Parma, 22th October 2020

Subject: VCT OZONE GENERATOR (O3-NEX OzonePRO) – Efficacy test against Coronavirus

The sanitization of premises and means of transport is of paramount importance in the fight against the Coronavirus, to stop the spread of COVID-19.

Numerous studies demonstrate the efficacy of ozone as a biocide, however, the Istituto Superiore di Sanità Italiano (the Italian National Institute of Health) in its ISS COVID-19 Report no. 56/2020 dated 23 July 2020, evaluating the situation, specifies that "no direct demonstrations of the efficacy against Sars-Cov2 obtained in controlled studies are available at the moment" and in its conclusions states that "further studies, carried out according to predefined standards, would be useful to define protocols for the effective and safe sanitization of premises/surfaces, so as to be able to evaluate essential parameters such as concentration and contact time".

MAHLE AFTERMARKET ITALY S.p.A.,

as manufacturer of the VCT OZONE GENERATOR device, has instructed a laboratory, accredited and authorized by the Italian Ministry of Health,to perform a specific evaluation, for its product VCT OZONE GENERATOR, to ascertain the disinfectant efficacy against the Sars-CoV-2 virus.

To avoid risks of spreading Sars-CoV-2 virus and infection to operators, the laboratory used the bovine coronavirus (BCoV), normally used as a surrogate for SARS-related viruses (e.g. SARSCoV or SARS-CoV-2) which is low pathogenic to humans, while SARS viruses are highly pathogenic, BCoV's resistance to chemical disinfection has been shown to be at least comparable to that of SARS virus, if not slightly higher.

The MAHLE VCT OZONE GENERATOR is equipped with automatic ozone treatments of different duration selectable by the user. As a further guarantee for the correct evaluation of the product's efficacy, it was chosen to perform the treatment with a shorter duration (**called P1**).

The evaluation methods applied by the laboratory refer to the following standards:

AFNOR NF T 72-281, 2014 – Methods of airborne disinfection of surfaces - Determination of bactericidal, yeasticidal, mycobactericidal, tuberculicidal, sporicidal and virucidal activity, including bacteriophages.

EN 17272:2020 / UNI EN 17272:2020 - Chemical disinfectants and antiseptics Methods of airborne room disinfection by automated process - determination of bactericidal, mycobactericidal, sporicidal, fungicidal, yeasticidal, virucidal and phagocidal activities.

Based on the results obtained, it has been verified that the P1 cycle performed by the MAHLE VCT OZONE GENERATOR causes a reduction in infectious activity of ≥ 99,683 %.

This result specifically confirms the effectiveness of the treatments carried out with the MAHLE VCT OZONE GENERATOR product and contributes in general to demonstrating the virucidal action of ozone against the Coronavirus.

The test report is attached.

CARLO ROCCHI
MANAGING DIRECTOR
MAHLE AFTERMARKET ITALY

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TITLE	**EVALUATION OF DISINFECTION EFFICACY OF VCT OZONE GENERATOR AGAINST *Bovine Coronavirus* – Surface virucidal activity**
SPONSOR	MAHLE AFTERMARKET ITALY S.P.A. VIA RUDOLF DIESEL 10/A 43122 PARMA ITALY
METHOD REFERENCE	AFNOR NF T 72-281, 2014 – Methods of airborne disinfection of surfaces - Determination of bactericidal, yeasticidal, mycobactericidal, tuberculicidal, sporicidal and virucidal activity, including bacteriophages. EN 17272:2020 / UNI EN 17272:2020 - Chemical disinfectants and antiseptics Methods of airborne room disinfection by automated process - determination of bactericidal, mycobactericidal, sporicidal, fungicidal, yeasticidal, virucidal and phagocidal activities.

