



Public Health Institute Ostrava
Center of clinical laboratories
Testing laboratory n. 1554 accredited according to
ISO/IEC 17025
702 00 Ostrava
VAT: CZ71009396
Laboratory for testing virucidal effect



L 1554

TEST REPORT n. 4/2014/SVU

Virucidal quantitative suspension test for the evaluation of virucidal activity in the medicinal area (phase 2/ step 1)
EN 14476

Customer:

BOCHEMIE a.s.
Lidická 326
735 95 Bohumín
CZ

Order number: 00024884

Reference number: ZU/15091/2014

Identification of disinfectant – sample:

Product name:	CHIROSAN PLUS
Lot number:	027A140418
Expiration:	4/2016
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 and surface disinfection
Date of delivery of the product:	16.5.2014
Date of test:	22.5. – 12.6.2014

Results - for details see annex

According to EN 14476 the test product CHIROSAN PLUS, designed for instrument and surface disinfection, diluted with hard water to 1% demonstrate virucidal activity after an exposure time 60 min, at temperature $20^{\circ}\text{C}\pm 1^{\circ}\text{C}$, under clean (0,3 g/l bovine serum albumin) and dirty conditions (3,0 g/l Bovine serum albumin and 3,0 ml erythrocytes), using viral titration on cell suspension on a microtitre plate by reduction of reference virus *Poliovirus typ 1, strain LScs-2ab* by more than 4 lg.

According to EN 14476 the test product CHIROSAN PLUS, designed for instrument and surface disinfection, diluted with hard water to 0,5% demonstrate virucidal activity after an exposure time 30 and 60 min, at temperature $20^{\circ}\text{C}\pm 1^{\circ}\text{C}$, under clean (0,3 g/l bovine serum albumin) and dirty conditions (3,0 g/l Bovine serum albumin and 3,0 ml erythrocytes), using viral titration on cell suspension on a microtitre plate by reduction of reference virus *Poliovirus typ 1, strain LScs-2ab* by more than 4 lg.

According to EN 14476 the test product CHIROSAN PLUS, designed for instrument and surface disinfection, diluted with hard water to 0,1% does not demonstrate virucidal activity after an exposure time 60 min, at temperature $20^{\circ}\text{C}\pm 1^{\circ}\text{C}$, under clean (0,3 g/l bovine serum albumin) and dirty conditions (3,0 g/l Bovine serum albumin and 3,0 ml erythrocytes), using viral titration on cell suspension on a microtitre plate by reduction of reference virus *Poliovirus typ 1, strain LScs-2ab* by more than 4 lg.

According to EN 14476 the test product CHIROSAN PLUS, designed for instrument and surface disinfection, diluted with hard water to 1% demonstrate virucidal activity after an exposure time 60 min, at temperature $20^{\circ}\text{C}\pm 1^{\circ}\text{C}$, under clean (0,3 g/l bovine serum albumin) and dirty conditions (3,0 g/l Bovine serum albumin and 3,0 ml erythrocytes), using viral titration on cell suspension on a microtitre plate by reduction of reference virus *Adenovirus type 5, strain Adenoid 75* by more than 4 lg.

According to EN 14476 the test product CHIROSAN PLUS, designed for instrument and surface disinfection, diluted with hard water to 0,5% demonstrate virucidal activity after an exposure time 30 min and 60 min, at temperature $20^{\circ}\text{C}\pm 1^{\circ}\text{C}$, under clean (0,3 g/l bovine serum albumin) and dirty conditions (3,0 g/l Bovine serum albumin and 3,0 ml erythrocytes), using viral titration on cell suspension on a microtitre plate by reduction of reference virus *Adenovirus type 5, strain Adenoid 75* by more than 4 lg.

According to EN 14476 the test product CHIROSAN PLUS, designed for instrument and surface disinfection, diluted with hard water to 0,1% demonstrate virucidal activity after an exposure time 60 min, at temperature $20^{\circ}\text{C}\pm 1^{\circ}\text{C}$, under clean (0,3 g/l bovine serum albumin) and dirty conditions (3,0 g/l Bovine serum albumin and 3,0 ml erythrocytes), using viral titration on cell suspension on a microtitre plate by reduction of reference virus *Adenovirus type 5, strain Adenoid 75* by more than 4 lg.

