Public Heath Institute Ostrava
Center of clinical laboratories
Workplace 1 - Ostrava
Laboratory for testing virucidal activity
Partyzánské nám.2633/7, Moravská Ostrava, 702 00 Ostrava
VAT: CZ71009396

L 1554

TEST REPORT n. 73/2020/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:
St. Crux Pharma, s.r.o.
053 22 Odorín 115
Slovakia

Order number: not specified
Reference number: ZU/12532/2020

Identification of desinfectant – sample:

Product name:	**HYG-DEZ ST. CRUX**
Lot number:	01 03 04 20
Expiration:	04/2022
Producer:	St. Crux Pharma, s.r.o.
Storage conditions:	cool place, out of heat
Diluent product recommended by the producer for use:	ready to use
Product appearance:	clear colourless liquid
The active substance(s) and its (their) concentration:	Ethanol 80% Hydrogen peroxide 0,125%
Purpose of product:	hygienic handrub
Date of delivery of the product:	21.5.2020
Date of test:	25.5. – 11.6.2020

Results - for details see annex:

According to CSN EN 14476+A2:2020 the test product **HYG-DEZ ST. CRUX** , lot.n. 01 03 04 20, designed for hygienic handrub, non diluted (real tested concetration 80%) reduced virus titre 4,000 ± 0,000 lg after an exposure time 30 s at temperature 20°C±1°C, under dirty conditions (3,0 g/l Bovine serum albumin + 3,0 ml erythrocytes), 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 **HYG-DEZ ST. CRUX**, lot.n. 01 03 04 20, designed for hygienic handrub, non diluted (real tested concetration 80%), demonstrated virucidal activity to enveloped viruses under the dirty conditions after exposure time 30 s.

In Ostrava, 15.6.2020

Authorized by: Mgr. Ludmila Porubová

Guarantor of testing

Annex to the protocol n.: 73/2020/SVU

Identification of product:	
Product name:	**HYG-DEZ ST. CRUX**
Lot number:	01 03 04 20
Expiratin:	04/2022
Producer:	St. Crux Pharma, s.r.o.
Date of delivery of product	21.5.2020
Storage conditions:	cool place, out of heat
Diluent:	ready to use
Product appearance:	clear colourless liquid
The active compound(s) and its (their) concentration:	Ethanol 80% Hydrogen peroxide 0,125%
Experimental conditions:	Quantitative suspension test for the evaluation of virucidal activity of disinfectants according to CSN EN 14476+A2:2020 (SOP n. 1901) /ACTS /ACTS: actualization of standard
Date of testing:	25.5. – 11.6.2020
Diluent:	ready to use, water
Testing concentration:	100% (real tested concentration 80%)
Other testing concetration:	50%, 10%
Appearance of dilution of the product:	clear solution
Contact times:	30 s
Testing temperature:	20 °C±1 °C
Interfering substance:	dirty conditions – 3,0 g/l Bovine serum albumin + 3,0 ml erythrocytes
Stability of mixture during testing:	precipitate formation
Incubation temperature:	37°C±1°C
Method of filtration:	MicroSpin
Test virus:	*Vaccinia virus, strain Modified Vaccinia virus Ankara* (ATCC), 4.passage, EMEM + 2% FBS
Cell line:	BHK-21 cells (ATCC), 64., 68. passage, DMEM + 10% FBS
Proces 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 calcullated by:	Spaerman – Kärber‘s method

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 inactivacion test of product
8. Control of susceptibility

Table n.1 The results and validation of the test for product HYG-DEZ ST. CRUX 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)	
				0,5	1	30	60	0,5	
HYG-DEZ ST. CRUX	100%*	3,0 g/l BSA + erythrocytes	2,5	≤2,500 ± 0,000	n.d.	n.d.	n.d.	≥4,000 ± 0,000	
HYG-DEZ ST. CRUX	50%	3,0 g/l BSA + erythrocytes	2,5	≤2,500 ± 0,000	n.d.	n.d.	n.d.	≥4,000 ± 0,000	
HYG-DEZ ST. CRUX	10%	3,0 g/l BSA + erythrocytes	1,5	6,000± 0,189	n.d.	n.d.	n.d.	0,500 ± 0,378	
Virus control	n.a.	3,0 g/l BSA + erythrocytes	n.a.	6,500 ± 0,000	n.d.	n.d.	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.	≥3,000 ± 0,000	≥3,000 ± 0,000
Virus control - MicroSpin	n.a.	PBS	n.a.	6,500 ± 0,000	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 HYG-DEZ ST. CRUX 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
HYG-DEZ ST. CRUX	100%*	3,0 g/l BSA + erythrocytes	30 s	CT	000000	000000	000000	000000	000000	000000
HYG-DEZ ST. CRUX	50%	3,0 g/l BSA + erythrocytes	30 s	CT	000000	000000	000000	000000	000000	000000
HYG-DEZ ST. CRUX	10%	3,0 g/l BSA + erythrocytes	30 s	444444	444444	444444	444444	023200	000000	000000
HYG-DEZ ST. CRUX cytotoxicity	100%*	3,0 g/l BSA + erythrocytes	n.a.	CT	000000	000000	n.d.	n.d.	n.d.	n.d.
HYG-DEZ ST. CRUX cytotoxicity	50%	3,0 g/l BSA + erythrocytes	n.a.	CT	000000	000000	n.d.	n.d.	n.d.	n.d.
HYG-DEZ ST. CRUX cytotoxicity	10%	3,0 g/l BSA + erythrocytes	n.a.	000000	000000	000000	n.d.	n.d.	n.d.	n.d.
Virus control	n.a.	3,0 g/l BSA + erythrocytes	30 s	444444	444444	444444	444334	443232	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	222232	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

Prepared by: Mgr. Ludmila Porubová

END OF THE PROTOCOL