SIENA UNIVERSITY

Department of Medical Biotechnology

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Prato (PO) – Italy

REPORT ON VIRUCIDAL PHOTO ACTIVE AND PHOTO ACTIVE AG

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INTRODUCTION

This study aims to demonstrate the virucidal activity of Photo Active (TiO_2) and Photo Active Ag (TiO_2+Ag) against SARS-Cov-2. The samples of Photo Active and Photo Active Ag were incubated at different dilutions with SARS-Cov-2 for 30 minutes at room temperature. After filtration on Micro-Spin S400 HR column, the filtrate was used to infect Vero E6 cells, according to UNI EN14476 (luglio 2019).

Sample identification:

Sample 1 (TiO_2)

- name: PhotoACTIVE®
- Lot No.: 200709
- manufactory by:
- storage: room temperature

Sample 2 (TiO_2 Ag)

- name: PhotoACTIVE® Ag
- Lot No.: 200731
- manufactory by:
- storage: room temperature

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CYTOTOXICITY TEST

Virus-sensitive Vero E6 cells (ATCC CRL-1586) were inoculated (2x 10^5/ml) into a 96 well plate and incubated at 37°C, 5% CO_2.
After 24 hours, Photo Active and Photo Active Ag samples were diluted to 40% in D-MEM (SS) culture medium and 100 µl were filtrated in a column Micro-Spin S400 HR (2' at 2000 rpm). The filtered solution (100 µl)were diluted with 130 µl of D-MEM (SS) (1/2,3) and tested, distributing 50µl of each sample per well (in quadruplicate) in the 96-well plate containing Vero E6 cells. Then, 50 µl of D-MEM (SS) were added to each well.
The plate was observed every day under an optical microscope (10x). The cells were observed after 3 days for the presence of cytotoxic effect.

CYTOTOXICITY RESULTS

SAMPLE 1 CYTOTOXICITY RESULTS:

Sample 1: PhotoACTIVE®	
TiO2 diluted as described	No Toxic

SAMPLE 2 CYTOTOXICITY RESULTS:

Sample 2: PhotoACTIVE® Ag	
TiO2 diluted as described	No Toxic

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Virucidal Activity

Virus-susceptible Vero E6 cells (ATCC CRL-1586) were inoculated (2x 10^5/ml) in a 96 well plate and incubated at 37 °C, 5% CO_2.
After 24 hours, Photo Active and Photo Active Ag samples (40%) were tenfold diluted in D-MEM (SS) medium. Sixty µl of SARS-Cov-2 (2x10^6 $TICD_{50}$/vial) were added to 40µl of each product for 30 minutes at room temperature. For the virus control, the test product was replaced with the culture medium, and the virus was treated in the same way as the tested samples.
Samples were filtered in a Micro-Spin S400 HR column (2' at 2000 rpm), collected and diluted with 130 µl of D-MEM (SS) (1/2,3).
Virus titration was performed after treatment. Fifty µl of the dilutions were distributed (in quadruplicate) in a 96 well plate containing Vero E6 cells. Then 50 µl of D-MEM (SS) were added in each well.
The plate was observed daily under an optical microscope (10x). The final virus titre was evaluated after 3 days, when a cytopathic effect was evident for the virus control. The viral titre was calculated according to the formula of "Reed and Muench" (Reed LJ, Muench H. A simple method of estimating fifty per cent endpoints. Am J Hyg. 1938; 27:493-497). The test was performed in triplicate on the 10/09/2020, 10/20/2020 e 11/10/2020.

RESULTS OF VIRUCIDAL ACTIVITY

Sample 1: PhotoACTIVE	**Time**	**Virus sample after treatment**	**Dilution 10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**	**10^{-6}**	**10^{-7}**	**Title ($TCID_{50}$/ml)**
TiO_2 diluted to 40%	30'	+ + + -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	75% infectivity in the undiluted sample
K Virus	30'	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + - -	- - - -	- - - -	2 x 10^6

+ = presence of cytopathic effect in each well

Sample 2: PhotoACTIVE Ag	**Time**	**Virus sample after treatment**	**Dilution 10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**	**10^{-6}**	**10^{-7}**	**Title ($TCID_{50}$/ml)**
TiO_2 Ag diluted to 40%	30'	+ + - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	50% infectivity in the undiluted sample
K Virus	30'	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + - -	- - - -	- - - -	2 x 10^6

+ = presence of cytopathic effect in each well

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CONCLUSION

Photo Active Ag and Photo Active have similar virucidal activity against SARS-Cov-2, as shown in the table. The treatment with both the products has been effective against the virus starting from the 10^{-1} dilution.

The product is considered virucidal when, after the contact time, it causes a reduction of the $TCID_{50}$ titre of at least 4 log10 compared to the virus control.

MATERIAL AND REAGENT USED

- Sars-Cov-2 Vero E6 (2) (SARS-CoV-2 Siena-1/2020, Accession no. MT531537. (Stock 27/07/2020)
- D-MEM Dulbecco's Modified Eagle Medium, Gibco (cat. No. 41965-039, Lot 21 S6422, Exp. 2020-12-31)
- FBS (Fetal Bovine Serum) Euroclone (cat. No. ECS0180L, Lot EUS00AY, Exp. 04/2024)
- Heraeus CO_2 incubator (Hera cell240)
- 96-well platelets, Cyto One (Cat. No. CC7682-7596, Lot 1481802)
- Microspin S-400 HR Columns (cat n. 27511400 I, Lot 171 S0989, GE Healthcare) EXP: 1.10.2022

Operator: Chiara Terrosi

Chiara Terrosi

November 13.2020

Prof. Maria Grazia Cusi

Maria Grazia Cusi

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