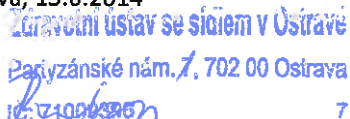
Conclusion:

According to EN 14476 the test product CHIROSAN PLUS after dilution with hard water to 1% demonstrate virucidal activity to non – enveloped viruses Adenovirus and Poliovirus under the clean and dirty conditions after exposure time 60 min.

According to EN 14476 the test product CHIROSAN PLUS after dilution with hard water to 0,5% demonstrate virucidal activity to non – enveloped viruses Adenovirus and Poliovirus under the clean and dirty conditions after exposure time 30 min and 60 min.

According to EN 14476 the test product CHIROSAN PLUS after dilution with hard water to 0,1% demonstrate virucidal activity to non – enveloped virus Adenovirus under the clean and dirty conditions after exposure time 60 min.

In Ostrava, 13.6.2014


Zdravotní ústav se sídlem v Ostravě
Pařížské nám. 7, 702 00 Ostrava
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Mgr. Ludmila Porubová
Guarantor of testing

Annex to the protocol n.: 4/2014/SVU

Identification of product:

Product name: CHIROSAN PLUS
Lot number: 27A140418
Expiration: 4/2016
Producer: Bochemie a.s.
Date of delivery of product: 16.5.2014
Storage conditions: -10 až +25°C
Diluent recommended by producer: domestic water
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 (SOP n. 1901)
Date of testing: 22.5.2014 – 12.6.2014
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: 60 in, 30 min only 0,5% solution
Testing temperature: 20°C±1°C
Interfering substance: clean conditions – 0,3 g/l bovine serum albumin
dirty conditions – 3,0 g/l bovine serum albumin (BSA) + 3,0 ml erythrocyte
Stability of mixture during teyting: stable, during 60 min incubation reaction with erythrocytes, change the colour of solution from red to yellow
Method of filtration: none
Test virus: *Poliovirus type 1, strain LSc-2ab* (NIBCS), 3.passage, DMEM + 2% FBS
Adenovirus type 5, strain Adenoid 75 (ATCC), 7.passage, EMEM + 2% FBS
Proces to stop action of product: virucidal activity of product is suppressed by transferring the sample into the ice cold diluent
Cell line: RD (ATCC), 15. passage, DMEM + 10% FBS
Hela (ATCC), 15.passage, EMEM + 10% FBS
Titration method: viral titration on cell culture in suspension on the microplate
Reference substance: Formaldehyde (Sigma-Aldrich, č.š. SZBC2290V)
Titers callulated 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 of the test for product CHIROSAN PLUS to Poliovirus type 1, strain LSc-2ab – clean conditions

Product	Concentration	Interfering substance	Level of cytotoxicity	log ₁₀ TCID ₅₀ / ml after ... min					Reduction factor (Δlog ₁₀ TCID ₅₀ / ml after ... min)	
				5	10	15	30	60	30	60
CHIROSAN PLUS	1,0%	0,3 g/l BSA	2,5	n.d.	n.d.	n.d.	n.d.	2,5	n.d.	4,2
CHIROSAN PLUS	0,5%	0,3 g/l BSA	2,5	n.d.	n.d.	n.d.	2,5	2,5	4,6	4,2
CHIROSAN PLUS	0,1%	0,3 g/l BSA	1,5	n.d.	n.d.	n.d.	n.d.	6,3	n.d.	0,4
Formaldehyde	0,7% (m/V)	PBS	4,5	n.d.	n.d.	n.d.	5,9	4,5		
Virus control	n.a.	0,3 g/l BSA	n.a.	6,9	n.d.	n.d.	7,1	6,7		
Susceptibility of cells - PBS	n.a.	0,3 g/l BSA	n.a.	n.d.	n.d.	n.d.	n.d.	7,1		
Susceptibility of cells - Chirosan Plus	0,0001%	0,3 g/l BSA	n.a.	n.d.	n.d.	n.d.	n.d.	7,5		

Table n.2 The results of the test for product CHIROSAN PLUS to Poliovirus type 1, strain LSc-2ab – dirty conditions

