Reference no. 20CN00179

STUDY OF THE "EINOVA MUNDUS PRO" MEDICAL DEVICE

CONTENTS

1 SCOPE AND FIELD OF APPLICATION

The purpose of this study was to test the efficacy of abatement of an active virus by means of treatment with the "EINOVA MUNDUS PRO" device whose active principle is based on a mixture of UV-C wavelengths combined with ozone and titanium dioxide. This treatment was applied to two different materials such as leather and plastic, artificially contaminated in the laboratory. The efficacy of the virucidal action of the EINOVA MUNDUS PRO device was tested after 8 minutes of treatment in the device (preset cycle) which had been heated in advance for 45 minutes.
The operating methods used in this study were agreed upon by the Laboratory with the contact persons at Eggtronic Engineering SpA, who had requested the experimentation in order to validate the deterioration of the virus after being placed on the materials in the device for the time spans specified by the client.
The target virus proposed and used for the laboratory experimentation was *Feline calicivirus* (ATCC VR782) which has an RNA genome, unsegmented and with positive polarity.
The study bases its principle on the comparison of recoveries of the target virus distributed on the surfaces of leather and plastic materials, untreated or treated for 8 minutes in the device after preheating for 45 minutes.
The test procedure was carried out at the Lachiver Alimenti laboratory located at in Via Liguria 26/28 - Sona (Verona) accredited pursuant to ACCREDIA No. 0403 L, in an ASALAIR EXCELSIOR biohazard hood with a volume of 0.38 m^3, under environmental conditions of 25±2°C temperature and 50±10% relative humidity.

2 MATERIALS AND METHODS

The study was carried out according to the principles envisaged in ISTISAN Reports 13/37 "Monitoring strategies of biological air pollution in indoor environment"; to complete the standard, the internal methods of this laboratory were applied.
For the tests of contamination, the active *Feline calicivirus* (FCV) ATCC VR-782 was chosen; this choice was motivated by the need to work in the laboratory with an RNA virus that was not dangerous for humans in its active form, but was similar - for infectiousness and symptomatology on the respiratory system of felines - to SARS-CoV-2 (currently available on the market only in disaggregate lysed form).

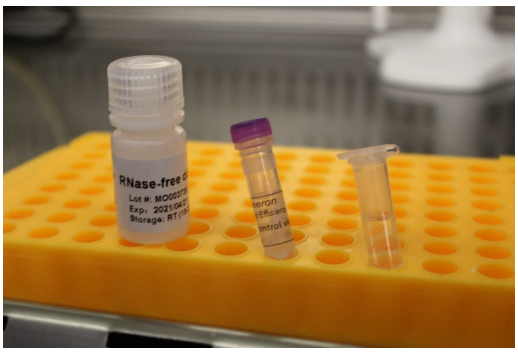
3 PROCEDURE

3.1 Preparation of the viral suspension in the laboratory

The certified material used for the test was the virus ATCC VR-782 VERYfinder FCV/IC RNA L.009-20 in a

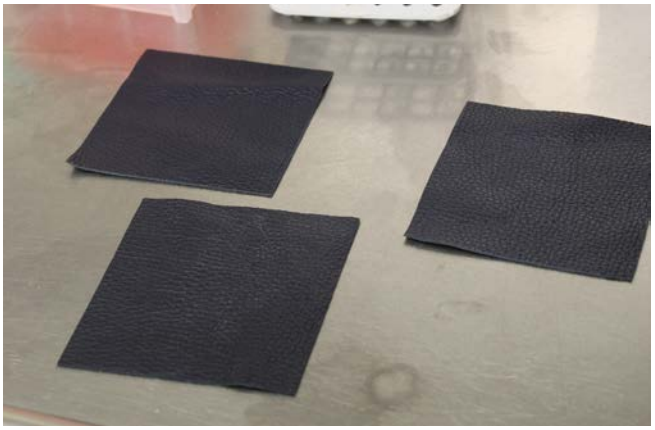
concentration of $1.04*10^4$ PFU/ml.

