Public Heath Institute Ostrava
Center of clinical laboratories
Testing laboratory n. 1554 accredited
accorrding to ISO/IEC 17025
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Laboratory for testing virucidal effect

L 1554

TEST REPORT n. 8/2013/SVU

Virucidal quantitative suspension test for chemical desinfectants and antiseptics used in human medicine - Test method and requirements (phase 2/ step 1)
EN 14476+A1

Customer:
BOCHEMIE a.s.
Lidická 326
735 95 Bohumín
CZ

Digitally signed by Grițaenco Andrei
Date: 2022.12.26 15:58:02 EET
Reason: MoldSign Signature
Location: Moldova

Order number: 00021571
Reference number: ZU/40510/2013

Identification of desinfectant – sample:

Product name:	CHIROSAN PLUS
Lot number:	001A130724
Expiration:	7/2015
Producer:	Bochemie a.s.
Storage conditions:	-10 až +25°C
Diluent product recommended by the producer for use:	dilution by water
Product appearance:	solid, white powder
Activation of solution:	15 min intensive blending
The active substance(s) and its (their) concentration:	peracetic acid generated in situ
Purpose of product:	instrument disinfection
Date of delivery of the product:	17.12.2013
Date of test:	22.1.2014 – 7.5.2014

Results - for details see annex

The test product CHIROSAN PLUS, designed for instrument disinfection, diluted with hard water to 2% demonstrate virucidal activity after an exposure time 10 min, at temperature 20°C±1°C, under dirty conditions (3,0 g/l Bovine serum albumin and 3,0 ml erythrocytes), using viral titration on monolayer cell culture on a microtitre plate by reduction of reference virus *Murine norovirus, strain S99* by more than 4 lg.

The test product CHIROSAN PLUS, designed for instrument disinfection, diluted with hard water to 1% demonstrate virucidal activity after an exposure time 30 min and 60 min, at temperature 20°C±1°C, under dirty conditions (3,0 g/l Bovine serum albumin and 3,0 ml erythrocytes), using viral titration on monolayer cell culture on a microtitre plate by reduction of reference virus *Murine norovirus, strain S99* by more than 4 lg.

The test product CHIROSAN PLUS, designed for instrument disinfection, diluted with hard water to 2% demonstrate virucidal activity after an exposure time 5 min, at temperature 20°C±1°C, under dirty conditions (3,0 g/l Bovine serum albumin and 3,0 ml erythrocytes), using viral titration on monolayer cell culture on a microtitre plate by reduction of reference virus *Human rotavirus, strain Wa* by more than 4 lg.

The test product CHIROSAN PLUS, designed for instrument disinfection, diluted with hard water to 1% demonstrate virucidal activity after an exposure time 10 min, at temperature 20°C±1°C, under dirty conditions (3,0 g/l Bovine serum albumin and 3,0 ml erythrocytes), using viral titration on monolayer cell culture on a microtitre plate by reduction of reference virus *Human rotavirus, strain Wa* by more than 4 lg.

The test product CHIROSAN PLUS, designed for instrument disinfection, diluted with hard water to 0,5% demonstrate virucidal activity after an exposure time 30 min, at temperature 20°C±1°C, under dirty conditions (3,0 g/l Bovine serum albumin and 3,0 ml erythrocytes), using viral titration on monolayer cell culture on a microtitre plate by reduction of reference virus *Human rotavirus, strain Wa* by more than 4 lg.

Conclusion:

The test product CHIROSAN PLUS after dilution with water to 2% demonstrate limited virucidal activity to Norovirus under the dirty conditions after exposure time 10 min.

The test product CHIROSAN PLUS after dilution with water to 1% demonstrate limited virucidal activity to Norovirus under the dirty conditions after exposure time 30 min and 60 min.

The test product CHIROSAN PLUS after dilution with water to 2% demonstrate limited virucidal activity to Rotavirus under the dirty conditions after exposure time 5 min.

The test product CHIROSAN PLUS after dilution with water to 1% demonstrate limited virucidal activity to Norovirus under the dirty conditions after exposure time 10 min.

The test product CHIROSAN PLUS after dilution with water to 0,5% demonstrate limited virucidal activity to Norovirus under the dirty conditions after exposure time 30 min.

In Ostrava, 22.5.2014

Zdravotní ústav se sídlem v Ostravě
Par[illegible]7, 702 00 Ostrava
IČ: 710[illegible]7

Mgr. Ludmila Porubová
Guarantor of testing

Note:

The test method was performed according to EN 14476+A1 when the test viruses were replaced by the Human rotavirus, strain Wa, as the representative of human rotaviruses, and Murine norovirus, strain S99 (MNV-1). MNV-1 is the only representative of genus Norovirus, which effectively propagate on cell culture. The cytophatic effect is observede during infection of murine dendritic cells or macrophades by norovirus (1,2). This property of MNV-1 allows to quantify the number of infectious particels, which is needed to calculate the virucidal activity of disinfectant according EN 14476+A1. For this reason, MNV-1 was chosen as the reference virus to determine of virucidal effect of disinfectant against human norovirus (3).