Product	Concentration	Interfering substance	Level of cytotoxicity	log ₁₀ TCID ₅₀ / ml after ... min					Reduction factor (Δlog ₁₀ TCID ₅₀ / ml after ... min)	
				5	10	15	30	60	30	60
CHIROSAN PLUS	1,0%	3,0 g/l BSA + erythrocyte	2,5	n.d.	n.d.	n.d.	n.d.	2,5	n.d.	4,2
CHIROSAN PLUS	0,5%	3,0 g/l BSA + erythrocyte	2,5	n.d.	n.d.	n.d.	1,5	1,5	5,0	5,2
CHIROSAN PLUS	0,1%	3,0 g/l BSA + erythrocyte	1,5	n.d.	n.d.	n.d.	n.d.	5,7	n.d.	1
Formaldehyde	0,7% (m/V)	PBS	4,5	n.d.	n.d.	n.d.	5,9	4,5		
Virus control	n.a.	3,0 g/l BSA + erythrocyte	n.a.	6,9	n.d.	n.d.	6,7	6,7		
Susceptibility of cells - PBS	n.a.	3,0 g/l BSA + erythrocyte	n.a.	n.d.	n.d.	n.d.	n.d.	7,9		
Susceptibility of cells - Chirosan Plus	0,0001%	3,0 g/l BSA + erythrocyte	n.a.	n.d.	n.d.	n.d.	n.d.	7,1		

Prepared by: Mgr. Ludmila Porubová



Table 3: Raw data of test for product CHIROSAN PLUS to Poliovirus type 1, strain LSc-2ab – dirty conditions

Product	Concentration	Interfering substance	Contact time	Dilution (log 10)								
				-1	-2	-3	-4	-5	-6	-7	-8	
Chirosan plus	1,0%	3 g/l BSA + erythrocyty	60 min	CT	00000	00000	00000	00000	00000	00000	00000	00000
Chirosan plus	0,5%	3 g/l BSA + erythrocyty	30 min	00000	00000	00000	00000	00000	00000	00000	00000	00000
Chirosan plus	0,5%	3 g/l BSA + erythrocyty	60 min	00000	00000	00000	00000	00000	00000	00000	00000	00000
Chirosan plus	0,1%	3 g/l BSA + erythrocyty	60 min	44444	44444	444444	44444	04000	00000	00000	00000	00000
Cytotoxicity Chirosan plus	1,0%	3 g/l BSA + erythrocyty	n.a.	CT	00000	00000	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Cytotoxicity Chirosan plus	0,5%	3 g/l BSA + erythrocyty	n.a.	00000	00000	00000	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Cytotoxicity Chirosan plus	0,1%	3 g/l BSA + erythrocyty	n.a.	00000	00000	00000	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Cytotoxicity Formaldehyde	0,7% (m/V)	PBS	n.a.	CT	CT	CT	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Formaldehyd e	0,7% (m/V)	PBS	30 min	CT	CT	CT	44444	00004	00004	00000	00000	00000
			60 min	CT	CT	CT	00000	00000	00000	00000	00000	00000
Susceptibility of cells - PBS	0,0001%	3 g/l BSA + erythrocyty	60 min	44444	44444	44444	44444	44444	44444	01003	00000	00000
Susceptibility of cells - Chirosan plus	n.a.	3 g/l BSA + erythrocyty	30 min	44444	44444	44444	44444	44444	24200	00000	00000	00000
Virus control	n.a.	3 g/l BSA + erythrocyty	60 min	44444	44444	44444	44444	44044	00004	00000	00000	00000
Virus control	1,0%	3 g/l BSA + erythrocyty	60 min	44444	44444	44444	44444	44444	04000	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

Prepared by: Mgr. Ludmila Porubová



Table 4: Raw data of test for product CHIROSAN PLUS to Poliovirus type 1, strain LSc-2ab – clean conditions

Product	Concentration	Interfering substance	Contact time	Dilution (log 10)								
				-1	-2	-3	-4	-5	-6	-7	-8	
Chirosan plus	1,0%	0,3 g/l BSA	60 min	CT	00000	00000	00000	00000	00000	00000	00000	00000
Chirosan plus	0,5%	0,3 g/l BSA	30 min	CT	00000	00000	00000	00000	00000	00000	00000	00000
Chirosan plus	0,5%	0,3 g/l BSA	60 min	CT	00000	00000	00000	00000	00000	00000	00000	00000
Chirosan plus	0,1%	0,3 g/l BSA	60 min	44444	44444	44444	44444	41440	00000	00000	00000	00000
Cytotoxicity Chirosan plus	1,0%	0,3 g/l BSA	n.a.	CT	00000	00000	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Cytotoxicity Chirosan plus	0,5%	0,3 g/l BSA	n.a.	CT	00000	00000	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Cytotoxicity Chirosan plus	0,1%	0,3 g/l BSA	n.a.	00000	00000	00000	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Cytotoxicity Formaldehyde	0,7% (m/V)	PBS	n.a.	CT	CT	CT	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Formaldehyde	0,7% (m/V)	PBS	30 min	CT	CT	CT	44444	00004	00004	00000	00000	00000
			60 min	CT	CT	CT	00000	00000	00000	00000	00000	00000
Susceptibility of cells - PBS	0,0001%	0,3 g/l BSA	60 min	44444	44444	44444	44444	44444	40330	00000	00000	00000
Susceptibility of cells - Chirosan plus	n.a.	0,3 g/l BSA	30 min	44444	44444	44444	44444	44444	44444	00000	00000	00000
Virus control	n.a.	0,3 g/l BSA	60 min	44444	44444	44444	44444	44444	04044	00000	00000	00000
Virus control	1,0%	0,3 g/l BSA	60 min	44444	44444	44444	44444	44444	00004	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