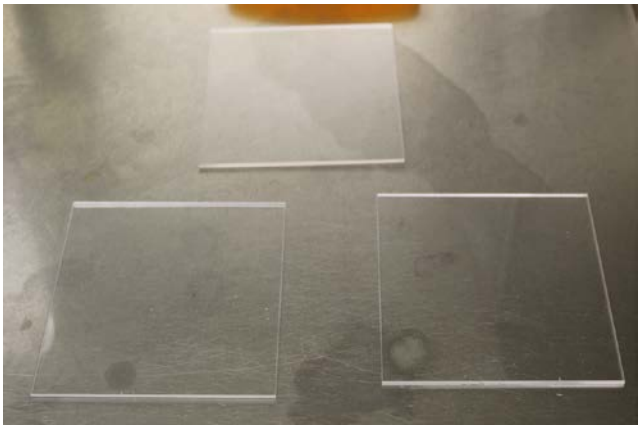
The suspension for contamination was prepared by diluting 330µl of initial suspension to 1000µl with RNase-free water. The dilution was calculated estimating an instrumental reading of 10% of the equivalent on the pure positive control.



3.2 Preparation of the surfaces prior to contamination

To prevent any interference on the materials used, supplied by Eggtronic Engineering SpA, the surfaces were treated with isopropyl alcohol and adequately dried by evaporation prior to contamination.



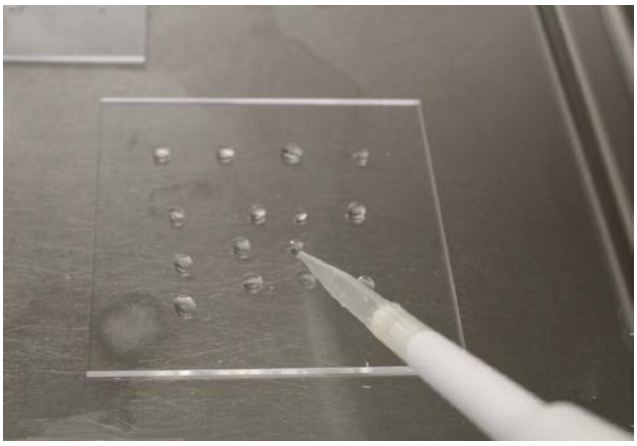


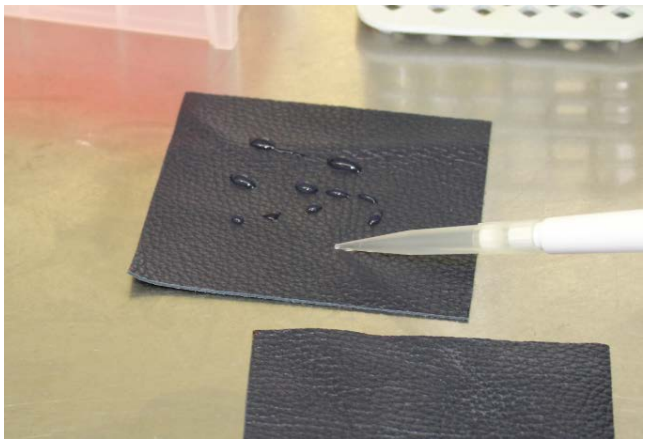
3.3 Preparation of the device

As agreed with Eggtronic Engineering SpA before proceeding with treatment of the contaminated materials, we provided to preheat the EINOVA MUNDUS PRO machine for 45 minute, that is, for five complete 8-minute cycles and one partial cycle of 5 minutes.

3.4 Contamination procedure

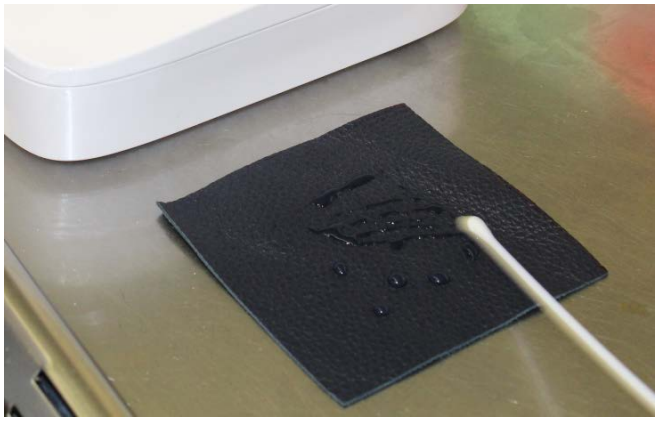
To verify the efficacy of the treatment for abatement of the viral load after 8 minutes, two samples of each of two test materials (including two contaminated surfaces not subjected to the treatment) were contaminated with identical volumes of viral suspension.

