

June 3, 2020

Rick Latella
DMR International, Inc
720 S. Eastwood Dr. Suite 243
Woodstock, IL 60098

Re: Final Report for IITRI Project # 2958001001001: Samaritan Shield Testing with SARS-CoV-2

Dear Mr. Latella,

The study investigation of the effectiveness of Samaritan Shield Copper and Samaritan Shield Zinc Clearcoat Cleaner Protectorant against SARS-CoV-2, the causative virus for COVID19 has been completed. Testing of the two Samaritan Shield Clearcoat Cleaner Protectorants occurred between May 8 and May 20, 2020 and used the sponsor-requested JIS Z2801 Standards Protocol that was modified for use with the SARS-CoV-2 virus.

Test Article and Test Substrate identification and preparation.

The Test Articles (TA) used for this study were Samaritan-Shield-Copper Clearcoat Cleaner Protectorant and Samaritan-Shield-Zinc Clearcoat Cleaner Protectorant (alternatively known as: Samaritan-Shield-ZN Clearcoat Cleaner Protectorant) and was provided by the Sponsor. The Test Articles were received as a liquid in spray bottles.

The Test Substrate (TS) was a Formica material (measuring approximately 7/8" x 7/8") to serve as a substrate for the TA. The Test Substrate as supplied by the Sponsor has two sides, one of which is a brown finely rippled surface and the other side is a white smooth surface. The TA was sprayed onto the smooth white surface of the substrate, and wiped-off leaving a layer of the TA on the substrate.

Test Virus and Cell Culture.

The Test Virus used for this study was 2019 Novel Coronavirus, Isolate USA-WA1/2020 (SARS-CoV-2). The virus was stored at approximately $\leq -65^{\circ}\text{C}$ prior to use. The multiplicity of infection (MOI) was 0.01 TCID₅₀/cell.

The Cell Culture used for the TCID₅₀ test was African Green Monkey Kidney Cells (Vero E6 cells) that were maintained in Dulbecco's Minimum Essential Medium with 10% fetal calf serum. All growth media contained heat-inactivated fetal calf serum and antibiotics.

Study Design:

The test design is shown below in Table 1. This test design assessed the TA on a substrate (Formica) in various conditions as shown in Table 1 using a modified JIS Z2801 Standards Protocol which was adapted for virus.

1. The Test Substrate was treated for sterility via 70% ethanol for approximately 5 minutes (Sponsor-supplied Formica material).
2. For Test Groups 1-3 (72 hour pre-virus exposure groups), either TA (Samaritan-Shield-Copper or Samaritan-Shield-Zinc Clearcoat Cleaner Protectorant) was applied to the Test Substrate so that the

surface was coated with TA. The TA was left on the substrate surface for approximately 5-10 seconds and then sterilely wiped with gauze or a similar sterile material to remove any excess TA. The surface was allowed to air dry for at least 5 minutes. The treated Test substrates were sterilely stored for approximately 72 hours prior to application of the SARS-CoV2 virus.

3. For Test Groups 4 and 5, either TA (Samaritan-Shield-Copper or Samaritan-Shield-Zinc Clearcoat Cleaner Protectorant) was applied to the Test Substrate so that the surface was coated with TA. The TA was left on the substrate surface for 5-10 seconds and then sterilely wiped with gauze or a similar sterile material to remove any excess TA. The surface was allowed to air dry for at least 5 minutes before application of the virus.
4. For Group 6 (5 minute wet group), the TA was sprayed onto the Test Substrate without drying.
5. Virus-only controls on substrate was added for each timepoint to verify that the TCID₅₀ assay was valid. A cell culture-only control was also included to indicate that cells without any TA or virus remain viable throughout the assay.
6. The treated Test Substrate plus TA was placed into a sterile 6 well cell culture plate and approximately 1×10^5 TCID₅₀ SARS-CoV-2 virus was layered onto the Test Substrate and covered with a sterile glass coverslip to assist in the spreading of the virus.
7. After application of the virus, the virus was in contact with the Test Substrate for approximately 5 minutes (Groups 3 and 6), 4 hours (Groups 2 and 5), and 24 hours (Groups 1 and 4) while incubating at 37°C with 5% CO₂ at $\geq 80\%$ humidity. Each Test Substrate per time per TA was performed in triplicate.
8. After the incubation time, the treated virus was washed with 1 mL of cell culture media (DMEM-2) for approximately 15 minutes within the 6 well cell culture plate and the glass cover slip was removed. This was equivalent to a 10-fold dilution. The plate was shaken to enhance recovery of the virus.
9. For the TCID₅₀, the cell culture media (DMEM-2) used to wash the Test Substrate was serially diluted 10 fold and transferred into respective wells of a 96-well plate which contained a monolayer of African Green Monkey Kidney Cells (Vero E6 cells) for titration. The TCID₅₀ assay was performed non-GLP according to IITRI Standard Operating Procedures for the assay. The TCID₅₀ titers was calculated using the method of Reed-Meunch. The study design used for this study is shown below in Table 1