Literature:

1. Wobus, Ch.E., Thackray, L.B., Virgin, H.W.: Murine norovirus: a Model Systém to Study Norovirus Biology and Pathogenesis, Journal of Virology, 2006, p. 5104 – 5112
2. Wobus, Ch.E., Karst, S.M., Thackray, L.B., Chang, K.-o., Sosnovtsev, S.v., Belliot, G., Krug,A., Mackenzie, J.,M., Green, K.Y., Virgin, H.W: Replication of Norovirus in Cell Culture Reveals a Tropism for Dendritic Cells and Macrophages, PLoS Biology, 2004: p. 2076 – 2084
3. PrEN 14476:2011: Chemical disinfectant and antiseptik – Quantitative suspension test for the evaluation of virucidal aktivity of chemical disinfectatn and antiseptik used in human medicine – test method and requirements (phase 2, step 1)

Annex to the protocol n.: 8/2013/SVU

Identification of product:

Product name:	CHIROSAN PLUS
Lot number:	001A130724
Expiratin:	7/2015
Producer:	Bochemie a.s.
Date of delivery of product	17.12.2013
Storage conditions:	-10 **až** +25°C
Product appearance:	solid, white powder
The active compound(s) and its (their) concentration:	peracetic acid generated in situ

Experimental conditions:	Quantitative suspension test for the determine of virucidal effect of disinfectant EN 14476+A1 (SOP n. 1901)
Date of testing:	22.1.2014 – 7.5.2014
Diluent:	hard water
Testing concentration:	2%, 1%, 0,5%
Activation of solution:	15 min intensive blending
Appearance of dilution of the product:	after dilution remain in solution small crystals of product
Contact times:	Norovirus - 2% - 10 min, 1% - 30 min, 60 min Rotavirus – 2% - 5 min, 1% - 10 min, 0,5% - 30 min
Testing temperature:	20°C±1°C
Interfering substance:	dirty conditions – 3,0 g/l bovine serum albumin (BSA) + 3,0 ml erythrocyte
Stability of mixture during teyting:	1% mixture after 60 min change the colour from red to yellow
Method of filtration:	MicroSpin S 400 HR
Test virus:	*Murine norovirus, strain S99* (FLI), 2.passage, DMEM + 2% FBS Human rotavirus, strain Wa (ATCC), 1.passage, EMEM + 2 µg/ml trypsin
Proces to stop action of product:	virucidal activity of product is suppressed by transferring the sample into the ice cold diluent
Cell line:	RAW 264.7 (FLI), 30.passage, DMEM + 10% FBS MA-104 (FLI), 28.passage, DMEM + 10% FBS
Titration method:	viral titration on monolayer cell culture on the microplate
Reference substance:	Formaldehyd (Sigma-Aldrich, č.š. SZBC2290V)
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 CHIROSAN PLUS na *Murine norovirus, strain S99* – 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	10	15	30	60	10	30	60
Chirosan Plus	2%	3 g/l BSA + erythrocyte	1,5	n.d.	1,5	n.d.	n.d.	n.d.	5,4	n.d.	n.d.
Chirosan Plus	1%	3 g/l BSA + erythrocyte	1,5	n.d.	n.d.	n.d.	1,5	1,5	n.d.	5,4	5,4
Formaldehyde	0,7% (m/V)	PBS	3,5	6,1	n.d.	5,7	3,5	2,5			
Virus control	n.a.	3 g/l BSA + erythrocyte	n.a.	6,9	n.d.	n.d.	n.d.	6,9			
Susceptibility of cells - PBS	n.a.	3 g/l BSA + erythrocyte	n.a.	n.d.	n.d.	n.d.	n.d.	6,9			
Susceptibility of cells - Chirosan Plus	0,001%	3 g/l BSA + erythrocyte	n.a.	n.d.	n.d.	n.d.	n.d.	6,7			

Table n.2 The results and validation of the test for product CHIROSAN PLUS na *Human rotavirus, strain Wa* – 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	10	15	30	60	5	10	30
Chirosan Plus	2%	3 g/l BSA + erytrocyty	1,5	1,7	n.d.	n.d.	n.d.	n.d.	4,0	n.d.	n.d.
Chirosan Plus	1%	3 g/l BSA + erytrocyty	1,5	n.d.	1,5	n.d.	n.d.	n.d.	n.d.	4,2	n.d.
Chirosan Plus	0,5%	3 g/l BSA + erytrocyty	1,5	n.d.	n.d.	n.d.	1,5	n.d.	n.d.	n.d.	4,2
Formaldehyde	0,7% (m/V)	PBS	3,5	4,5	n.d.	4,1	3,5	3,5			
Virus control	n.a.	3 g/l BSA + erytrocyty	n.a.	5,7	n.d.	n.d.	n.d.	5,7			
Susceptibility of cells - PBS	n.a.	3 g/l BSA + erytrocyty	n.a.	n.d.	n.d.	n.d.	n.d.	5,5			
Susceptibility of cells - Chirosan Plus	0,001%	3 g/l BSA + erytrocyty	n.a.	n.d.	n.d.	n.d.	n.d.	5,3			

