Public Health Institute Ostrava
Centre of Clinical Laboratories
Location 1 - Ostrava
Partyzánské náměstí 2633/7, Moravská Ostrava, 702 00 Ostrava
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L 1554

TEST REPORT n. 71/2022/V

Customer:
Vzduchotechnik, s.r.o.
Tovární ulice 548
463 31 Chrastava

Order number: 34835
Date of delivery: 16.11.2022
Reference number: ZU/34835/2022

Identification of sample:

Number of sample:	**71/2022**
Name of sample [i]:	Microbicidal and virucidal nanofiber filter membrane type XC130
Manufacturer [i]:	Vzduchotechnik, s.r.o.
Batch number [i]:	/
Manufacturing date/ Expiry date [i]:	/
Sample composition [i]:	nanofiber polymer membrane, functionalized with a natural, harmless substance
Storage conditions [i]:	refrigerator (no light)
Appearance of the sample:	grey coloured fabric
Date of delivery:	3.2.2023
Test method:	**ČSN ISO 18184:2020**
Date(s) of tests (period of analysis):	6.2.2023 – 16.2.2023
Location of tests:	Location 1 - Ostrava

[i] Data provided by customer.

Test procedure:

Test method:	ČSN ISO 18184:2020 Textiles – Determination of antiviral aktivity of textile products

Tested virus strain:	*Vaccinia virus, strain Modified Vaccinia virus Ankara (ATCC-VR-1508)*
Cell line:	BHK-21 (ATCC-CCL-10)
Growth medium:	DMEM + 10% FBS
Maintenance medium:	EMEM + 2% FBS

Neutralizer:	maintenance medium
Control material:	nanofiber filter membrane supplied by the customer
Method of sterilization:	samples and control material delivered sterile
Sample size:	0,200 ± 0,025 g
Volume of viral suspension:	100 µl
Volume of neutralizer:	10 ml
Special light source:	ordinary laboratory lighting
Contact time(s):	1 hour, 2 hours, 24 hours
Testing temperature:	25 °C ± 1 °C
Incubation temperature:	37 °C ± 1 °C
Method of filtration:	not used
Titration method:	viral titration on monolayer cell culture on the microplates
Titers calculated by:	Spaerman – Kärber´s method

Description of the test:

Under aseptic conditions, samples of control material and test material weighing 0,200 ± 0,025 g were prepared. The samples were transferred to sterile tubes. A given volume of viral suspension was applied to both control and tested samples. The samples were cultured at 25 °C ± 1 °C and a relative humidity of 90 %. After 24 hours, 2 hours and 1 hour of incubation, a given amount of neutralising solution was added to each sample and the samples were shaken on a vortex. After that, a series of tenfold dilutions of this mixture was prepared. Each dilution was titrated on a single-layer cell culture on a microtitre plate.

Three samples of both control and test material were used to verify the cytotoxicity of the material. A volume of neutralising medium corresponding to the viral suspension on the previous samples was applied to the samples. The samples were incubated at 25 °C ± 1 °C and a relative humidity of 90 %. After a contact period of 24 hours, 2 hours and 1 hour, a given amount of neutralising solution was added to each sample and the samples were shaken vigorously on the vortex. After that, a series of tenfold dilutions of this mixture was prepared. Each dilution was titrated on a single-layer cell culture on a microtitre plate.

Three samples of both control and test material were used to check the effectiveness of suppression of the activity of the test sample. A given amount of maintenance medium was applied to the samples. Immediately after application, a given quantity of neutralising solution was added to each sample and the samples were shaken vigorously. 50 µl of viral suspension was added to 5 ml of this suspension. After 30 minutes of incubation, a series of tenfold dilutions of this mixture is prepared. After 30 minutes of incubation, a series of tenfold dilutions of this mixture is prepared. Each dilution was titrated on a single-layer cell culture on a microtitre plate.

The virus titer values of all tests were determine 6th day of cultivation and calculated according to the Spaerman-Kärber method. The titer value is expressed as lg $TCID_{50}$ - negative logarithm of 50% end point (50% infectious dose of virus suspension or such dilution of virus suspension that induces CPE in 50% of cell culture units).

Test results:

The test results are shown in the tables. These results are the average of three determinations of each sample and are expressed as the average of the calculated logarithms of the virus titres. The value of antiviral activity (M_V) is given in logarithms, it is expressed as the difference between the virus titre on the test sample (V_C) and the virus titre on the control (V_a).

$M_V = lg(V_a) - lg(V_C)$

Table n.1: The results for Vaccinia virus, strain Modified Vaccinia virus Ankara

Sample	Level of cytotoxicity	lg $TCID_{50}$/ml 1 hour	lg $TCID_{50}$/ml 2 hours	lg $TCID_{50}$/ml 24 hours	Value of antiviral activity (M_V) 1 hour	Value of antiviral activity (M_V) 2 hours	Value of antiviral activity (M_V) 24 hours
Microbicidal and virucidal nanofiber filter membrane XC130 (V_C)	1,20	4,49	3,78	1,49	1,21	1,92	3,50
Control (V_a)	1,20	5,70	5,70	4,99			

Table n.2: Verificacion of cell sensitivity to virus and the inactivation of antiviral activity for Vaccinia virus, strain Modified Vaccinia virus Ankara

lg $TCID_{50}$/ml Microbicidal and virucidal nanofiber filter membrane XC130	lg $TCID_{50}$/ml Control material	Δ g $TCID_{50}$/ml	Control valid (Δ < 1)
5,96	5,96	0,00	Yes

Table n.3: Control of test homogenity

Contact time	lg $TCID_{50}$/ml max	lg $TCID_{50}$/ml min	lg $TCID_{50}$/ml mean	max – min / mean	Control valid (≤0,2)
0 hour	6,07	5,45	5,74	0,11	Yes
1 hour	5,82	5,57	5,70	0,04	Yes
2 hours	5,82	5,57	5,70	0,04	Yes
24 hours	5,32	4,82	4,99	0,10	Yes

All conditions for a valid test were satisfied.
Prepared by: Mgr. Markéta Tietzová

Conclusion:

According to the ČSN ISO 18184:2020 - contact of tested sample **71/2022** of the material **Microbicidal and virucidal nanofiber filter membrane XC130** with virus *Vaccinia virus, strain Modified Vaccinia virus Ankara* caused:

- Reduction of virus titer by 1,21 lg after contact time 1 hour
- Reduction of virus titer by 1,92 lg after contact time 2 hours
- Reduction of virus titer by 3,50 lg after contact time 24 hours

By testing according to the ČSN ISO 18184:2020, the sample **71/2022** of the material **Microbicidal and virucidal nanofiber filter membrane XC130** demonstrate excellent antiviral effect against the tested virus *Vaccinia virus, strain Modified Vaccinia virus Ankara* after 24 hours of contact.

Conclusion prepared by: Mgr. Markéta Tietzová

In Ostrava 7.4.2023

Zdravotní ústav se sídlem v Ostravě
Centrum klinických laboratoří
Oddělení virologie
Laboratoř pro testování virucidního účinku
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Authorized by: Mgr. Markéta Tietzová
Laboratory for testing virucidal activity

END OF THE PROTOCOL