



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. 24/2014/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:** 00029477  
**Reference number:** ZU/406911/2014

### 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:	12.1. – 23.2.2015

**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%, 0,5% and 0,1% demonstrate virucidal activity after an exposure time 30 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 monolayer on a microtitre plate by reduction of reference virus *Murine norovirus, strain S99* by more than 4 lg.

**Conclusion:**

According to EN 14476 the test product CHIROSAN PLUS after dilution with hard water to 1%, 0,5% and 0,1% demonstrate virucidal activity to non – enveloped viruses Norovirus under the clean and dirty conditions after exposure time 30 min.

In Ostrava, 24.2.2015.

zdravotní ústav se sídlem v Ostravě  
Partyzánské nám. 7, 728 92 Ostrava  
IČ: 71009396

Mgr. Ludmila Porubová  
Guarantor of testing

## Annex to the protocol n.: 24/2014/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: 12.1. – 23.2.2015  
Diluent: hard water  
Testing concentration: 1%, 0,5%, 0,1%  
Activation of solution: 15 min intensive blending  
Appearance of dilution of the product: after dilution remain in solution small crystals of product  
Contact times: 30 min  
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 30 min incubation reaction with erythrocytes, change the colour of solution from red to yellow  
Method of filtration: none  
Test virus: *Murine norovirus, strain S99 (FLI)*, 6.passage, DMEM + 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: RAW 264.7 (FLI), 35. passage, DMEM + 10% FBS  
Titration method: viral titration on cell monolayer on the microplate  
Reference substance: Formaldehyde (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 of the test for product CHIROSAN PLUS to Murine norovirus, strain S99 – clean conditions

Product	Concentration	Interfering substance	Level of cytotoxicity	log 10 TCID50/ ml after ... min					Reduction factor ( $\Delta\log_{10}$ TCID <sub>50</sub> / ml after ... min)
				0,25	5	15	30	60	
CHIROSAN PLUS	1,0%	0,3 g/l BSA	2,5	n.d.	n.d.	n.d.	2,5	n.d.	30
CHIROSAN PLUS	0,5%	0,3 g/l BSA	2,5	n.d.	n.d.	n.d.	2,5	n.d.	4,2
CHIROSAN PLUS	0,1%	0,3 g/l BSA	1,5	n.d.	n.d.	n.d.	1,7	n.d.	4,2
Formaldehyde	0,7% (m/V)	PBS	3,5	n.d.	n.d.	n.d.	4,3	3,7	5,0
Virus control	n.a.	0,3 g/l BSA	n.a.	n.d.	n.d.	n.d.	6,7	6,1	
Susceptibility of cells - PBS	n.a.	0,3 g/l BSA	n.a.	n.d.	n.d.	n.d.	n.d.	5,9	
Susceptibility of cells - Chirosoan Plus	0,001%	0,3 g/l BSA	n.a.	n.d.	n.d.	n.d.	n.d.	6,5	

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 TCID50/ ml after ... min					Reduction factor ( $\Delta\log_{10}$ TCID <sub>50</sub> / ml after ... min)
				0,25	5	15	30	60	
CHIROSAN PLUS	1,0%	3,0 g/l BSA + erythrocyte	2,5	n.d.	n.d.	n.d.	2,5	n.d.	30
CHIROSAN PLUS	0,5%	3,0 g/l BSA + erythrocyte	2,5	n.d.	n.d.	n.d.	2,5	n.d.	4,4
CHIROSAN PLUS	0,1%	3,0 g/l BSA + erythrocyte	1,5	n.d.	n.d.	n.d.	1,5	n.d.	4,4
Formaldehyde	0,7% (m/V)	PBS	3,5	n.d.	n.d.	n.d.	4,3	3,7	5,4
Virus control	n.a.	3,0 g/l BSA + erythrocyte	n.a.	n.d.	n.d.	n.d.	6,9	6,1	
Susceptibility of cells - PBS	n.a.	3,0 g/l BSA + erythrocyte	n.a.	n.d.	n.d.	n.d.	n.d.	5,9	
Susceptibility of cells - Chirosoan Plus	0,001%	3,0 g/l BSA + erythrocyte	n.a.	n.d.	n.d.	n.d.	n.d.	6,3	

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 laboratories - testing laboratory n.1554 accredited according to ISO/IEC 17025. The sample was examined by SOP No.1901

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

Product	Concentration	Interfering substance	Contact time	Dilution (log 10)								
				-1	-2	-3	-4	-5	-6	-7	-8	
Chirosan plus	1,0%	3,0 g/l BSA + erythrocyte	30 min	CT	00000	00000	00000	00000	00000	00000	00000	00000
Chirosan plus	0,5%	3,0 g/l BSA + erythrocyte	30 min	CT	00000	00000	00000	00000	00000	00000	00000	00000
Chirosan plus	0,1%	3,0 g/l BSA + erythrocyte	30 min	00000	00000	00000	00000	00000	00000	00000	00000	00000
Cytotoxicity Chirosan plus	1,0%	3,0 g/l BSA + erythrocyte	n.a.	CT	00000	00000	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Cytotoxicity Chirosan plus	0,5%	3,0 g/l BSA + erythrocyte	n.a.	CT	00000	00000	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Cytotoxicity Chirosan plus	0,1%	3,0 g/l BSA + erythrocyte	n.a.	CT	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	44044	00000	00000	00000	00000	00000	00000
			60 min	CT	CT	00020	00000	00000	00000	00000	00000	00000
Susceptibility of cells - PBS	0,001%	3,0 g/l BSA + erythrocyte	60 min	44444	44444	44444	44444	30003	00000	00000	00000	00000
Susceptibility of cells - Chirosan plus	n.a.	3,0 g/l BSA + erythrocyte	60 min	44444	44444	44444	44444	32444	00000	00000	00000	00000
Virus control	n.a.	3,0 g/l BSA + erythrocyte	30 min	44444	44444	44444	44444	33344	30000	00000	00000	00000
Virus control	n.a.	3,0 g/l BSA + erythrocyte	60 min	44444	44444	44444	44444	44040	00000	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 *Murine norovirus, strain S99* – 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	30 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	0,1%	0,3 g/l BSA	30 min	00004	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	44044	00000	00000	00000	00000	00000	00000
			60 min	CT	CT	00020	00000	00000	00000	00000	00000	00000
Susceptibility of cells - PBS	0,0001%	0,3 g/l BSA	60 min	44444	44444	44444	44444	30003	00000	00000	00000	00000
Susceptibility of cells - Chirosan plus	n.a.	0,3 g/l BSA	60 min	44444	44444	44444	44444	32444	00000	00000	00000	00000
Virus control	n.a.	0,3 g/l BSA	30 min	44444	44444	44444	44444	33344	30000	00000	00000	00000
Virus control	n.a.	0,3 g/l BSA	60 min	44444	44444	44444	44444	44040	00000	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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