To whom it may concern,

The novel human coronavirus (SARS-CoV-2) has become a global health concern affecting your employees, customers, and company liability. Safety programs cause logistic issues including social distancing, temperature monitoring, sanitation process, etc., all of which are difficult to implement. Sanitation processes that include surface wipe down are impossible to do effectively during business operation. Surfaces can then be re-contaminated immediately and the sanitation needs to be perpetually repeated to be moderately effective.

Our proprietary ROS (Reactive Oxygen Species) technology sanitizes all surfaces & air safely and continuously 24/7 with an intelligent control system. Our ROS system is fully organic, requires no added chemicals, and is ultra-low in energy consumption. This technology has been applied through various industries including food processing, horticulture, & transportation since 2005 and has a well proven operation history.

The attached Research Brief regarding AirROS by SAGE test results confirms our effectiveness in eradicating HCoV (Coronavirus) on various surface types at our normally low ozone level of 30ppb with full reduction in 45min (36,000,000 CFU to <1 CFU). In 15min, we had a 99.98% reduction (36,000,00CFU down to 5,200CFU) and in 30min, we had a 99.99997% reduction (down to 10CFU). This test further confirms the previous testing of our proprietary technologies ability to destroy the following:

- Bacteria
  - Staph aureus (mRSA)
  - Citrobacter
  - Pseudomonas
- Yeast
  - Candida (Fungus)
- Viruses
  - Corona Virus (CoVid-19)
  - Influenza A (including Bird Flu)
  - Norovirus (stomach flu)
  - Rhinovirus (colds)

In summary, if you have a plastic or stainless-steel surface, studies show that the virus is detectable for up to three days. With our system alone, the virus is undetectable in 45min without using any other mitigation process on all things the air touches. However, as with all sanitation processes, we suggest a multi-layered approach and you should follow all CDC and related guidelines to protect the spread of the virus.

Our 4000 series purification systems can be applied from 500 cubic feet to multi million cubic feet areas with our full range of products including world-power options.

Feel free to email sales@sageindustrial.com with information regarding your application to have a more extensive discussion on how our product can meet your specific needs.

For more information, visit our website at www.sageindustrial.com or www.airros.com.

Research Brief

Effect of *AirROS* Series 4000 for Control of *Human Coronavirus* on Various Inoculated Surfaces†

ABSTRACT

The novel human coronavirus SARS-CoV-2 has become a global health concern causing severe respiratory tract infections in humans. Transmissions, Human-to human have been described, perhaps via coughing/sneezing droplets but also possibly via contaminated hands or surfaces. In a recent review on the persistence of human and veterinary coronaviruses on inanimate surfaces it was shown that human coronaviruses such as Sever Acute Respiratory Syndrome (SARS) coronavirus, Middle East Respiratory Syndrome (MERS) coronavirus or endemic human coronavirus (HCoV) can persist on inanimate surfaces like metal, glass or plastic for up to 9 days.

Human coronavirus is an enveloped, positive-sense, single-stranded RNA virus which enters its host cell by the ACE2 receptor. Infection with the virus has been confirmed worldwide and has an association with many common symptoms and diseases. Associated diseases include mild to moderate upper respiratory tract infections, severe lower respiratory tract infection, croup and bronchiolitis. The virus originated from infected palm civets, also called a toddy cat, which is not a cat but a small to medium-sized mammal in the Viverrid family and bats. HCoV-OC43 is one of seven known coronaviruses to infect humans, including HCoV-229E, HCoV-OC43, HCoV-HKU1, MERS-CoV, the original SARS-CoV (or SARS-CoV-1) and SARS-CoV-2.

The virus that causes coronavirus disease 2019 (COVID-19) is stable for several hours to days in aerosols and on surfaces, according to an earlier study from National Institutes of Health, CDC, UCLA and Princeton University scientists in The New England Journal of Medicine. The scientists found that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was detectable in aerosols for up to three hours, up to four hours on copper, up to 24 hours on cardboard and up to two to three days on plastic and stainless steel. The results provide key information about the stability of SARS-CoV-2, which causes COVID-19 disease, and suggests that people may acquire the virus through the air and after touching contaminated objects.

