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Research contract between the University of Modena and Reggio Emilia, Department of Surgical, Medical, Dental and Morphological Sciences with Transplant, Oncological and Regenerative Medicine Interest and the DEMOCENTER-Sipe Foundation of Modena

Evaluation of antiviral activity against AdV and HCoV-OC43 of a germicidal system based on UV radiation on a material (+ control) for a single exposure time and at a single distance)

FINAL REPORT

INTRODUCTION

The virus used in this study is the human Coronavirus HCov-OC43 which has an extremely high homology of structure with the virus responsible for CoViD-19, HCoV-SARS-2, both from a phylogenetic and a molecular point of view. In fact, they both belong to the β-Coronavirus group in an extremely close position in the phylogenetic tree. The homology is such that some antibodies, highly specific against HCoV-OC43 too, also recognize SARS-2. This indicates that proteins, which are the main constituent of the viral particle and determine its resistance, are extremely similar between the two viruses. Since germicidal treatments act with non-specific mechanisms, morphologically similar viruses respond to inactivation in a similar way. Therefore, HCoV-OC43 has been used in several studies on viral persistence / inactivation as a substitute for the highly pathogenic Coronaviruses SARS-1, SARS-2 and MERS. In fact, HCoV-OC43 can be more easily manipulated, not requiring a laboratory with a biosafety level of 3 but 2, as the UNIMORE laboratory is.

In addition, AdenoVirus-5 (AdV) has also been used in this study. AdV is a virus with a much higher resistance than that of HCoV-OC43 to the point that it is mandatory for certification tests of virucidal systems according to the UNI EN standards. AdV as well is transmitted both through saliva droplets and by contact with surfaces contaminated by respiratory secretions.

PURPOSE

The purpose of this investigation was to evaluate the efficacy of a germicidal treatment based on the emission of UVc radiation applied to a glass surface.

EXPERIMENTAL PROTOCOL

1) The treatment was applied to a glass slide

2) Viral contamination

Before each experiment, the slides were exposed to UVa light overnight to eliminate any microbial contamination that would have interfered with the results by preventing subsequent analyses.

An aliquot of 50µl of HCoV-OC43 viral suspension was deposited on 1x2 cm portion of the slide (title of the viral stock:$10^{4,5}$ $TCID_{50}$) or of AdV-5 (viral stock title: 10^{8} $TCID_{50}$). The slides were then left to dry for about 30 minutes and then subjected to UVc radiation for 1.5 seconds using the device supplied by the company. In parallel, a control sample equal to the one treated, was not subjected to treatment. At the end of the treatment, the residual virus was collected, both from the untreated control slide and from that subjected to UVc by rubbing the contaminated area for 1 minute with a swab which was then diluted in 1 ml of cell culture medium.

3) Titration of the viral load

The limit dilution method was used to titrate the virus present in the various samples. After vortex shaking, the supernatants of the samples contaminated with the virus were subjected to serial 10-fold dilutions up to 10^{-7}. One hundred µl of the undiluted supernatant and all dilutions were inoculated on HCoV-OC43 permissive HTC-8 cells in 96-well cell culture plates and allowed to incubate at 33° C for 11 days. Whereas, VERO cells were used for AdV-5. Cell cultures were examined daily under an inverted light optical microscope to rule out any microbial contamination. On the eleventh day for HCoV-OC43 and on the third day for AdV-5, every single well of cells was checked to evaluate the appearance of the typical signs of viral growth. The reciprocal number of the dilution factor of the last cell culture well in which this effect appeared (end point) was considered as the viral titer, i.e. the amount of virus recovered from the material, expressed as total cells infectious dose 50 ($TCID_{50}$). Each experiment was repeated twice, each sample tested in duplicate.

RISULTATI

Table 1 shows the results obtained with HCoV-OC43 while table 2 shows those with AdV-5. These tables reports: the initial title of the stock used (therefore the quantity of virus put in contact with the slide), that found on the surface of the control slide and the one on the treated slide. Titles are expressed as $TCID_{50}$. The reduction, calculated with respect to the untreated control, is expressed as Log.

Table 1

Results of viral titration of the residual virus on slides experimentally contaminated with HCoV-OC43

Initial Inoculum	**Ctrl slide**	**Treated slide**
$10^{4,5}$	$10^{2,5}$	Neg
Logarithmic reduction	2,5	

The values shown represent the average of the duplicates of two experiments

Table 2

Results of viral titration of the residual virus on slides experimentally contaminated with AdV-5

Initial Inoculum	**Ctrl slide**	**Treated slide**
10^{8}	$10^{4,25}$	10^{3}
Logarithmic reduction	1,25	

The values shown represent the average of the duplicates of two experiments

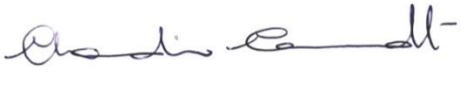
COMMENTS

Virucidal treatment resulted in a 2.5 log reduction in viral load for Coronavirus and 1.25 for Adenovirus. This difference between the two viruses was expected as Adenoviruses are extremely more resistant.

Chief of the Convention
Prof.ssa Elisabetta Blasi

Modena, July 27th 2020,

Trial Supervisor
Prof. Claudio Cermelli



Trial Manager
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