Prepared by: Mgr. Ludmila Porubová

Table 3: Raw date of test for product CHIROSAN PLUS na *Murine norovirus, strain S99* – dirty conditions

Product	Concentration	Interfering substance	Level of cytotoxicity	Dilution (log 10)							
				-1	-2	-3	-4	-5	-6	-7	-8
Chirosan plus	2%	3 g/l BSA + erythrocyte	10 min	00000	00000	00000	00000	00000	00000	00000	00000
Chirosan plus	1%	3 g/l BSA + erythrocyte	30 min	00000	00000	00000	00000	00000	00000	00000	00000
Chirosan plus	1%	3 g/l BSA + erythrocyte	60 min	00000	00000	00000	00000	00000	00000	00000	00000
Cytotoxicity Chirosan plus	2%	3 g/l BSA + erythrocyte	n.a.	00000	00000	00000	00000	n.a.	n.a.	n.a.	n.a.
Cytotoxicity Chirosan plus	1%	3 g/l BSA + erythrocyte	n.a.	00000	00000	00000	00000	n.a.	n.a.	n.a.	n.a.
Cytotoxicity Formaldehyde	0,7% (m/V)	PBS	n.a.	CT	CT	00000	00000	n.a.	n.a.	n.a.	n.a.
Formaldehyde	0,7% (m/V)	PBS	5 min	CT	CT	44444	43444	30330	00000	00000	00000
			15 min	CT	CT	44444	44444	00004	00000	00000	00000
			30 min	CT	CT	44444	00000	00000	00000	00000	00000
			60 min	CT	CT	00000	00000	00000	00000	00000	00000
Susceptibility of cells - PBS	n.a.	3 g/l BSA + erythrocyte	60 min	44444	44444	44444	44444	44444	03300	00000	00000
Susceptibility of cells - Chirosan plus	0,0001%	3 g/l BSA + erythrocyte	60 min	44444	44444	44444	44444	44444	30000	00000	00000
Virus control	n.a.	3 g/l BSA + erytrocyty	10 min	44444	44444	44444	44444	44444	10030	00000	00000
Virus control	n.a.	3 g/l BSA + erytrocyty	60 min	44444	44444	44444	44444	44444	00044	00000	00000

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

Table 4: Raw date of test for product CHIROSAN PLUS na *Human rotavirus, strain Wa* – vyšší znečištění

Product	Concentration	Interfering substance	Level of cytotoxicity	Dilution (log 10)							
				-1	-2	-3	-4	-5	-6	-7	-8
Chirosan plus	2%	3 g/l BSA + erytrocyty	5 min	01000	00000	00000	00000	00000	00000	00000	00000
Chirosan plus	1%	3 g/l BSA + erytrocyty	10 min	00000	00000	00000	00000	00000	00000	00000	00000
Chirosan plus	0,5%	3 g/l BSA + erytrocyty	30 min	00000	00000	00000	00000	00000	00000	00000	00000
Cytotoxicity Chirosan plus	2%	3 g/l BSA + erytrocyty	n.a.	00000	00000	00000	00000	n.a.	n.a.	n.a.	n.a.
Cytotoxicity Chirosan plus	1%	3 g/l BSA + erytrocyty	n.a.	00000	00000	00000	00000	n.a.	n.a.	n.a.	n.a.
Cytotoxicity Chirosan plus	0,5%	3 g/l BSA + erytrocyty	n.a.	00000	00000	00000	00000	n.a.	n.a.	n.a.	n.a.
Cytotoxicity Formaldehyde	0,7% (m/V)	PBS	n.a.	CT	CT	00000	00000	n.a.	n.a.	n.a.	n.a.
Formaldehyde	0,7% (m/V)	PBS	5 min	CT	CT	22222	00000	00000	00000	00000	00000
			15 min	CT	CT	11010	00000	00000	00000	00000	00000
			30 min	CT	CT	00000	00000	00000	00000	00000	00000
			60 min	CT	CT	00000	00000	00000	00000	00000	00000
Susceptibility of cells - PBS	n.a.	PBS	60 min	44444	44444	44444	22322	00000	00000	00000	00000
Susceptibility of cells - Chirosan plus	0,0001%	PBS	60 min	44444	44444	44444	22330	00000	00000	00000	00000
Virus control	n.a.	3 g/l BSA + erytrocyty	5 min	44444	44444	44444	32333	10000	00000	00000	00000
Virus control	n.a.	3 g/l BSA + erytrocyty	60 min	44444	44444	44444	20212	00110	00000	00000	00000

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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