Prepared by: Mgr. Ludmila Porubová



Table n.5 The results of the test for product CHIROSAN PLUS to Adenovirus type 5, strain Adenoid 75 – clean conditions

Product	Concentration	Interfering substance	Level of cytotoxicity	log ₁₀ TCID ₅₀ / ml after ... min					Reduction factor (Δlog ₁₀ TCID ₅₀ / ml after ... min)		
				5	10	15	30	60	30	60	
CHIROSAN PLUS	1,0%	0,3 g/l BSA	2,5	n.d.	n.d.	n.d.	n.d.	2,5	n.d.	n.d.	4,8
CHIROSAN PLUS	0,5%	0,3 g/l BSA	2,5	n.d.	n.d.	n.d.	2,5	2,5	4,6	n.d.	4,8
CHIROSAN PLUS	0,1%	0,3 g/l BSA	1,5	n.d.	n.d.	n.d.	n.d.	1,5	n.d.	n.d.	5,8
Formaldehyde	0,7% (m/V)	PBS	3,5	n.d.	n.d.	n.d.	4,1	3,5	n.d.	n.d.	n.d.
Virus control	n.a.	0,3 g/l BSA	n.a.	6,9	n.d.	n.d.	7,1	7,3	n.d.	n.d.	n.d.
Susceptibility of cells - PBS	n.a.	0,3 g/l BSA	n.a.	n.d.	n.d.	n.d.	n.d.	7,5	n.d.	n.d.	n.d.
Susceptibility of cells - Chiroosan Plus	0,0001%	0,3 g/l BSA	n.a.	n.d.	n.d.	n.d.	n.d.	7,1	n.d.	n.d.	n.d.

Table n.6 The results of the test for product CHIROSAN PLUS to Adenovirus type 5, strain Adenoid 75 – dirty conditions

Product	Concentration	Interfering substance	Level of cytotoxicity	log ₁₀ TCID ₅₀ / ml after ... min					Reduction factor (Δlog ₁₀ TCID ₅₀ / ml after ... min)		
				5	10	15	30	60	30	60	
CHIROSAN PLUS	1,0%	3,0 g/l BSA + erythrocyte	2,5	n.d.	n.d.	n.d.	n.d.	2,5	n.d.	n.d.	4,6
CHIROSAN PLUS	0,5%	3,0 g/l BSA + erythrocyte	1,5	n.d.	n.d.	n.d.	1,5	1,5	6,0	n.d.	5,4
CHIROSAN PLUS	0,1%	3,0 g/l BSA + erythrocyte	2,5	n.d.	n.d.	n.d.	n.d.	1,5	n.d.	n.d.	5,4
Formaldehyde	0,7% (m/V)	PBS	3,5	n.d.	n.d.	n.d.	4,5	3,5	n.d.	n.d.	n.d.
Virus control	n.a.	3,0 g/l BSA + erythrocyte	n.a.	6,9	n.d.	n.d.	7,5	6,9	n.d.	n.d.	n.d.
Susceptibility of cells - PBS	n.a.	3,0 g/l BSA + erythrocyte	n.a.	n.d.	n.d.	n.d.	n.d.	7,1	n.d.	n.d.	n.d.
Susceptibility of cells - Chiroosan Plus	0,0001%	3,0 g/l BSA + erythrocyte	n.a.	n.d.	n.d.	n.d.	n.d.	7,5	n.d.	n.d.	n.d.