This study will analyze the impact of AirROS technology against Human Coronavirus HCoV at 20°C (68°F) and a relative humidity (RH) 40% with treatment times of 0, 15, 30, 45-minutes, 1, 2, 4, 8, and 12-hours. A selection of three surfaces have been studied using response surface methodology (RSM). In these tests the log reduction of the above-mentioned virus demonstrates the inactivation effects delivered with the AirROS treatment. The statistical analysis of developed predictive model suggested that concentration, RH and treatment time all significantly ($P<0.01$) increased the rate of log reduction. Among the three (3) factors, the effect of treatment concentration on inactivation was the greatest, while effect of RH was the least. The interaction between concentration and RH exhibited a significant and synergistic effect ($P<0.05$).

† Mention of trade names or commercial products does not imply recommendation or endorsement to the exclusion of other products.

A. Current Trial

This trial was designed to demonstrate the benefits of using the AirROS technology to reduce/preclude this specific virus on various surfaces: plastic, stainless steel, and floor tile coupons (all used in commercial/hospitals).

For this trial *Human Coronavirus OC43 HCoV* (ATCC # VR-1558D™) was studied.

Previously, the AirROS 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 OC43 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 AirROS Series 4002 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 0.03 ppm O_3 concentration for 0, 15, 30, 45-minutes, 1, 2, 4, 8 and 12-hour increments at 20°C (68°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 AirROS and the other was not subjected to the treatment. Each treatment sample and the 3 controls were prepared in triplicate.

E. Recovery of *virus* 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 AirROS unit, the average reductions of the Human Coronavirus OC43 was 3.85-$\log_{10}$ on floor tile, 3.84-$\log_{10}$ on stainless steel and 3.85-$\log_{10}$ on plastic following 15-minute treatments, based on the infectious virus recovery.

Following treatment times of 0, 15, 30, 45-minutes, 1, 2, 4, 8 and 12-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 an average reduction on Human Coronavirus OC43 at 3.84-$\log_{10}$.
   - The 30-minute treatment results again shows a further average reduction of 6.57-$\log_{10}$.

2. **Impact on the organism**
   - The largest reduction 3.85-$\log_{10}$ was seen after the first 15-minute exposure on stainless steel, a 99.99% decrease.
   - The second largest reduction 6.65-$\log_{10}$ was seen after 30-minute exposure on stainless steel, a 99.9999% reduction.

3. **Impact on surfaces**
   - 15-minute exposure on all coupons showed the greatest reduction of 3.84-$\log_{10}$
   - 30-minute exposure on the stainless-steel and plastic coupons showed the greatest reduction of 6.65-$\log_{10}$ followed by the floor tile coupons at 6.51-$\log_{10}$.
   - 45-minute exposure showed all coupons to be completely reduced, showing a 7.56-$\log_{10}$ reduction.

