9
	20 minutes	B	1.58E+00	3.00	99.9
	20 minutes	C	1.58E+00	3.00	99.9
	60 minutes	A	1.58E+00	3.00	99.9
	60 minutes	B	1.58E+00	3.00	99.9
	60 minutes	C	3.41E+00	2.67	99.8
D-2	20 minutes	A	1.58E+00	3.00	99.9
	20 minutes	B	1.58E+00	3.00	99.9
	20 minutes	C	1.58E+00	3.00	99.9
	60 minutes	A	1.58E+00	3.00	99.9
	60 minutes	B	1.58E+00	3.00	99.9
	60 minutes	C	1.58E+00	3.00	99.9
D-8	20 minutes	A	1.58E+03	0.00	0.00
	20 minutes	B	1.58E+00	3.00	99.9
	20 minutes	C	1.58E+00	3.00	99.9
	60 minutes	A	1.58E+00	3.00	99.9
	60 minutes	B	1.58E+00	3.00	99.9
	60 minutes	C	1.58E+00	3.00	99.9
Positive Control			1.58E+03		
Negative Control			No CPE		

Table 1. Residual concentration and log-reduction of SARS-CoV-2 on glass surfaces coated with SD90+/SD-Pro. 1.58 TCID<sub>50</sub>/mL is the maximum viral concentration that could be expected if no CPE is observed in the assay. Therefore 1.58E+00 is used when no CPE is observed.

SD90+/SD Pro Coating Validation - Petri Dish					
Coating	Contact Time	Replicate	Titer (TCID50/mL)	Log Reduction	% Reduction
D0	20 minutes	A	1.58E+01	3.00	99.9
	20 minutes	B	1.58E+01	3.00	99.9
	20 minutes	C	1.58E+01	3.00	99.9
D-1	20 minutes	A	1.58E+01	3.00	99.9
	20 minutes	B	1.58E+01	3.00	99.9
	20 minutes	C	1.58E+01	3.00	99.9
D-7	20 minutes	A	1.58E+01	3.00	99.9
	20 minutes	B	1.58E+01	3.00	99.9
	20 minutes	C	1.58E+01	3.00	99.9
Positive Control (From Dish Harvest)			1.58E+04		
Positive Control (Neat)			7.34E+03		
Negative Control			No CPE		

Table 2. Residual concentration and log-reduction of SARS-CoV-2 on plastic surfaces coated with SD90+.

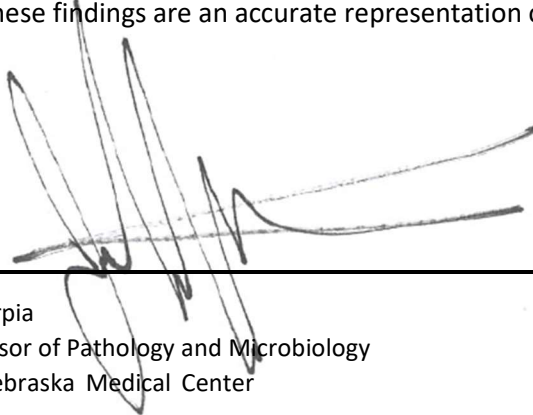
## Conclusion

All Samples of SD90+/SD-Pro at the 20-minute and 60-minute exposure times on day zero through day 7-8 post coating showed a 3-log reduction, against human coronavirus SARS-CoV-2. These experiments, performed under idealized conditions, demonstrate the antiviral potential of SD90+/SD Pro against SARS-CoV-2. Further testing to more extensively characterize the antimicrobial activities of this product is warranted.

## References

Zhang, Huajun, Chen Peng, Bobo Liu, Jun Liu, Zhiming Yuan, and Zhengli Shi. "Evaluation of MICRO-CHEM PLUS as a disinfectant for Biosafety Level 4 laboratory in China." *Applied Biosafety* 23, no. 1 (2018): 32-38.

These findings are an accurate representation of the experiments performed to date.




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