EQUIPMENT AND PROCESS			
EQUIPMENT IDENTIFICATION (*)	VCT OZONE GENERATOR		
EQUIPMENT TYPOLOGY (*)	Ozone generator - Vehicles and room application		
MANUFACTURER (*)	MAHLE Aftermarket Italy S.p.A.		
MATERIAL ITEM ALIQUOT	LV-MAT-IJE2-20-211-0G25:a		
PARCEL REGISTRATION N.	IP-LV-2020190-AEJ	RECEIVING DATE	08-July-2020
(*) INFORMATION PROVIDED BY THE SPONSOR			
ANALYSIS STARTING DATE	02-Oct-2020	**ANALYSIS ENDING DATE**	07-Oct-2020

EXPERIMENTAL CONDITIONS	
NOTE	Bovine coronavirus (BCoV) is used as a surrogate virus for SARS-related viruses (eg. SARSCoV or SARS-CoV-2) as it is closely related to SARS viruses (including SARS-CoV-2) and it is low pathogenic to humans whilst SARS viruses are highly pathogenic BSL-3 high containment viruses. BCoV belongs to the same genus of Betacoronavirus as SARS viruses and showed similar susceptibility to WHO formulations in published studies. In fact, its resistance to chemical disinfection proved to be at least comparable to the one of SARS virus, if not slightly higher.
TESTED CYCLE	P1
DURATION	35 minutes
SURFACE	Cotton carriers of 4 cm diameter
TEST TEMPERATURE	Room Temperature (18°C-25°C)
INTERFERING SUBSTANCE	Bovine serum albumin (BSA) with a final concentration of 0.3 g/L (0.03% - simulating clean conditions).
TEST VIRUS	*Bovine Coronavirus (Betacoronavirus 1), strain S379 Riems* (RVB-0020)
CELL LINE	*PT* (CCLV-RIE 11)

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EXPERIMENTAL PROCEDURE (SUMMARY DESCRIPTION)	
TITRATION OF THE VIRUS SUSPENSION	The virus suspension showing concentration in about $1x10^7$ and $1x10^9$ $TCID_{50}$/ml (or sufficiently high to at least enable a titre reduction of 4 Log) was diluted by means serial dilutions 1:10 up to 10^{-8} with ice-cold maintenance Medium performed after filtration with the S400 HR columns MicroSpin™, starting from the virus stock suspension. Each dilution was placed six-fold, transferring 0.1 ml in 96 wells microtiter plates containing the cellular confluent monolayer (>90%) without any ice-cold maintenance Medium. In parallel at least 6 wells in the microplate did not receive the viral inoculum, but only ice-cold maintenance Medium and was used as control of cellular line. After 1 hour of incubation at 37±1°C, 0.1ml of ice-cold maintenance Medium was added. At the end of the required incubation period, the cellular culture was observed with inverted microscope to detect any cytopathic effect (CPE) due to viral suspension. After this detection the infecting activity ($TCID_{50}$ evaluation) was calculated by means of Spearman – Karber method.
PREPARATION OF THE INOCULUM SUSPENSION	Nine volumes of the test virus suspension were added to one volume of interfering substance. Just before use, the inoculum suspension was mixed.
INOCULUM OF THE TEST SURFACE	Each carrier was inoculated with 50 µl of inoculum suspension that was left to dry until visible drying at room temperature under the laminar air flow cabinet, for a maximum time of 1 hour.
VIRUS CONTROL TITRATION ON THE CARRIER (T)	Two carriers were inoculated and the virus recovery performed for each of the following time points: - T 1: virus recovery just after inoculum - T 2: virus recovery just after the drying step (time 0) - T 3: virus recovery after the scheduled cycle duration (maximum contact time) At the end of the set condition, the carriers were transferred into a container with 20 ml of ice-cold maintenance Medium. For each carrier, the inoculum was recovered by mixing. Starting from the recovered solution, eight serial dilutions 1:10 were performed with the ice-cold maintenance Medium. 0.1 ml of each dilution was transferred into a 96-wells microplate containing the cellular confluent monolayer (>90%) without any culture Medium. Each dilution was placed in six replicates. In parallel at least 6 wells in the microplate received only ice-cold maintenance Medium and were used as control of cellular line. After 1 hour of incubation at 37±1°C, 0.1 ml of ice-cold maintenance Medium was added. After the incubation period, the cellular culture was observed with inverted microscope to detect any cytopathic effect (CPE) due to viral suspension. After this detection, the infecting activity ($TCID_{50}$ evaluation) was calculated by means of Spearman – Karber method and the mean virus titration on the carriers (**T1 – T2 – T3**) for every set condition was calculated.