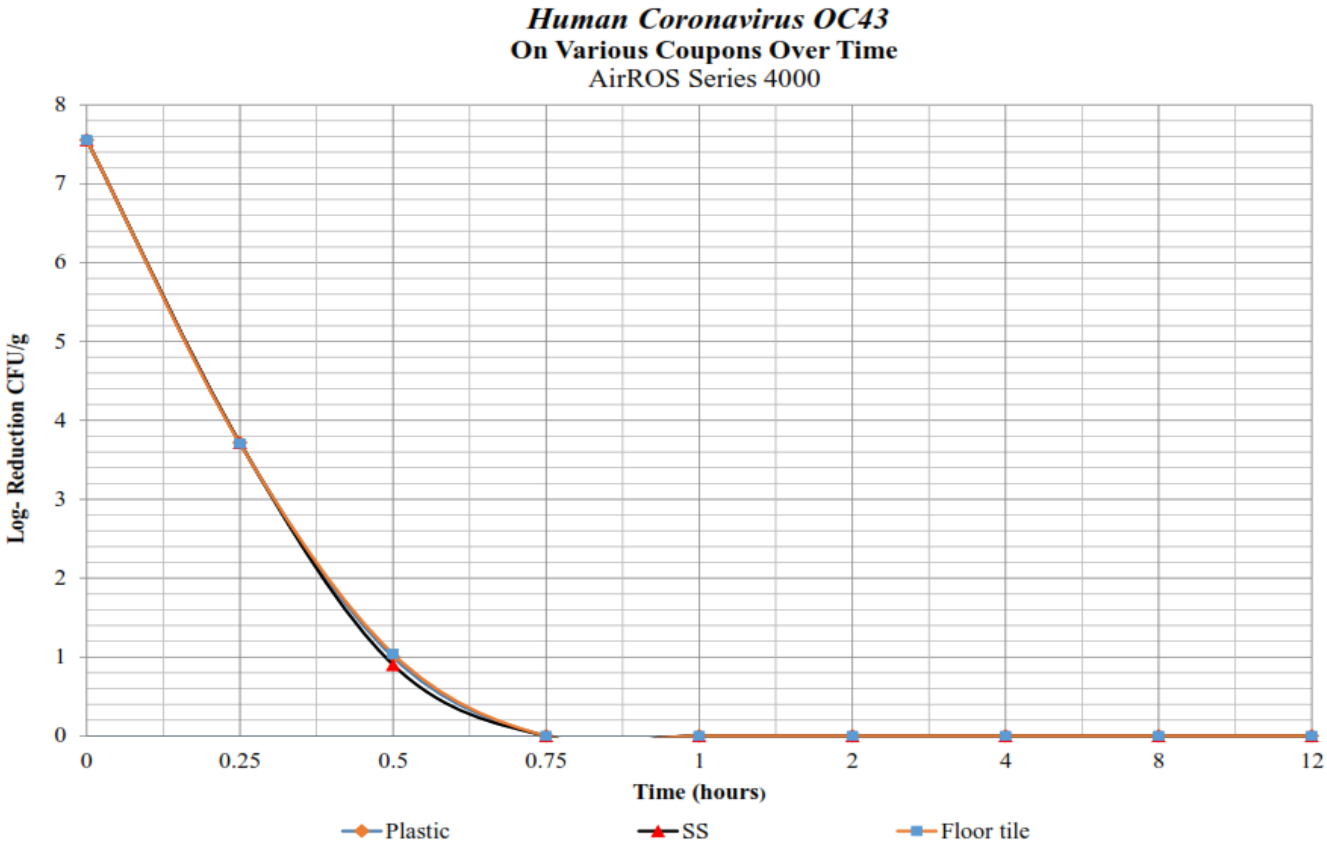
G. Conclusion

This study shows the substantial effect of the AirROS treatment in reducing HCoV OC43 viral cultures on three specific surfaces. The process carried out by this proprietary technology which inactivates viruses by breaking the protein envelope and inactivating the RNA strand. These results are explicit and indicate a strong correlation between AirROS treatment at the indicated concentrations and the stated log reductions of the virus on all surfaces tested.

TABLE 1. Human Coronavirus *OC43* populations following treatment with AirROS system.

	Plastic			Stainless Steel			Floor Tile		
Time	CFU/g Log_{10} CFU	Standard Deviation	Reduction	CFU/g Log_{10} CFU	Standard Deviation	Reduction	CFU/g Log_{10} CFU	Standard Deviation	Reduction
0	36,000,000 7.56	0.3	-	36,000,000 7.56	0.3	-	36,000,000 7.56	0.2	-
15-m	5,200 3.72	0.1	3.84	5,000 3.70	0.2	3.84	5,100 3.71	0.1	3.85
30-m	10 1.00	0.1	6.56	8 0.90	0.1	6.65	11 1.04	0.1	6.51
45-m	<1 0	0.1	7.56	<1 0	0.1	7.56	<1 0	0.1	7.56
1-h	<1 0	0.1	7.56	<1 0	0.1	7.56	<1 0	0.1	7.56
2-h	<1 0	0.1	7.56	<1 0	0.1	7.56	<1 0	0.1	7.56
4-h	<1 0	0.1	7.56	<1 0	0.1	7.56	<1 0	0.1	7.56
8-h	<1 0	0.1	7.56	<1 0	0.1	7.56	<1 0	0.1	7.56
12-h	<1 0	0.1	7.56	<1 0	0.1	7.56	<1 0	0.1	7.56

GRAPHIC REPRESENTATION OF RESULTS



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