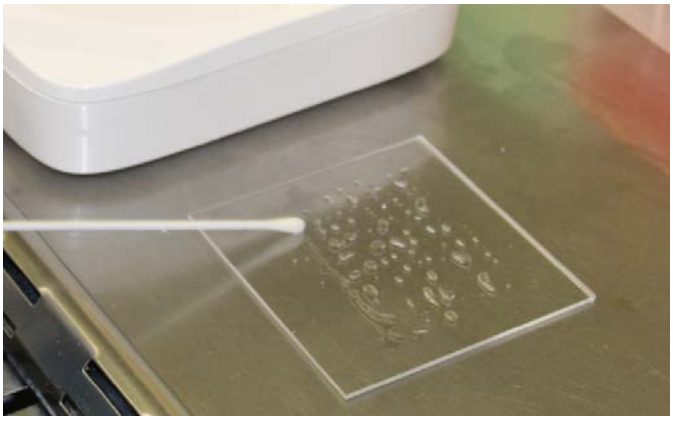




3.5 Treatment and sampling

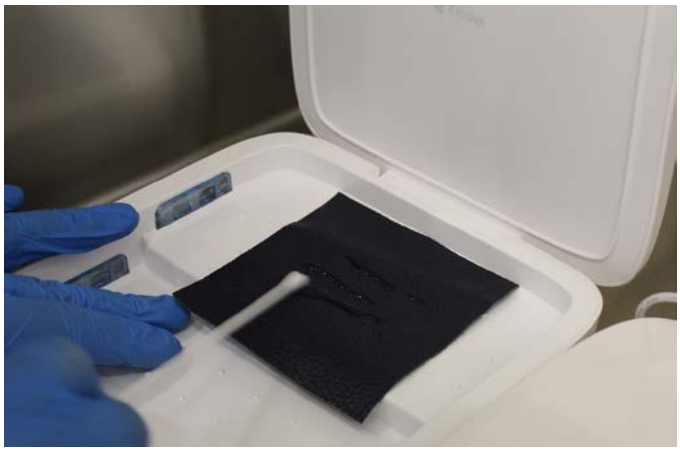
We proceed to the recover target virus on the contaminated and untreated samples, one of leather and one of plastic, using a pad moistened with RNase-free water.





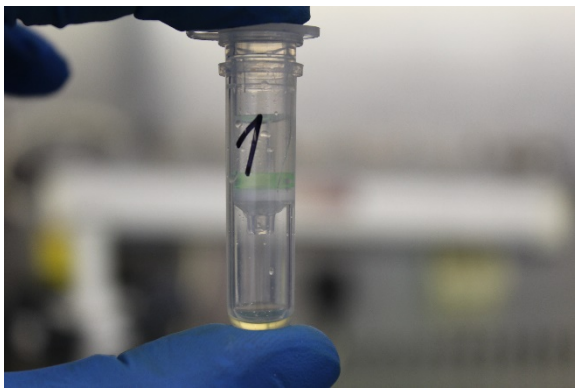
One material per type of each contaminated sample (leather and plastic) was placed in the EINOVA MUNDUS PRO for 8 minutes

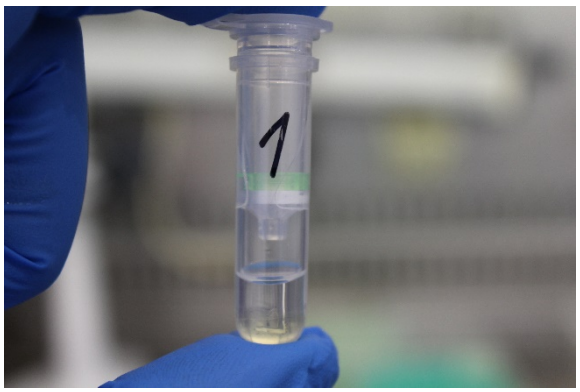

After 8 minutes of treatment, the two contaminated surfaces were sampled using pads moistened in RNase-free water.