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TEST PROCEDURE	The test has been performed inside a 1 m^3 airlock chamber, and the ozone level was measured during the entire cycle. Three inoculated carriers were placed in vertical position on the opposite side of the disinfection device. Ozone detector was positioned inside the chamber, and then the chamber has been locked and the selected cycle started. After the end of the set cycle, the chamber has been opened and the forced ventilation was applied for few minutes to reduce the residual ozone level in the air, in order to enter in the room safely. Successively the test carriers were got back and transferred into a container with 20 ml of ice-cold maintenance Medium and the inoculum was recovered from the carrier by mixing. Starting from the recovered solution, eight serial dilutions 1:10 were performed with the ice-cold maintenance Medium. 0.1 ml of each dilution was transferred into a 96-wells microplate containing the cellular confluent monolayer (>90%) without any culture Medium. At least 6 wells in the microplate did not receive the viral inoculum but only culture Medium and were used as control of cellular line. After 1 hour of incubation at at 37±1°C, 0.1ml of ice-cold maintenance Medium was added. At the end of the required incubation period, the cellular culture was observed with inverted microscope to detect any cytopathic effect (CPE) due to viral suspension. After this detection, the mean infecting activity ($TCID_{50}$ evaluation) was calculated by means of Spearman – Karber method in the cellular culture treated with the test item.
VALIDITY CRITERIA	The test of virucidal activity is valid if the following criteria are fulfilled: *Assay of viral activity (virus titration)* The minimum titre of the virus suspensions is at least $1x10^7$ and $1x10^9$ $TCID_{50}$/ml; in any case, it shall be sufficiently high to at least enable a titre reduction of 4 Log to verify the method.
CALCULATION OF LOG REDUCTION	The reduction (*R*) was calculated as the difference between the Log titre of the virus control at the maximum contact time (a) and the Log titre of residual virus (rest virus) after exposure to the set cycle of the device (b). The reduction (*R*) was so calculated as follows: $R = a - b$ Where: RF = reduction factor from the test run a = Log $TCID_{50}$/ml of virus control titration on the carrier (T3) b = Log $TCID_{50}$/ml of rest virus titration at the end of the test run The corresponding % value of killing was calculated as follows: % kill = (Aa – Bb)/Aa *100 Where: Aa = antiLog $TCID_{50}$/ml of virus control titration on the carrier (T3) Bb = antiLog $TCID_{50}$/ml of rest virus titration at the end of the test run

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RESULTS	**Log reduction and % kill after the set cycle on the device VCT OZONE GENERATOR**		
	Cycle P1 - 35 min		
	Bovine Coronavirus, strain S379 Riems	≥ 2.50 ± 0.000	99,683%
	Maximum Ozone level measured	≥ 7.5 ppm	
	See Addenda N. 1-2		
CONCLUSIONS	On the basis of the obtained results and in compliance with Sponsor requirements, the cycle P1 performed by VCT OZONE GENERATOR **CAUSES a reduction ≥2.50±0.000 Log (99.683%)** against *Bovine Coronavirus (Betacoronavirus 1),* in the adopted test conditions, using bovine serum albumin (BSA) with a final concentration of 0.3 g/L (0.03% - simulating clean conditions). Accordingly to the standard reference, the log reduction is calculated against the non-treated control recovered after the set contact time. However, the tested virus shows a significant loss in viability due to its sensitivity to desiccation on textile surfaces. Considering the initial amount of virus dried on the test surface and not subjected to the entire holding time, it can be stated that the cycle P1 performed by VCT OZONE GENERATOR **CAUSES a reduction ≥ 3.92 ± 0.000 Log (99,987%)** against *Bovine Coronavirus (Betacoronavirus 1),* in the adopted test conditions, using bovine serum albumin (BSA) with a final concentration of 0.3 g/L (0.03% - simulating clean conditions).		
ADDENDA	N. 1: RAW DATA ELABORATION (*5 pages*) N. 2: OZONE LEVEL DETECTION (*12 pages*)		

Reviewed and electronically signed for Study Technical Supervisor Approval by
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