Table 1: Study Design

Group	Test Group	Samaritan-Shield-Copper	Samaritan-Shield-Zinc
1	72 hour pre-exposure: 24 hour post-exposure	3 Test Substrates	3 Test Substrates
2	72 hour pre-exposure: 4 hour post-exposure	3 Test Substrates	3 Test Substrates
3	72 hour pre-exposure: 5 minute post-exposure	3 Test Substrates	3 Test Substrates
4	Samaritan (Wipe after 5-10 sec): 24 hr post exposure	3 Test Substrates	3 Test Substrates
5	Samaritan (Wipe after 5-10 sec): 4 hr post exposure	3 Test Substrates	3 Test Substrates
6	Samaritan (wet): 5 min post exposure	3 Test Substrates	3 Test Substrates
Control Group	Virus Control on Test Substrate	3 Test Substrates	3 Test Substrates

Results:

The Test Articles, Test Substrates and virus (SARS- CoV-2) were prepared according to protocol and each preparation was noted in the study notebook for this study.

After coating the Test Substrates with either TA (Samaritan-Shield-Copper or Samaritan-Shield-Zinc Clearcoat Cleaner Protectorant) for 72 hrs or on the day of virus exposures (Groups shown in Table 1 above), a TCID₅₀ was performed at various timepontos (5 minutes, 4 hours, and 24 hours). For groups that were assessed at 4 hours after virus exposure (Groups 2 and 5) and 24 hours after virus exposure (Groups 1 and 4), both the controls (on substrate) associated with those timepoints and the experimental groups did not show any viral titers. This indicated that the modified JIS Z2801 Standards Protocol, which was originally for assessment of surface treatments with bacteria, appeared to not be appropriate for SARS-CoV-2 over an extended incubation/exposure period under these experimental conditions. While bacteria are able to survive a prolonged period up to at least 24 hours, it was found that the SARS-CoV-2 virus that was used for both the controls and test groups appeared to be at least partially inactivated when placed on

a surface of the substrate for the 4 hour and 24 hour tests. An additional virus-only control (in DMEM media without Test Substrate) did show that the virus survived to a 4.75 TCID₅₀Log₁₀/mL. The purpose of this control was to show that active virus was present. This further indicated that the modified JIS Z2801 Standards Protocol, as performed, was not sufficient for the extended incubation periods used for this study. As a result, further modifications to the JIS Z2801 protocol may be needed to assess longer exposure periods for the SARS-CoV-2 virus.

However, using the modified JIS Z2801 Standards Protocol on the shorter 5 minute virus exposures (Groups 3 and 6), results observed did indicate an effectiveness in reducing infectious virus titers under these experimental conditions. The results are shown below in Table 2.

Table 2: Samaritan-Shield-Copper and Samaritan-Shield-Zinc Results -72 Hour and 5 Minute Pretreatment.

Group	Test Article	Replicate	Pretreatment [#]	TCID ₅₀ Log ₁₀ /mL*	Averaged TCID ₅₀ Log ₁₀ /mL	St. Dev.	log difference [^]
3	Samaritan Copper	1	72 hr	4.25	2.58	1.46	-0.83
	Samaritan Copper	2	72 hr	2.00			
	Samaritan Copper	3	72 hr	≤1.50			
3	Samaritan Zinc	1	72 hr	2.75	3.00	0.43	-0.42
	Samaritan Zinc	2	72 hr	2.75			
	Samaritan Zinc	3	72 hr	3.50			
6	Samaritan Copper	1	5 min (WET)	≤1.50	2.08	0.52	-1.33
	Samaritan Copper	2	5 min (WET)	2.50			
	Samaritan Copper	3	5 min (WET)	2.25			
6	Samaritan Zinc	1	5 min (WET)	≤1.50	1.50	0.00	-1.92
	Samaritan Zinc	2	5 min (WET)	≤1.50			
	Samaritan Zinc	3	5 min (WET)	≤1.50			
	Virus Control on substrate	1	N/A	3.50	3.42	0.38	N/A
	Virus Control on substrate	2	N/A	3.75			
	Virus Control on substrate	3	N/A	3.00			

[#]Pretreatment is defined as the period of treatment with the TA prior to the exposure to the SARS-CoV-2 virus.

