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TITLE	**EVALUATION OF DISINFECTION EFFICACY OF THE OZONE GENERATOR MACHINE *ELOZO H800* AGAINST *Bovine Coronavirus* – Surface virucidal activity**		
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METHOD REFERENCE	EN 16777:2018 / UNI EN 16777:2019 - Chemical disinfectants and antiseptics — Quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity of chemical disinfectants used in the medical area — Test method and requirements (phase 2/step 2)		
EQUIPMENT AND PROCESS			
EQUIPMENT IDENTIFICATION	Elozo H800		
EQUIPMENT TYPOLOGY	Ozone cleaning system		
MANUFACTURER	HTT-Group Oy		
MATERIAL ITEM ALIQUOT	LV-MAT-F5PH-20-302-0827:a		
PARCEL REGISTRATION N.	IP-LV-2020162-ADP	RECEIVING DATE	10-June-2020
ANALYSIS STARTING DATE	29-Oct-2020	**ANALYSIS ENDING DATE**	03-Nov-2020
EXPERIMENTAL CONDITIONS			
CYCLE	Customized cycle: Power level 6 - 124 minutes		
SURFACE	Stainless steel carriers with 2 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 10^8 $TCID_{50}$/ml (or sufficiently high to at least enable a titre reduction of 4 Log) was diluted by means serial dilutions 1:10 with maintenance Medium, starting from the virus stock suspension. Each dilution was placed six-fold, transferring 0.1 ml in 96 wells microplates containing the cellular confluent monolayer (>90%) without any culture Medium. After 1 hour of incubation at the indicated temperature, 0.1ml of maintenance Medium was added. The outline of the microplate did not receive the viral inoculum but only culture Medium and was used as control of cellular line. 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-Kärber 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.
PREPARATION OF THE TEST SURFACE	Each carrier was inoculated with 10 µl of inoculum suspension that was left to dry until visible dry at room temperature under the laminar air flow cabinet, for a maximum time of 1 hour.
TEST	Two carriers were inoculated with 10 µl of the inoculum suspension previously prepared. Immediately after drying, the inoculated carriers were positioned inside the machine with the inoculum placed upwards. The result was the mean value obtained by the two carriers. At the end of the set cycle, each test surface was transferred in a 6-well plate and the inoculum was recovered with 1 ml of ice-cold culture Medium to re-suspend the residual virus. Immediately after elution, eight serial dilutions 1:10 were prepared in ice-cold maintenance Medium and each dilution was placed six-fold, transferring 0.1 ml in 96 wells microplates containing the cellular confluent monolayer (>90%) without any culture Medium. After 1 hour of incubation at the indicated temperature, 0.1 ml of maintenance Medium was added. The outline of the microplate did not receive the viral inoculum but only culture Medium and was used as control of cellular line. At the end of the required incubation period, the cellular culture were observed with inverted microscope to detect any cytopathic effect (CPE) due to the residual virus and the corresponding $TCID_{50}$ was calculated by means of Spearman-Kärber method.
VIRUS CONTROL	Two carriers have been inoculated and dried, and the viral recovery has been performed immediately after the drying time, in order to determine the virus control at time 0. Other two carriers have been inoculated and dried but not subjected to the ozone cycle. The viral recovery has been performed after the treatment time provided by the cycle set on the device, in order to determine the virus control at the maximum contact time. For the viral recovery, each test surface was transferred in a 6-well plate and the inoculum was recovered with 1 ml of ice-cold culture Medium to re-suspend the residual virus. Immediately after elution, eight serial dilutions 1:10 were prepared in ice-cold maintenance Medium and each dilution was placed six-fold, transferring 0.1 ml in 96 wells microplates containing the cellular confluent monolayer (>90%) without any culture Medium. After 1 hour of incubation at the indicated temperature, 0.1 ml of maintenance Medium was added. The outline of the microplate did not receive the viral inoculum but only culture Medium and was used as control of cellular line. Each virus control was performed on two carriers and the result was the mean value. At the end of the required incubation period, the cellular culture were 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-Kärber method. The reduction of virus titre was calculated from titre differences between treated carriers and not-treated carriers (virus control at the maximum contact time).

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VALIDITY AND EFFICACY CRITERIA	In compliance with EN 16777:2018 / UNI EN 16777:2019, the minimum titre of the virus suspensions is at least 10^8 $TCID_{50}$/ml; in any case, it shall be sufficiently high to at least enable a titre reduction of 4 Log to verify the method. The test item is considered virucidal when, within 5 or 60 minutes of contact, it causes a reduction of viral titre of at least 4 Log compared to control virus when the test organisms are *Adenovirus Type 5* and *Murine norovirus (MNV, strain S99).* In case of specific use conditions for which other contact times, temperatures, test organisms and interfering substances are applied instead of or in addition to the standard ones, the test item shall demonstrate at least 4 Log reduction under the chosen test conditions

RESULTS	**Log reductions after the set cycle on the device Elozo H800**	
	Customized cycle: 124 min – power level 6	
	Bovine Coronavirus, strain S379 Riems	≥4.00±0.000
	Ozone level measured	See Addendum N. 2
	See Addenda N. 1-2	

CONCLUSIONS	On the basis of the obtained results in compliance with the assay validity criteria of EN 16777:2018 / UNI EN 16777:2019 and Sponsor requirements, the tested cycle performed by *Elozo H800* device is considered **EFFECTIVE** in reducing the titre of *Bovine Coronavirus* by at least 4 log, in the adopted test condition, 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 (*4 pages*) N. 2: OZONE LEVEL DETECTION (*27 pages*)

Reviewed and electronically signed for Study Technical Supervisor Approval by
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