3.6 Performance of the analysis

A portion of the liquid in which the pads used for recovery were soaked, to which the process control had been added, was purified and concentrated. This was done using solid state extraction columns, based on the principle that under certain conditions (polarity, pH, etc.) the nucleic acids bind to the compress material, making it possible, by means of solvents and centrifuging, to eliminate all that which is extraneous for the purpose of the analysis (see photos below of extraction columns). After that, by changing the conditions of the column, the target RNA is released in a given volume of eluent, to permit the subsequent steps.



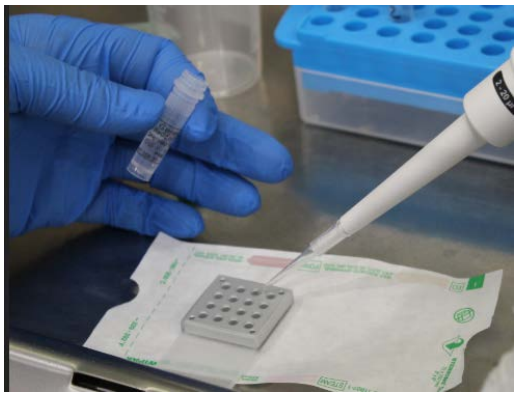


We proceed at that point to prepare the amplification plate in which, in addition to a liquid that acts as substrate (mastermix) we add aliquots of:

- positive control (target RNA at 100%, 10% and 1%) – Amplification check
- negative control – Exclusion of false positives
- process control (Intype IC-RNA at 100%) - Recovery check
- extract of samples – with the addition of the process control

The plate is placed in the thermal cycler (Hyris Bcube) which, according to a preset thermal profile, proceeds to amplify the target RNA and possible marker by a reading of the fluorescent signal.

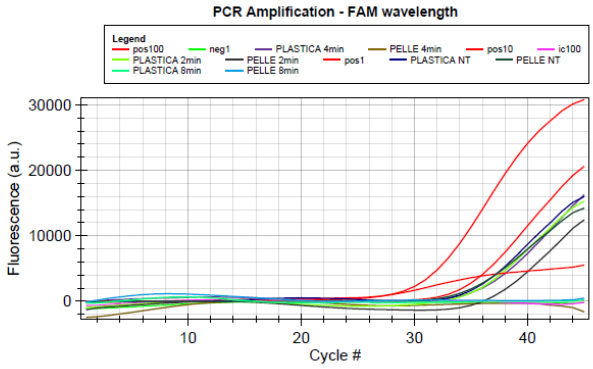
STEP		T (°C)	Duration	Cycles
Reverse transcription		55	10 min	1
Preheating		95	3 min	1
Amplification	Denaturing	95	15 sec	45
	Annealing/Extension + Reading	58	30 sec	



4 ANALYSIS OF RESULTS

As can be seen from the illustration below, relative to the FAM channel, the process came back positive on the three positive controls (pos100, pos10 and pos1) and on the two samples not treated with EINOVA MUNDUS PRO (NTplastic and NTleather), while both of the samples that were treated for 8 minutes (plastic 8min and leather 8min) do not exhibit a curve of amplification and therefore can be considered negative (no detectable target virus genetic material).

PCR Amplification



PCR Amplification	
pos100 (PosCtrl)	32.86
neg1 (NegCtrl)	N/A
PLASTICA 4min (Sample)	37.83
PELLE 4min (Sample)	N/A
pos10 (PosCtrl)	36.12
ic100 (Sample)	N/A
PLASTICA 2min (Sample)	36.67
PELLE 2min (Sample)	38.18
pos1 (PosCtrl)	38.46
PLASTICA NT (Sample)	36.47
PELLE NT (Sample)	36.33
PLASTICA 8min (Sample)	N/A
PELLE 8min (Sample)	N/A

The second illustration, relative to the HEX channel, displays the process controls of each sample which, compared with the value of Ic100 (recovery at 100%) report the recovery of each separate test. The laboratory considers a test to have been successful if it reports a recovery of 6.25% (4 cycles) or better.

