



Public Health Institute Ostrava
Center of clinical laboratories
Testing laboratory n. 1554 accredited
according to ISO/IEC 17025
Partyzánské nám.7, 702 00 Ostrava
VAT: CZ71009396
Laboratory for testing virucidal effect



L 1554

TEST REPORT n. 7/2014/SVU

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

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

Order number: 00025032
Reference number: ZU/15824/2014

Identification of disinfectant – sample:

Product name:	CHLORAMIX DT+
Lot number:	000A131106
Expiration:	11/2018
Producer:	Bochemie a.s.
Storage conditions:	-20 až +30°C
Diluent product recommended by the producer for use:	dilution by domestic water
Product appearance:	solid, white tablets, weight of tablet 3,3 -3,5 g
The active substance(s) and its (their) concentration:	Sodium dichloroisocyanurate, dihydrate, active chlorine content min.43% = 1.5 g active Cl ₂ /tablet
Purpose of product:	surface disinfection
Date of delivery of the product:	21.5.2014
Date of test:	22.5. – 7.7.2014

Results - for details see annex

According to EN 14476 the test product CHLORAMIX DT, designed for surface disinfection, working solution 1 tablet in 1,5 l hard water, demonstrate virucidal activity after an exposure time 15 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 plus 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.

According to EN 14476 the test product CHLORAMIX DT, designed for surface disinfection, working solution 1 tablet in 5 l hard water and 1 tablet in 5l hard water demonstrate virucidal activity after an exposure time 15 min at temperature $20^{\circ}\text{C}\pm 1^{\circ}\text{C}$, under clean conditions (0,3 g/l bovine serum albumin), 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.

According to EN 14476 the test product CHLORAMIX DT, designed for surface disinfection, working solution 1 tablet in 5 l hard water and 1 tablet 5l hard water does not demonstrate virucidal activity after an exposure time 15 min at temperature $20^{\circ}\text{C}\pm 1^{\circ}\text{C}$, under dirty conditions (3,0 g/l bovine serum albumin plus 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.

According to EN 14476 the test product CHLORAMIX DT, designed for surface disinfection, working solution 1 tablet in 10 l hard water does not demonstrate virucidal activity after an exposure time 15 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 + erythrocyte), 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.

Conclusion:

According to EN 14476 the test product CHLORAMIX DT working solution 1 tablet in 1,5 l water demonstrate virucidal activity to Norovirus under the clean and dirty conditions after exposure time 15 min.

According to EN 14476 the test product CHLORAMIX DT working solution 1 tablet in 5 l water demonstrate virucidal activity to Norovirus under the clean conditions after exposure time 15 min.

In Ostrava, 8.7.2014.

Čiřivostní ústav se sídlem v Ostravě

Partyzánské nám. 7, 728 92 Ostrava

IČ: 71009396

Mgr. Ludmila Porubová

Guarantor of testing



Annex to the protocol n.: 9/2013/SVU

Identification of product:

Product name:	CHLORAMIX DT
Lot number:	000A131106
Expiration:	11/2018
Producer:	Bochemie a.s.
Date of delivery of product	21.5.2014
Storage conditions:	-20 až +30°C
Diluent:	domestic water
Product appearance:	solid, white tablet
The active compound(s) and its (their) concentration:	Sodium dichloroisocyanurate, dihydrate, active chlorine content min.43% = 1.5 g active Cl ₂ /tablet

Experimental conditions:

	Quantitative suspension test for the determine of virucidal effect of disinfectant EN 14476 (SOP n. 1901)
Date of testing:	22.5. – 7.7.2014
Diluent:	hard water
Testing concentration:	1%, 0,5%, 0,1%
Appearance of dilution of the product:	clear solution
Contact times:	15 min
Testing temperature:	20°C±1°C
Interfering substance:	clean conditions – 0,3 g/l bovine serum albumin (BSA) dirty conditions – 3,0 g/l bovine serum albumin (BSA) + 3,0 ml erythrocyte
Stability of mixture during testing:	stable
Incubation temperature:	37°C±1°C
Method of filtration:	none
Test virus:	<i>Murine norovirus, strain S99</i> (FLI), 4.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), 23.passage, DMEM + 10% FBS
Titration method:	viral titration on monolayer cell culture on the microplate
Reference substance:	Formaldehyd (Sigma-Aldrich, č.š. SZBC2290V)
Titers calculated 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 inactivation test of product
8. Control of susceptibility

Table n.1 The results and validation of the test for product CHLORAMIX DT to Murine norovirus, strain S99 – 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
CHLORAMIX DT	1 tbi/1,5l	0,3 g/l BSA	2,5	n.d.	n.d.	2,5	n.d.	n.d.	15
CHLORAMIX DT	1 tbi/5l	0,3 g/l BSA	1,5	n.d.	n.d.	1,5	n.d.	n.d.	4,0
CHLORAMIX DT	1 tbi/10l	0,3 g/l BSA	1,5	n.d.	n.d.	2,5	n.d.	n.d.	5,0
Formaldehyde + MicroSpin	0,7% (m/V)	PBS	4,5	n.d.	n.d.	n.d.	4,1	3,5	4,0
Virus control	n.a.	0,3 g/l BSA	n.a.	n.d.	n.d.	6,5	n.d.	6,5	
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 - CHLORAMIX DT	1 tbi/1500 l	0,3 g/l BSA	n.a.	n.d.	n.d.	n.d.	n.d.	5,5	

