Dr. Markus Eickmann, Institut für Virologie, Hans-Meerwein-Str 2, 35043 Marburg

An
Bioclimatic GmbH
Im Niedernfeld 4
D-31542 Bad Nenndorf

Fachbereich Medizin
Institut für Virologie
Dr. Markus Eickmann

Tel.: 06421 2864315
Fax: 06421 2865482
E-Mail: eickmann@staff.uni-marburg.de

Anschrift: Hans-Meerwein-Straße 2
35043 Marburg
Web: www.uni-marburg.de/FB20/virologie
Az.:

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Expertise for the evidence of efficacy Of the Bioclimatic product series Viroxx Regarding the H1N1 Influenza virus (A/Hamburg/05/09)

The Air Sterilizer Viroxx manufactured by the company bioclimatic GmbH, D-31542 Bad Nenndorf was examined regarding the virus inhibiting effect to the Influenza virus H1N1 (Strain A/Hamburg/05/09). The efficiency of the UV-source of the Viroxx-unit was evaluated. The virus was exposed in a culture medium with 0.1% bovine Serum albumen (BSA) for 60 seconds, 1 second and 0.5 seconds to UV-radiation.

Recapitulatory it can be said that the UV-source of the Viroxx-unit manufactured by the company Bioclimatic, based on the product-given distance, already after 0.5 seconds shows inhibiting capacities to H1N1 Influenza viruses, whose infectivity by this treatment sank below the detection limit.

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Identification of the device:

Product identification	Viroxx
Manufacturer	Bioclimatic GmbH Im Niedernfeld 4 D-31542 Bad Nenndorf

Test conditions:

Period of testing	October to November 2009
Temperature during testing	20.0 +/- 0.5°C
Impact time of UV-Rays at device-given distance	60, 1, 0.5 seconds
Protein charge	0.2% BSA
Diluting agent	Minimum Essential Medium (MEM) (Company: GIBCO)
Virus Strain	Influenza Virus H1N1 (A/Hamburg/05/09)

Preparation of the Virus-suspension
For the preparation of the virus-suspension MDCK-cells were used, which were kept in 175cm² Roux-bottles (Nunc GmbH& co, Wiesbaden) with minimum essential medium (MEM), with additives of 10% fetal calf serum (FCS), sodium pyruvate, penicillin, streptomycin and glutamine. Cultivated and by 80% confluent MDCK-cells were infected with the influenza virus H1N1 and a multiplicity of infection (moi) of 0.1 in a 175m² cell culture bottle. The cells were inoculated with 10ml of a corresponding virus dilution and incubated in an incubator for one hour at 37°C (5%CO_2). Subsequent to this 30ml of MEM with additives of glutamine, penicillin, streptomycin, 1µg/ml TPCK-Trypsine and BSA with an end concentration of 0.2% were kept in the incubator at 37°C (5% CO_2) till the occurrence of a cytopathic effect. 72 hours after infection the supernatants were harvested. The virus containing supernatant was freed of cell debris by ten minutes of centrifugation at 3000 rounds per minute (rpm) in a Megafuge 1.0 R (Heraeus) and stored at 4°C.

Execution of the Inactivation test
The suspension of the test virus (80µl) was applied on a object holder and placed at the device given distance to the UV-source for 60, 1 or 0.5 seconds. The adherence of the short incubation times (1 and 0.5 seconds) was ensured by the release mechanism of a light exposure unit.
The virus control was treated analog to the product test solution. The test method proceeded at 20.0 +/- 2 °C. Subsequent to the UV irradiation a titration of the remaining infectiousness in steps of 10 with MEM wit penicillin, streptomycin, glutamine, BSA and trypsine was prepared immediately and a dilution series on a semi-confluent grown cell culture in 96-hole plate conducted. The incubation of the cell culture occurred at 37°C in the CO_2-Incubator with CO_2-concentration of 5%.

Determination of the infectiousness
The infectiousness was determined by a quantal test (end point titration): For this test 96-hole plates with confluent MDCK-cell layers were used. Four dents each were inoculated with 100µl of the dilution series. After this the cell culture plates were incubated at 37°C in a humid chamber in the CO_2-Incubator with a CO_2-concentration of 5% for three days. The cytopathic effects were documented by means of the analysis at the light optical microscope. Following the virus dilution was determined where 50% of the inoculated cultures of a dilution batch were infected ($TCID_{50}$/ml). The calculation of the $TCID_{50}$/ml was done by the Spearman-Kärber-method (BR. J. Psychol. 2(1908): 227-42, Arch. Exp. Path. Pharmak. 162 (1931): 480-87).

Calculation of the virus inhibiting effectiveness
The evaluation of the virus inhibiting effect occurred by calculation of the titer waste (Δ lg $TCID_{50}$) compared to the parallel done control titration respectively.

Result
Test of Bioclimatic Viroxx H1N1-inhibition with UV-source of the device

Parameters of the tests:
- Virus used: H1N1 (A/Hamburg/05/09) (H1N1)
- Dilution medium: MEM with 0.2% BSA

Test of reduction of the infectiousness after UV-irradiation

(a) Without UV-irradiation – 7,5 $TCID_{50}$/ml
(b) 60 seconds of UV-irradiation – no infectiousness
(c) 1 second of UV-irradiation – no infectiousness
(d) 0.5 seconds of UV-irradiation – no infectiousness

	10-1	10-2	10-3	10-4	10-5	10-6	10-7	10-8
(a)	X	X	X	X	X			
H1N1	X	X	X	X	X			
Without	X	X	X	X	X			
UV	X	X	X	X	X			

	10-1	10-2	10-3	10-4	10-5	10-6	10-7	10-8
(b)								
H1N1								
60 sec.								
UV								

	10-1	10-2	10-3	10-4	10-5	10-6	10-7	10-8
(c)								
H1N1								
1 sec.								
UV								

	10-1	10-2	10-3	10-4	10-5	10-6	10-7	10-8
(d)								
H1N1								
0.5 sec.								
UV								