The formula used to calculate recovery is as follows:

$Delta = C_t^{Unknown} - C_t^{IC\text{-}100}$ $\quad$ $Yield\ \% = 100 / 2^{Delta}$

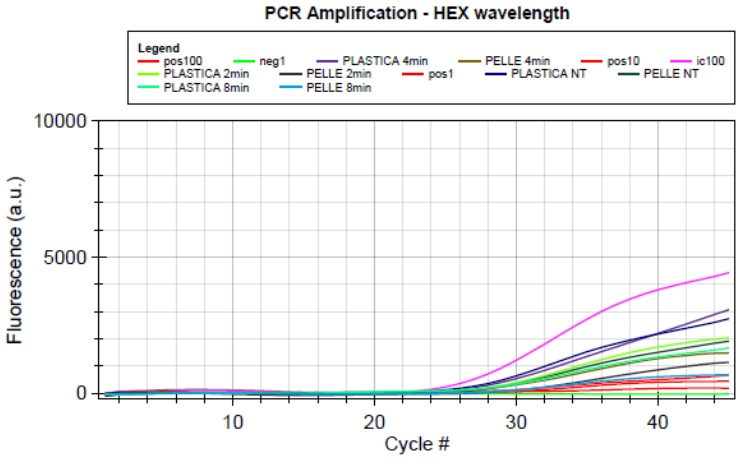
Recoveries obtained:

Sample – NTplastic: 64%

Sample – Plastic 8 min: 30%

Sample – NTleather: 30%

Sample – Leather 8 min: 28%



PCR Amplification	
pos100 (PosCtrl)	N/A
neg1 (NegCtrl)	N/A
PLASTICA 4min (Sample)	30.04
PELLE 4min (Sample)	29.55
pos10 (PosCtrl)	N/A
ic100 (Sample)	27.92
PLASTICA 2min (Sample)	29.58
PELLE 2min (Sample)	30.83
pos1 (PosCtrl)	N/A
PLASTICA NT (Sample)	28.57
PELLE NT (Sample)	29.68
PLASTICA 8min (Sample)	29.64
PELLE 8min (Sample)	29.76

Having ascertained that the positive controls, the negative control and the recovery control were successful, the results obtained for the study of the presence or absence of the virus on the two treated and untreated materials are to be considered reliable.

5 CONCLUSIONS

For both of the materials (plastic and leather) contaminated with the target virus *Feline calicivirus* (ATCC VR782), the tests carried out demonstrated that after treatment for 8 minutes in the Einova Mundus Pro

machine (preset cycle) we could find no recovery of the virus's genetic material (absence of *Feline calicivirus*).

6 Bibliography:

- PG 11 Rev. 26:2018 "Performance of laboratory test analyses"
- MPI107/M *Feline calicivirus* (FCV) research
- ISTISAN Report no. 13/37 "Monitoring strategies of biological air pollution in indoor environment"
- IO 11.01 Rev. 12:2017 "Sample Preparation"
- Microbiological contamination of surfaces in workplaces - INAIL 2017
- Microbiological monitoring in the workplace - Sampling and analysis – INAIL 2010
- ISS COVID-19 report no. 25/2020 "Interim recommendations on cleaning and disinfection of non-healthcare settings during COVID-19 health emergency: surfaces, indoor environments and clothing"
- VETfinder Real-Time RT-PCR kit for FCV + IC Detection assay – Rev 1 update of 01/07/2020
- DNA/RNA Patho Gene-spin Extraction Kit – rev.0 update of 07/04/2020

7 ENCLOSURES:

Test Report	20LA24529	MACHINE PREHEATED FOR 45 MINUTE CYCLE RUNNING 8 MINUTES
	20LA24529/1	PAD ON SURFACE OF PLASTIC MATERIAL
	20LA24529/2	PAD ON SURFACE OF LEATHER MATERIAL

Report Bcube "FCV test for 8 minutes with preheated machine"

Dr. Alma Carlotta Rui