Prepared by: Mgr. Ludmila Porubová

Without written consent of the testing laboratory, the protocol shall not be reproduced except in full. The result of test relate only to the tested sample. The center of clinical laboratory - testing laboratory n. 1554. The sample was examined by SOP No.1901

Table 7: Raw data of test for product CHIROSAN PLUS to Adenovirus type 5, strain Adenoid 75 – clean conditions

Product	Concentration	Interfering substance	Contact time	Dilution (log 10)								
				-1	-2	-3	-4	-5	-6	-7	-8	
Chirosan plus	1,0%	0,3 g/l BSA	60 min	CT	00000	00000	00000	00000	00000	00000	00000	00000
Chirosan plus	0,5%	0,3 g/l BSA	30 min	CT	00000	00000	00000	00000	00000	00000	00000	00000
Chirosan plus	0,5%	0,3 g/l BSA	60 min	00000	00000	00000	00000	00000	00000	00000	00000	00000
Chirosan plus	0,1%	0,3 g/l BSA	60 min	CT	00000	00000	00000	00000	00000	00000	00000	00000
Cytotoxicity Chirosan plus	1,0%	0,3 g/l BSA	n.a.	CT	00000	00000	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Cytotoxicity Chirosan plus	0,5%	0,3 g/l BSA	n.a.	CT	00000	00000	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Cytotoxicity Chirosan plus	0,1%	0,3 g/l BSA	n.a.	00000	00000	00000	00000	n.a.	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.	n.a.
Formaldehyde	0,7% (m/V)	PBS	30 min	CT	CT	44400	00000	0000	00000	00000	00000	00000
			60 min	CT	CT	00000	00000	00000	00000	00000	00000	00000
Susceptibility of cells - PBS	0,0001%	0,3 g/l BSA	60 min	44444	44444	44444	44444	33233	22021	00000	0000	0000
Susceptibility of cells - Chirosan plus	n.a.	0,3 g/l BSA	30 min	44444	44444	44444	44444	44444	00211	00000	00000	00000
Virus control	n.a.	0,3 g/l BSA	60 min	44444	44444	44444	44444	43444	00340	20000	00000	00000
Virus control	1,0%	0,3 g/l BSA	60 min	44444	44444	44444	44444	44444	44303	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

Prepared by: Mgr. Ludmila Porubová

Table 8: Raw data of test for product CHIROSAN PLUS to Adenovirus type 5, strain Adenoid 75 – dirty conditions

Product	Concentration	Interfering substance	Contact time	Dilution (log 10)								
				-1	-2	-3	-4	-5	-6	-7	-8	
Chirosan plus	1,0%	3 g/l BSA + erythrocyty	60 min	CT	00000	00000	00000	00000	00000	00000	00000	00000
Chirosan plus	0,5%	3 g/l BSA + erythrocyty	30 min	00000	00000	00000	00000	00000	00000	00000	00000	00000
Chirosan plus	0,5%	3 g/l BSA + erythrocyty	60 min	00000	00000	00000	00000	00000	00000	00000	00000	00000
Chirosan plus	0,1%	3 g/l BSA + erythrocyty	60 min	44444	44444	444444	44444	04000	00000	00000	00000	00000
Cytotoxicity Chirosan plus	1,0%	3 g/l BSA + erythrocyty	n.a.	CT	00000	00000	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Cytotoxicity Chirosan plus	0,5%	3 g/l BSA + erythrocyty	n.a.	00000	00000	00000	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Cytotoxicity Chirosan plus	0,1%	3 g/l BSA + erythrocyty	n.a.	00000	00000	00000	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Cytotoxicity Formaldehyde	0,7% (m/V)	PBS	n.a.	CT	CT	CT	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Formaldehyde	0,7% (m/V)	PBS	30 min	CT	CT	44400	00000	00000	00000	00000	00000	00000
			60 min	CT	CT	00000	00000	00000	00000	00000	00000	00000
Susceptibility of cells - PBS	0,0001%	3 g/l BSA + erythrocyty	60 min	44444	44444	44444	44444	33233	10000	02020	0000	0000
Susceptibility of cells - Chirosan plus	n.a.	3 g/l BSA + erythrocyty	30 min	44444	44444	44444	44444	44444	00111	00110	00000	00000
Virus control	n.a.	3 g/l BSA + erythrocyty	60 min	44444	44444	44444	44444	44444	43231	00000	00000	00000
Virus control	1,0%	3 g/l BSA + erythrocyty	60 min	44444	44444	44444	44444	44444	40020	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

Prepared by: Mgr. Ludmila Porubová

