For this trial *Human Coronavirus NL63 HCoV* (ATCC # VR-3263SD) was studied.

Previously, the ADB technology has been demonstrated to have beneficial effects on the reduction of bacteria, mold and food pathogens in refrigerated and non-refrigerated environments. In a growing number of commercial applications, these benefits have enabled perishable product processors, growers/shippers, wholesalers and retailers of perishable commodities to significantly expand their marketing window, reduce losses due to decay and disease and reduce operational risk and costs.

B. Materials and Methods

Viral culture

Human Coronavirus NL63 HCoV (ATCC # VR-3263SD), was acquired from ATCC, Manassas, VA., USA. and maintained on ATCC complete growth medium and minimum essential medium (ATCC, Manassas, VA., USA) with 2 µM L- glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 µM non-essential amino acids, and 1.0 µM sodium pyruvate, 90%; fetal bovine serum, 10% and cultured in Trypticase Soy Agar with added; sodium bicarbonate, non-essential amino acids, and combination of sodium pyruvate and fetal bovine serum, in aerobic growth conditions at 33-35°C.[1]

Cells from both of the above (approx. $1x10^7$ CFU/ml) from a 24-hour static culture incubated at 33-35°C were used to inoculate various 5 cm x 5 cm plastic, stainless steel, and floor tile coupons.

The inoculum suspensions were enumerated by surface plating in duplicate samples on TSA after serial dilution in 0.1% peptone solution. The plates were incubated for 24-hours at 37°C.

C. Inoculation of various media surface areas

A 100 µl droplet from the initial inoculum suspension of the virus culture was used to inoculate the external surface (5 cm x 5 cm) on plastic, stainless steel and floor tile coupons, with the final inoculum level to be approximately 7.5-$\log_{10}$ CFU/g sample. The inoculated samples were dried by air-blowing for 1-hour at 22°C prior to the treatment being initiated. The 1-hour drying allows the inoculated cells to attach to the surface host and minimize the growth of inoculated cells during drying.

D. Treatment

Treatment was carried out using an ADB Technology 4007 unit installed in a testing chamber. The chamber was monitored by the built-in smart controller and gas sensors to monitor H_2O_2 and O_3 (indicators of reactive oxygen species production) as well as temperature and relative humidity.

The plastic, stainless steel and floor tile (5 cm x 5 cm) coupon surfaces were inoculated with the virus and were treated with a setpoint of 0.10 ppm H_2O_2 (calculated) and 0.04 ppm O_3 concentration for 15, 30-minutes, 1, 2, 4, 8, 12 and 24-hour increments at 24°C (75°F) at 40% RH. After the treatment, the samples were subjected to enumeration by surface plating. The log reduction of the virus was evaluated with and without the consideration of resuscitation of injured cells after treatment.

[1] *Cells expressing heteroresistance grow more slowly than the oxacillin-susceptible population and may be missed at temperatures above 35°C. This is why CLSI recommends incubating isolates being tested against oxacillin, methicillin, or nafcillin at 33-35° C (maximum of 35°C) for a full 24 hours before reading.*

Three different controls were prepared in each treatment. For a positive control, a 5 cm x 5 cm area of the three coupons were inoculated with virus cells and dried for 1-hour but not exposed to the treatment. There were three negative controls, in which the 5 cm x 5 cm coupons were inoculated with 100 µl droplet of sterile water and dried for 1 hour.

One negative control was treated with ADB Technology and the other was not subjected to the treatment. Each treatment sample and the 3 controls were prepared in triplicate.

E. Recovery of *pathogens* from the surface samples

After the treatment, each of the 5 cm x 5 cm coupons were transferred into a 400 ml stomacher bag (Fisher Scientific Inc., PA., USA) combined with 50 ml sterile 0.1% peptone solution, and then blended with a AES Easy Mix Stomacher (AES Laboratories, Princeton, NJ., USA) for 2-min at normal speed. Wash fluid was serially diluted, followed by surface plating for enumeration.

A centrifugation method was used to recover low populations of injured virus. The centrifugation method (Mossel and others 1991) was modified and used to concentrate the virus populations in the wash fluid so that less than 250 CFU/ml of bacteria can be enumerated by the surface plating.

F. Study Results and Discussion Following treatment with the ADB Technology unit, the average reductions of the Human Coronavirus was 4.21-$\log_{10}$ in plastic, 4.32-$\log_{10}$ in stainless steel and 4.22-$\log_{10}$ in floor tile following 15-minute treatments, based on the infectious virus recovery. Following treatment times of 30- minutes, 1, 2, 4, 8, 12 and 24-hours on the inactivation of the virus on a selection of surface samples is noticeable from the Table attached.

1. **Overall log reduction**
   - The 15-minute treatment results show a slightly greater average reduction on Human Coronavirus at 4.32-log10.
   - The 30-minute treatment results again shows a greater average reduction on Human Coronavirus at 6.63-log10.

2. **Impact on the organism**
   - The largest reduction 4.32-$\log_{10}$ was seen after the first 15-minute exposure on stainless steel. A 99.99% decrease.
   - The second largest reduction 6.63-$\log_{10}$ was seen after 30-minute exposure on stainless steel. A 99.9999% decline.

