Public Heath Institute Ostrava
Centre of Clinical Laboratories
Location 1 - Ostrava
Laboratory for testing virucidal activity
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L 1554

TEST REPORT n. 68/2021/SVU

Quantitative suspension test for the evaluation of virucidal activity of disinfectants
Test method and requirements (phase 2/ step 1) according to CSN EN 14476+A2: 2020

Customer:
RETECH, s.r.o.
Vackova 1541/4
155 00 Praha 5 - Stodůlky

Order number: not provided
Date of delivery: 21.10.2021
Reference number: ZU/30184/2021

Identification of disinfectant – sample:

Name of the product [i]:	**ULTRASONIC CLEANING SOLUTION**
Batch number [i]:	not provided
Expiry date [i]:	24 months from manufacturing date
Manufacturing date [i]:	not provided
Storage conditions [i]:	+5 to +30 °C
Product diluent recommended by the manufacturer [i]:	ready to use
Active substance(s) and concentration(s) [i]:	ethanol: 0,558g Quaternary ammonium compounds, benzyl-C12-16-alkyldimethyl, chlorides: 0,5 g Didecyl(dimethyl)ammonium-chloride: 0,125 g
Purpose of product [i]:	PT2 - surface disinfection outside medical area and professional use
Appearance of the product:	clear colourless liquid
Date of delivery:	21.10.2020
Date(s) of tests (period of analysis):	8.11. – 22.11.2021

[i] Data provided by customer.

Results - for details see annex:

According to CSN EN 14476+A2:2020 the test product **ULTRASONIC CLEANING SOLUTION**, lot. n. not provided, designed for surface disinfection, nondiluted (really tested concentration 80%), reduced virus titre 4,000± 0,000 lg after an exposure time 45 min at temperature 20°C±1°C, under dirty conditions (3,0 g/l Bovine serum albumin), using viral titration on monolayer cell culture on a microtitre plate by reduction of reference virus *Vaccinia virus, strain Modified Vaccinia virus Ankara,* **i.e. demonstrated virucidal activity to *Vacciniavirus* by more than 4 lg.***

**The statement of compliance is based on a 95% coverage probability for the expanded uncertainty.*

Conclusion and interpretation:

According to CSN EN 14476+A2:2020 the test product **ULTRASONIC CLEANING SOLUTION**, lot. n. not provided, designed for surface disinfection, nondiluted (really tested concentration 80%), demonstrated virucidal activity to enveloped viruses under the dirty conditions after exposure time 45 min.

In Ostrava, 22.12.2021

Authorized by: Mgr. Ludmila Porubová

Guarantor of testing

Annex to the protocol n.: 68/2021/SVU

Identification of product:

Name of the product [i]:	**ULTRASONIC CLEANING SOLUTION**
Batch number [i]:	not provided
Expiry date [i]:	18.2.2023
Manufacturing date [i]:	24 months from manufacturing date
Date of delivery:	21.10.2021
Storage conditions [i]:	5 to 30 °C
Product diluent recommended by the manufacturer [i]:	ready to use
Appearance of the product:	clear colourless liquid
Active substance(s) and concentration(s) [i]:	ethanol: 0,558g Quaternary ammonium compounds, benzyl-C12-16-alkyldimethyl, chlorides: 0,5 g Didecyl(dimethyl)ammonium-chloride: 0,125 g
Purpose of product [i]:	PT2 - surface disinfection outside medical area and professional use
Experimental conditions:	Quantitative suspension test for the evaluation of virucidal activity of disinfectants according to CSN EN 14476+A2:2020 (SOP n. 1901)
Date(s) of tests (period of analysis):	8.11. – 22.11.2021
Diluent:	ready to use, distilled water
Testing concentration [i]:	100% (really tested concentration 80%)
Other testing concentration:	50%, 10%
Appearance of dilution of the product:	clear liquid
Contact times [i]:	45 min
Testing temperature [i]:	20 °C±1 °C
Interfering substance [i]:	dirty conditions – 3,0 g/l Bovine serum albumin
Stability of mixture during testing:	stable
Incubation temperature:	37°C±1°C
Method of filtration:	MicroSpin
Test virus:	*Vaccinia virus, strain Modified Vaccinia virus Ankara* (ATCC), 6. passage, EMEM + 2% FBS
Cell line:	BHK-21 cells (ATCC), 14., 26. passage, DMEM + 10% FBS
Process to stop action of product:	virucidal activity of product is suppressed by transferring the sample into the ice cold diluent
Titration method:	viral titration on monolayer cell culture on the microplates
Reference substance:	Formaldehyde (Sigma-Aldrich, lot. n. MKCH0868)
Titers calculated by:	Spaerman – Kärber‘s method

[i] Data provided by customer

Test detail:

1. Preparation of tissue culture testing
2. Preparation of the test virus suspension
3. Test infectivity of the virus
4. Titration of the virus with the conditions
5. The cytotoxic effect of the product
6. Reference viral inactivation test
7. Viral inactivation test of product
8. Control of susceptibility

Table n.1 The results and validation of the test for product ULTRASONIC CLEANING SOLUTION to *Vaccinia virus, strain Modified Vaccinia virus Ankara - dirty conditions*

