CHELAB S.R.L.

REPORT N. X ANNEX TO TEST REPORT AA/XXXXXXXXX

Chemical disinfectants and antiseptics Quantitative suspension test for the evaluation of virucidal activity in the medical area (phase 2, step 1) based on UNI EN 14476+A1:2015

SAMPLE INFORMATION

ID Sample:	19.528357.0001
Sample description:	Product Name: NANOKSIA Protocol no: 1002019020352 Receiving date: 11/09/2019 Product appearance: liquid, colourless
Sponsor	NANOKSIA BIYOTEKNOLOJI SAN.VE TIC.A.S. GOZTEPE MH. SAKAYIK CD. GOKSU EVLERI NO:8 IC KAPI NO: 1 BEYKOZ

METHOD

The method is based on UNI EN 14476+A1:2015, using Poliovirus type 1 Lsc-2ab according to sponsor required.

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EXPERIMENTAL CONDITIONS:

- Test virus and number of passages:
  Poliovirus (passage number 4)
- Cell line and number of passages:
  LLC-MK2 ATCC CCL-7.1 for the propagation o*f Poliovirus* type 1 Lsc-2ab (passage number 51+5)
- Product test concentrations: 97%
- Diluent used for product test solution: Distillated water
- Appearance of product dilutions: Liquid and colourless
- Stability and appearance of the mixture during the procedure: Stable, without precipitates
- Interfering substance: Albumin Bovine 1,5 g/l (clean conditions)
- Contact time: 60 min ± 10 sec
- Test temperature: 20 °C ± 1°C
- Neutralization method: Filtration method with Microspin™ S400HR columns
- Growth medium: EMEM 10% FCS
- Manteinance medium: EMEM 2% FCS

TEST RESULTS

See table n° 1 and 2.

CONCLUSION

Based on UNI EN 14476+A1:2015, under the test conditions applied, the test product resulted to have virucidal activity (R ≥ 4) against Poliovirus at concentration 97% after a contact period of 60 min ± 10 sec, at temperature of 20 °C ± 1°C.

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Table 1: Results of the test UNI EN 14476+A1:2015 on Poliovirus contact time 60 minutes

VIRUS	TEST	SAMPLE	VIRUS TITRATION $logTCID_{50}$	ACCEPTANCE CRITERIA	RESULT
Poliovirus	TITRATION OF VIRUS CONTROL	0 min	6.75	/	/
		60 min	6.625	/	/
	PRELIMINARY CYTOTOXICITY EFFECT	DONE			
	CELL SUSCEPTIBILITY	CONTROL	6.375	< 1	R = 0.25 PASS
		0.097%*	6.125		
	EFFICIENCY FOR SUPPRESSION OF DISINFECTANT ACTIVITY	97%	6.875	≤ 0.5	R = 0.25 PASS
	VIRUCIDAL ACTIVITY	97%	2.5	R ≥ 4	R = 4.125 ACTIVE
	REFERENCE VIRUS INACTIVATION TEST	30 MINUTES	4.25	0.5 ≤ R ≤ 2.5	R = 2.375
		60 MINUTES	3	2 ≤ R ≤ 4.5	R = 3.625

* lowest apparently non cytotoxic dilution

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Table 2: Raw Data UNI EN 14476+A1:2015 on Poliovirus contact time 60 minutes

Test	Contact Time	Interfering substance	Concentration	dilutions log_{10}							
				2	3	4	5	6	7	8	9
Virus Titration	0 min	BSA 1,5 g/l	/	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4400 0000	0000 0000	0000 0000
	60 min		/	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4000 0000	0000 0000	0000 0000
Efficiency of Suppression of Product's Activity	60 min	BSA 1,5 g/l	97%	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4440 0000	0000 0000	0000 0000
Virucidal Activity	60 min	BSA 1,5 g/l	97%	4444 4444	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
Reference Inactivation Test	30 min	PBS	0.7%	CCCC CCCC	4444 4444	4444 4400	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	60 min		0.7%	CCCC CCCC	4444 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
Susceptibilty (Control)	60 min	PBS	/	4444 4444	4444 4444	4444 4444	4444 4444	4444 4440	0000 0000	0000 0000	0000 0000
Susceptibility (Product)	60 min	PBS	0.097%	4444 4444	4444 4444	4444 4444	4444 4444	4444 4000	0000 0000	0000 0000	0000 0000

Test	Contact Time	Interfering substance	Concentration	Product dilutions (log_{10})							
				1	2	3	4	5	6	7	8
Cytotoxicity (Product)	n.a.	BSA 1,5 g/l	97%	CCCC CCCC	CCCC CCCC	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000

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