*limit of detection is 1.5 TCID₅₀ Log₁₀/mL

[^]Log difference is defined as the averaged TCID₅₀ Log₁₀/mL from virus control on substrates - TCID₅₀ Log₁₀/mL from replicate test group. Log difference indicates amount of reduction in infectious virus when comparing the virus control on substrate to the test group.

To summarize, for Group 3-Samaritan Copper, when averaging the 72 hour pretreatment replicates, was 2.58 ± 1.46 TCID₅₀ Log₁₀/mL with a log difference of -0.83 when compared to the averaged virus control on substrate. However, due to the large difference from the first replicate to replicates 2 and 3, excluding replicate 1, the averaged TCID₅₀ Log₁₀/mL of replicates 2 and 3 would be 1.75 ± 0.35 with a log difference of -1.67. For Group 3-Samaritan Zinc, when averaging the 72 hour pretreatment replicates, there was a TCID₅₀ Log₁₀/mL of 3.00 ± 0.43 with a log difference of less than a -0.5 when compared to the virus control on substrate. For the Group 6-Samaritan Copper with the 5 minute (wet) pretreatment, there was an average of TCID₅₀ Log₁₀/mL of approximately 2.08 ± 0.52 with an average log drop of -1.33 when compared to the virus control on substrate. For the Group 6-Samaritan Zinc with the 5 minute wet pretreatment, there was an average TCID₅₀ Log₁₀/mL of approximately 1.5 ± 0.00 with an average log drop of -1.92 when compared to the virus control on substrate.

Summary:

Using the modified JIS Z2801 Standards Protocol, the Test Articles, Samaritan-Shield-Zinc and Samaritan-Shield-Copper, appeared to show some effectiveness in reducing infectious virus titers in the experimental condition shown in the protocol after the 72 hour pretreatment and a 5 minute virus exposure when compared to the virus control on substrate (between a 0.42 to 0.83 log reduction, respectively). Additionally, excluding the first replicate of the 72 hour Samaritan Copper (which appears to be an aberration when compared to replicates 2 and 3), may actually indicate a more significant log difference at 1.75, as well.

As shown in Group 6 Samaritan Copper and Samaritan Zinc, the ability to reduce infectious virus appeared to be even greater with the 5 minute wet pretreatment for both Test Articles which ranged between 1.33

and 1.92 log reduction when compared to the virus control on substrate. A point to mention is that the limit of detection for the assay performed for this study is 1.5 TCID₅₀ Log₁₀/mL meaning that the maximum achievable log difference in relation to the virus control on substrate is only about 2 logs due to the low titer of the stock virus and the built-in dilutions of the study design. Therefore, the Group 6 Samaritan Zinc (5 minute wet) may actually be able to reduce the viral titers greater than 2 logs that is detectable by the assay performed for this study. The use of a higher titer virus, which was not available at the time to conduct the study, may be able to determine if the virus titer can be reduced greater than 2 logs by the Test Articles. Additionally, by using larger numbers of Test Substrates and pooling the recovered virus for titration, dilutions can be minimized increasing the ability to detect even greater inactivation effects.

Results were not able to be obtained for the 4 hour and 24 hour post-virus exposure in both the test groups and control group (specifically the virus control on substrate group). An additional control virus group run with the study, in which the virus was not applied to a substrate, had a 4.75 TCID₅₀ Log₁₀/mL suggesting that the virus was inactivated over the longer incubation periods (4 hour and 24 hour exposures) while on the test substrate.

Overall, these results show that both the Samaritan-Shield-Copper Clearcoat Cleaner Protectorant and Samaritan-Shield-Zinc Clearcoat Cleaner Protectorant when applied either at 72 hour or 5 minute (wet) prior to SARS-CoV-2 exposure can reduce infectious virus performance on a substrate within a short period of time (5 minute exposure). Additionally, within these testing conditions, even greater effectiveness against this virus is seen when both the Samaritan-Shield-Copper and Samaritan-Shield-Zinc Test Articles were applied immediately and in wet form.

Respectfully Submitted,

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