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Translation from Bulgarian language into English language

Testing for virocidal action of an air disinfection module UV-V100 against human respiratory syncytial virus (HRSV-2) and human adenovirus (HAdV-5) in 60 minutes

PROTOCOL

Test was conducted in Department of Virology at the Institute of Microbiology "Stefan Angelov" of the Bulgarian Academy of Sciences
Test supervisor: Associate Professor, Dr. Ivanka Nikolova

Identification of the provided device:

Air disinfection module UV-V100

Serial number: 01464

Power: 15V

Manufacturer: Medical Education Technology

Test carried out on request of:

Samokontrol OOD, Plovdiv

Experimental conditions:

Test date: 12.04.2021 – 28.04.2021

Method for determination of residual viral infectivity: titration by virus-specific CPE

Test virus: HRSV-2 (human respiratory syncytial virus) and HAdV-5 (human adenovirus)

Test: Quantitative suspension test

2021
Sofia

Materials and methods

Cell culture

Cell line Hep-2 (permanent human cell line of throat carcinoma) from the collection of the Institute of Microbiology “Stefan Angelov”.

The cells are grinded once or twice a week in growth environment, consisting of DMEM (Dulbecco Modified Eagle’s Medium, DMEM, ATCC-30-2002) with 10% fatal veal serum (ATCC-30-2021), 10mM HEPES and antibiotics (100 UI/mL penicillin and 100 mg/mL streptomycin). Cell cultures were incubated at 37^0C and 5% CO_2.

Virus

Human adenovirus HAdV-5

A working viral suspension is prepared by culturing in cell culture HEp-2 and a supportive environment (DMEM with 0,5% fatal veal serum, 10mM HEPES and antibiotics) at 37^0C and 5% CO_2 for 48 hours until 90-100% deployment of the viral cytopathic effect (CPE) and subsequent freezing. The obtained viral suspension is released from cell debris by low speed centrifugation (120g/10 min) and distributed in aliquots, stored at -80^0C.

Human respiratory syncytial virus HRSV-2

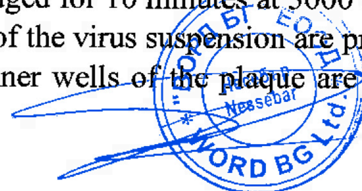
A working viral suspension is prepared by culturing in cell culture HEp-2 and a supportive environment (DMEM with 0,5% fatal veal serum, 10mM HEPES and antibiotics) at 37^0C and 5% CO_2 for 48-72 hours until 90-100% deployment of the viral cytopathic effect (CPE) and subsequent freezing. The obtained viral suspension is released from cell debris by low speed centrifugation (120g/10 min) and distributed in aliquots, stored at -80^0C.

Experimental staging

Air disinfection module UV-V100 Self-control is installed in a closed room with a total volume 24,75 m^3 (2,5 m x 3 m x 3,3 m), which is pre-sterilized by irradiation with a UV bactericidal lamp. The virus suspension is left in the closed room, in which air disinfection module UV-V100 operates. Samples were taken after 60 minutes, whereas the viral suspension is used for control, which is not exposed to the impact of the studied modular system.

Viral titration

Infectious viral titer is determined by the method of the final dilution and is read microscopically on 24th, 48th and 72nd hour. For titration are sown with HEp-2 cells in the inner 60 wells of 96-well plates and are incubated 24 hours before a complete monolayer is obtained. The corresponding virus stock solution was thawed and centrifuged for 10 minutes at 3000 rotations per minute directly before work. Successive ten-fold dilutions of the virus suspension are prepared of it, in a supportive environment. From each dilution in the inner wells of the plaque are instilled 0.1



mL/well, in the outer wells and control cells are instilled 0.1 mL/well supportive environment. After 1 hour (for adenovirus) and 2 hours (for respiratory syncytial virus) adsorption in the thermostat at 37^0C and 5% CO_2 the non-adsorbed virus is removed and to each well, including the control cell is added 0.1 mL supportive environment. In such a way, the treated cell cultures are incubated in a thermostat at 37^0C in the presence of 5% CO_2. The cytopathic effect, caused by viruses, is read on 24th, 48th and 72nd hour after infection. Viral titer is expressed as CCID50mL (cell culture infectious doses 50%).

Cytopathic effect is registered by the method of staining with the dye neutral red. Controls consist of cells that are incubated only with DMEM. After 48 hour (for adenovirus) and 72 hours (for respiratory syncytial virus) supportive environment is removed, the cells are washed and 0.1 mL/well supportive environment is added, containing 0.005% neutral red and the plates are incubated at 37^0C for 3 hours. After incubation, the dye is removed, the cells are washed with PBS and is added 0.15 mL/well extracting solution (1% vinegar acid, 49% ethanol, 50% distilled water). The optical density of each well is reported at wavelength 540 nm on ELISA reader. Cell survival is calculated as % relative to cell control by the formula: % cell survival = (number of live cells number of live cells in control) x 100.

Results

Titer of working virus suspension

HRSV-2 – 10^4CCID$_{50}$/0,1 mL

HAdV-5 – $10^{6,3}$CCID$_{50}$/0,1 mL

Viral titer of samples determined by serial dilutions in monolayer cell cultures Hep-2.

Table 1. *Virocidal activity at 60 minutes.*

Virus	Δlog 60 min
HRSV-2	0.2
HAdV-5	0

Conclusion

1. The results of the control sample show that the initial virus titers are respectively 104CCID50/0,1 mL for HRSV-2 and 106,3CCID50/0,1 mL for HAdV-5.

2. The results of samples taken after 60 minutes of exposure to the air disinfection module UV-V100 Self-control impact over HRSV-2 viral suspension show that the infectious viral titer is reduced by 0.2 logarithms (Δlg = 0,2), as compared with initial controls.

3. The results of samples taken after 60 minutes of exposure to the air disinfection module UV-V100 impact over HAdV-5 viral suspension show that no reduction in infectious viral titer was achieved compared to initial controls.

28.04.2021 Team leader: *signed by hand*

Sofia city /Associate Professor, Dr. Ivanka Nikolova/

Director of IMiBi: *signed by hand*

/Professor P. Petrova, Dr.BSc/

Seal: Bulgarian Academy of Science

Institute of Microbiology "Stefan Angelov"

The undersigned, Word BG EOOD, represented by Andrey Bryukhov, guarantees the accuracy of the translation from Bulgarian to English of the following document:

Testing protocol for virocidal action of an air disinfection module UV-V100.

Translation consists of 4(four) pages.