3. **Impact on surfaces**
   After 30-minute exposure the stainless-steel coupons showed the greatest reduction of 6.63-$\log_{10}$ followed by the plastic and the floor tile coupons at 6.52-$\log_{10}$ and 6.48-$\log_{10}$, respectively.

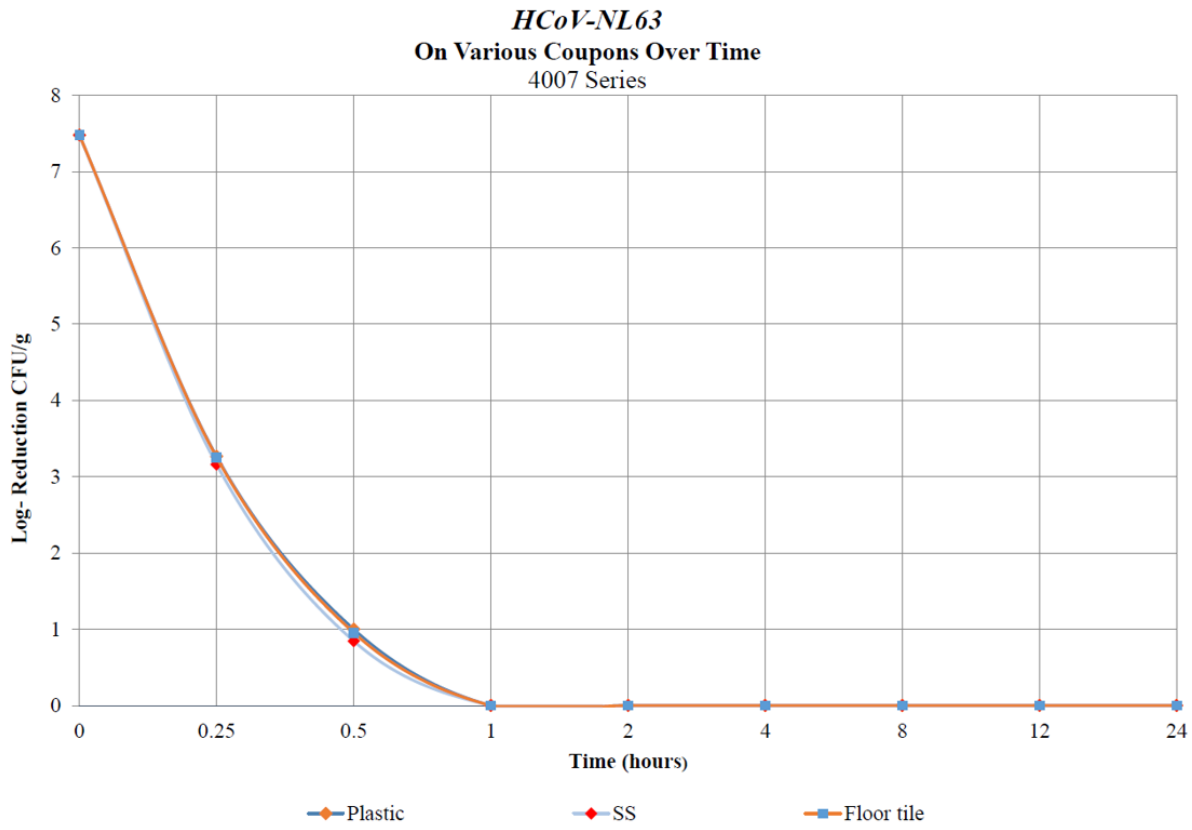
G. Conclusion

This study shows the significant effect of the ADB Technology treatment in reducing HCoV viral cultures on surfaces. The process carried out by this technology inactivates viruses by breaking the protein envelope and inactivating the RNA strand. The results are unambiguous and indicate a clear correlation between ADB Technology treatment at the indicated concentrations and the stated log reductions of the virus on all surfaces tested.

TABLE 1. Human Coronavirus *NL63* populations following treatment with ADB Technology system.

	Plastic			Stainless Steel			Floor Tile		
Time	Log_{10} variance	Standard Deviation	Reduction	Log_{10} variance	Standard Deviation	Reduction	Log_{10} variance	Standard Deviation	Reduction
0	7.48	0.4	-	7.48	0.3	-	7.48	0.2	-
15-m	3.27	0.2	4.21	3.16	0.3	4.32	3.26	0.3	4.22
30-m	<1	0.1	6.48	0.85	0.2	6.63	0.95	0.2	6.52
1-h	<1	0.1	7.48	<1	0.1	7.48	<1	0.1	7.48
2-h	<1	0.1	7.48	<1	0.1	7.48	<1	0.1	7.48
4-h	<1	0.1	7.48	<1	0.1	7.48	<1	0.1	7.48
8-h	<1	0.1	7.48	<1	0.1	7.48	<1	0.1	7.48
12-h	<1	0.1	7.48	<1	0.1	7.48	<1	0.1	7.48
24-h	<1	0.1	7.48	<1	0.1	7.48	<1	0.1	7.48

GRAPHIC REPRESENTATION OF RESULTS



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