Table n.2 The results and validation of the test for product CHLORAMIX DT to Murine norovirus, strain S99 – 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
CHLORAMIX DT	1 tbi/1,5l	3 g/l BSA + erythrocyte	2,5	n.d.	n.d.	2,5	n.d.	n.d.	15
CHLORAMIX DT	1 tbi/5l	3 g/l BSA + erythrocyte	1,5	n.d.	n.d.	2,9	n.d.	n.d.	4,0
CHLORAMIX DT	1 tbi/10l	3 g/l BSA + erythrocyte	1,5	n.d.	n.d.	3,5	n.d.	n.d.	3,6
Formaldehyde	0,7% (m/V)	PBS	4,5	n.d.	n.d.	n.d.	4,1	3,5	3,0
Virus control	n.a.	3 g/l BSA + erythrocyte	n.a.	n.d.	n.d.	6,5	n.d.	6,5	
Susceptibility of cells - PBS	n.a.	3 g/l BSA + erythrocyte	n.a.	n.d.	n.d.	n.d.	n.d.	5,9	
Susceptibility of cells - CHLORAMIX DT	1 tbi/1500 l	3 g/l BSA + erythrocyte	n.a.	n.d.	n.d.	n.d.	n.d.	5,5	

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 3: Raw data of test for product CHLORAMIX DT to *Murine norovirus, strain S99* – clean conditions

Product	Concentration	Interfering substance	Contact time	Dilution (log 10)								
				-1	-2	-3	-4	-5	-6	-7	-8	
CHLORAMIX DT	1 tbl/1,5l	0,3 g/l BSA	15 min	CT	00000	00000	00000	00000	00000	00000	00000	00000
CHLORAMIX DT	1 tbl/5l	0,3 g/l BSA	15 min	00000	00000	00000	00000	00000	00000	00000	00000	00000
CHLORAMIX DT	1 tbl/10l	0,3 g/l BSA	15 min	44444	00000	00000	00000	00000	00000	00000	00000	00000
Cytotoxicity CHLORAMIX DT	1 tbl/1,5l	0,3 g/l BSA	n.a.	CT	00000	00000	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Cytotoxicity CHLORAMIX DT	1 tbl/5l	0,3 g/l BSA	n.a.	00000	00000	00000	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Cytotoxicity CHLORAMIX DT	1 tbl/10l	0,3 g/l BSA	n.a.	00000	00000	00000	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Cytotoxicity Formaldehyde + MicroSpin	0,7% (m/V)	PBS	n.a.	CT	CT	00000	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Formaldehyd + MicroSpin	0,7% (m/V)	PBS	30 min	CT	CT	40044	00000	00000	00000	00000	00000	00000
			60 min	CT	CT	00000	00000	00000	00000	00000	00000	00000
Susceptibility of cells - PBS	n.a.	0,3 g/l BSA	60 min	44444	44444	44444	44444	00440	00000	00000	00000	00000
Susceptibility of cells - CHLORAMIX DT	1 tbl/1500l	0,3 g/l BSA	60 min	44444	44444	44444	04444	00004	00000	00000	00000	00000
Virus control	n.a.	0,3 g/l BSA	15 min	44444	44444	44444	44444	44444	00000	00000	00000	00000
Virus control	n.a.	0,3 g/l BSA	60 min	44444	44444	44444	44444	44044	00440	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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Table 4: Raw data of test for product CHLORAMIX DT to *Murine norovirus, strain S99* – dirty conditions

Product	Concentration	Interfering substance	Contact time	Dilution (log 10)								
				-1	-2	-3	-4	-5	-6	-7	-8	
CHLORAMIX DT	1 tbl/1,5l	3 g/l BSA + erythrocyte	15 min	CT	00000	00000	00000	00000	00000	00000	00000	00000
CHLORAMIX DT	1 tbl/5l	3 g/l BSA + erythrocyte	15 min	44444	02200	00000	00000	00000	00000	00000	00000	00000
CHLORAMIX DT	1 tbl/10l	3 g/l BSA + erythrocyte	15 min	44444	34444	00000	00000	00000	00000	00000	00000	00000
Cytotoxicity CHLORAMIX DT	1 tbl/1,5l	3 g/l BSA + erythrocyte	n.a.	CT	00000	00000	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Cytotoxicity CHLORAMIX DT	1 tbl/5l	3 g/l BSA + erythrocyte	n.a.	00000	00000	00000	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Cytotoxicity CHLORAMIX DT	1 tbl/10l	3 g/l BSA + erythrocyte	n.a.	00000	00000	00000	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Cytotoxicity Formaldehyde + MicroSpin	0,7% (m/V)	PBS	n.a.	CT	CT	00000	00000	n.a.	n.a.	n.a.	n.a.	n.a.
Formaldehyd + MicroSpin	0,7% (m/V)	PBS	30 min	CT	CT	40044	00000	00000	00000	00000	00000	00000
			60 min	CT	CT	00000	00000	00000	00000	00000	00000	00000
Susceptibility of cells - PBS	n.a.	3 g/l BSA + erythrocyte	60 min	44444	44444	44444	44044	04044	00000	00000	00000	00000
Susceptibility of cells - CHLORAMIX DT	1 tbl/1500l	3 g/l BSA + erythrocyte	60 min	44444	44444	44444	44444	00000	00000	00000	00000	00000
Virus control	n.a.	3 g/l BSA + erythrocyte	15 min	44444	44444	44444	44444	44444	00000	00000	00000	00000
Virus control	n.a.	3 g/l BSA + erythrocyte	60 min	44444	44444	44444	44444	44044	00440	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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