Product	Concentration	Interfering substance	Level of cytotoxicity	log 10 TCID50/ ml after … min				Reduction factor (Δlog_{10} $TCID_{50}$/ ml after … min)	
				5	30	45	60	45	
ULTRASONIC CLEANING SOLUTION	100%*	3 g/l BSA	4,5	n.d.	n.d.	4,500 ± 0,000	n.d.	2,000 ± 0,000	
ULTRASONIC CLEANING SOLUTION - MicroSpin	100%*	3 g/l BSA	2,5	n.d.	n.d.	2,500 ± 0,000	n.d.	4,000 ± 0,000	
ULTRASONIC CLEANING SOLUTION	50%	3 g/l BSA	4,5	n.d.	n.d.	4,500 ± 0,000	n.d.	2,000 ± 0,000	
ULTRASONIC CLEANING SOLUTION - MicroSpin	50%	3 g/l BSA	2,5	n.d.	n.d.	2,500 ± 0,000	n.d.	4,000 ± 0,000	
ULTRASONIC CLEANING SOLUTION	10%	3 g/l BSA	3,5	n.d.	n.d.	3,500 ± 0,000	n.d.	3,000 ± 0,000	
ULTRASONIC CLEANING SOLUTION - MicroSpin	10%	3 g/l BSA	2,5	n.d.	n.d.	2,500 ± 0,000	n.d.	4,000 ± 0,000	
Virus control	n.a.	3 g/l BSA	n.a.	n.d.	n.d.	6,000 ± 0,000	n.d.		
Virus control - MicroSpin	n.a.	3 g/l BSA	n.a.	n.d.	n.d.	6,000 ± 0,000	n.d.		
				5	15	30	60	5	15
Formaldehyde - MicroSpin	0,7% (m/V)	PBS	3,5	≤3,500 ± 0,000	≤3,500 ± 0,000	n.d.	n.d.	2,833 ± 0,282	2,833 ± 0,282
Virus control - MicroSpin	n.a.	PBS	n.a.	6,333 ± 0,141	n.d.	n.d.	n.d.		

*Product is not possible to test concentrated, due to dilution by adding of interference substance and virus suspension. The final test concentration of product was 80%.

Prepared by: Mgr. Ludmila Porubová

Table n.2 Raw date of test for product ULTRASONIC CLEANING SOLUTION to *Vaccinia virus, strain Modified Vaccinia virus Ankara - dirty conditions*

Product	Concentration	Interfering substance	Contact time	Dilution (log 10)						
				-1	-2	-3	-4	-5	-6	-7
ULTRASONIC CLEANING SOLUTION	100%*	3 g/l BSA	45 min	CT	CT	CT	000000	000000	000000	000000
ULTRASONIC CLEANING SOLUTION - MicroSpin	100%*	3 g/l BSA	45 min	CT	000000	000000	000000	000000	000000	000000
ULTRASONIC CLEANING SOLUTION	50%	3 g/l BSA	45 min	CT	CT	CT	000000	000000	000000	000000
ULTRASONIC CLEANING SOLUTION - MicroSpin	50%	3 g/l BSA	45 min	CT	000000	000000	000000	000000	000000	000000
ULTRASONIC CLEANING SOLUTION	10%	3 g/l BSA	45 min	CT	CT	000000	000000	000000	000000	000000
ULTRASONIC CLEANING SOLUTION - MicroSpin	10%	3 g/l BSA	45 min	CT	000000	000000	000000	000000	000000	000000
cytotoxicity ULTRASONIC CLEANING SOLUTION -	100%*	3 g/l BSA	n.a.	CT	CT	CT	n.d.	n.d.	n.d.	n.d.
cytotoxicity ULTRASONIC CLEANING SOLUTION - MicroSpin	100%*	3 g/l BSA	n.a.	CT	000000	000000	n.d.	n.d.	n.d.	n.d.
cytotoxicity ULTRASONIC CLEANING SOLUTION	50%	3 g/l BSA	n.a.	CT	CT	CT	n.d.	n.d.	n.d.	n.d.
cytotoxicity ULTRASONIC CLEANING SOLUTION - MicroSpin	50%	3 g/l BSA	n.a.	CT	000000	000000	n.d.	n.d.	n.d.	n.d.
cytotoxicity ULTRASONIC CLEANING SOLUTION	10%	3 g/l BSA	n.a.	CT	CT	000000	n.d.	n.d.	n.d.	n.d.
cytotoxicity ULTRASONIC CLEANING SOLUTION - MicroSpin	10%	3 g/l BSA	n.a.	CT	000000	000000	n.d.	n.d.	n.d.	n.d.
Virus control	n.a.	3 g/l BSA	45 min	444444	444444	444444	444444	323322	000000	000000
Virus control - MicroSpin	n.a.	3 g/l BSA	45 min	444444	444444	444444	444444	333332	000000	000000
Cytotoxicity Formaldehyde - MicroSpin	0,7% (m/V)	PBS	n.a.	CT	CT	000000	n.d.	n.d.	n.d.	n.d.
Formaldehyde - MicroSpin	0,7% (m/V)	PBS	5 min	CT	CT	000000	000000	000000	000000	000000
			15 min	CT	CT	000000	000000	000000	000000	000000
Virus control - MicroSpin	n.a.	PBS	5 min	444444	444444	444444	444444	433220	000000	000000

*Product is not possible to test concentrated, due to dilution by adding of interference substance and virus suspension. The final test concentration of product was 80%.

1 to 4 virus detectable (1 = 25% CPE, 4 = 100% CPE)
0 no virus/ no cytotoxicity
n.a. not applicable
n.d. not done
CT Cytotoxicologic effect
CPE Cytopathogenic effect

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END OF THE PROTOCOL