



Public Health Institute Ostrava
Centre of Clinical Laboratories
Location 1 - Ostrava
Laboratory for testing virucidal activity
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TEST REPORT n. 158/2020/SVU

Quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity of disinfectants
Test method and requirements (phase 2/ step 2) according to EN 16777:2018

Customer:
SCHULKE CZ
Lidická 445
735 81 Bohumín

Order number: not provided
Date of delivery: 23.10.2020
Reference number: ZU/30414/2020

Identification of disinfectant – sample:

Name of the product ⁱ :	chloramix DT
Batch number ⁱ :	604453
Expiry date ⁱ :	10/2023
Manufacturing date ⁱ :	not provided
Manufacturer ⁱ :	SCHULKE CZ, s.r.o.
Storage conditions ⁱ :	-20 °C to +30 °C
Product diluent recommended by the manufacturer ⁱ :	water
Active substance(s) and concentration(s) ⁱ :	Sodium dichloroisocyanurate, dihydrate 750g / kg
Purpose of product ⁱ :	PT2 – surface disinfection in medical area
Appearance of the product:	white tablet, slight chlorine odor
Date of delivery:	22.1.2021
Date(s) of tests (period of analysis):	25.1. – 19.4.2021

ⁱ Data provided by customer.

Results - for details see annex:

According to EN 16777:2018 the test product **chloramix DT**, lot. n. 604453, designed for surface disinfection in medical area, diluted 1 tablet to 1,5 l of hard water, reduced virus titre $4,333 \pm 0,267$ lg 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 *Adenovirus type 5, strain Adenoid 75*, i.e. **demonstrated virucidal activity to Adenovirus by more than 4 lg.***

According to EN 16777:2018 the test product **chloramix DT**, lot. n. 604453, designed for surface disinfection in medical area, diluted 1 tablet to 5 l of hard water, reduced virus titre $4,250 \pm 0,351$ lg 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*, i.e. **demonstrated virucidal activity to Norovirus by more than 4 lg.***

According to EN 16777:2018 the test product **chloramix DT**, lot. n. 604453, designed for surface disinfection in medical area, diluted 1 tablet to 5 l of hard water, reduced virus titre $4,667 \pm 0,367$ lg 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 + 3,0 ml/l erythrocytes), using viral titration on monolayer cell culture on a microtitre plate by reduction of reference virus *Murine norovirus, strain S99*, i.e. **demonstrated virucidal activity to Norovirus by more than 4 lg.***

According to EN 16777:2018 the test product **chloramix DT**, lot. n. 604453, designed for surface disinfection in medical area, diluted 1 tablet to 1,5 l of hard water, reduced virus titre $4,583 \pm 0,397$ lg 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*, i.e. **demonstrated virucidal activity to Norovirus by more than 4 lg.***

According to EN 16777:2018 the test product **chloramix DT**, lot. n. 604453, designed for surface disinfection in medical area, diluted 1 tablet to 5 l of hard water, reduced virus titre $4,500 \pm 0,333$ lg 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 + 3,0 ml/l erythrocytes), using viral titration on monolayer cell culture on a microtitre plate by reduction of reference virus *Murine norovirus, strain S99*, i.e. **demonstrated virucidal activity to Norovirus by more than 4 lg.***

**The statement of compliance is based on a 95% coverage probability for the expanded uncertainty.*

Conclusion and interpretation:

According to EN 16777:2018 the test product **chloramix DT**, lot. n. 604453, designed for surface disinfection in medical area, diluted 1 tablet to 1,5 l of hard water demonstrated limited spectrum virucidal activity under the clean conditions after exposure time 15 min.

In Ostrava, 28.4.2021

Authorized by: Mgr. Ludmila Porubová

Guarantor of testing

Zdravotní ústav se sídlem v Ostravě
Centrum klinických laboratoří
Oddělení virologie
Laboratoř pro testování virucidního účinku
Partyzánské náměstí 2633/7
Moravská Ostrava 702 00 Ostrava
Telefon: 596 200 400

No part of this report may be reproduced in any form without the written permission of the testing laboratory. The test results relate only to the test sample as received. The laboratory is not responsible for the data provided by the customer. Centre of Clinical Laboratories - Testing Laboratory No. 1554 accredited by ČIA according to ČSN EN ISO / IEC 17025: 2018. The list of accredited methods is available at www.zuova.cz. The sample was examined according to SOP No. 1902.

Annex to the protocol n.: 158/2020/SVU

Identification of product:

Name of the product ⁱ: **chloramix DT**
Batch number ⁱ: 604453
Expiry date ⁱ: 10/2023
Manufacturing date ⁱ: not provided
Manufacturer ⁱ: SCHULKE CZ, s.r.o.
Date of delivery: 22.1.2021
Storage conditions ⁱ: -20 °C to +30 °C
Product diluent recommended by the manufacturer ⁱ: water
Appearance of the product: white tablet, slight chlorine odor
Active substance(s) and concentration(s) ⁱ: Sodium dichloroisocyanurate, dihydrate 750g / kg

Purpose of product ⁱ: PT2 – surface disinfection in medical area

Experimental conditions: Quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity of disinfectants according to EN 16777:2018 (SOP n. 1902)

Date(s) of tests (period of analysis): 25.1. – 19.4.2021
Diluent: hard water
Testing concentration ⁱ: 1 tbl/ 5l water; 1 tbl/1,5l water
Other testing concentration: 1 tbl/ 15l water
Appearance of dilution of the product: clear liquid
Contact times ⁱ: 5 min, 15 min, 30 min
Testing temperature ⁱ: 20 °C±1 °C
Interfering substance ⁱ: clean conditions – 0,3 g/l Bovine serum albumin
dirty conditions – 3,0 g/l Bovine serum albumin + 3,0 ml/l sheep erythrocyte
Stability of mixture during testing: stable
Incubation temperature: 37°C±1°C
Method of filtration: MicroSpin
Test virus: *Adenovirus type 5, strain Adenoid 75* (ATCC), 5., 7. passage, EMEM + 2% FBS
Murine norovirus, strain S99 /FLI), 9. passage, DMEM + 2% FBS
Cell line: - HeLa cells (ATCC), 53., 64., 28. passage, EMEM + 10% FBS
RAW 264.7 (FLI), 35., 37. Passage, DMEM + 10% FBS
Process to stop action of product: virucidal activity of product is suppressed by transferring the sample into the ice cold diluent
Titration method: viral titration on monolayer cell culture on the microplates
Reference substance: Glutaraldehyde (Alfa-Aesar, lot. n. 10204382)
Titers calculated by: Spaerman – Kärber's method

ⁱ Data provided by customer

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 Adenovirus type 5, strain Adenoid 75

Product	Concentration	Interfering substance	Level of cytotoxicity	log 10 TCID ₅₀ / ml after ... min				Reduction factor ($\Delta\log_{10}$ TCID ₅₀ / ml after ... min)	
				1	5	15	60	5	15
chloramix DT	1 tbl/1,5 l	0,3 g/l BSA	1,5	n.d.	4,417 ± 0,188	2,083 ± 0,070	n.d.	2,000 ± 0,440	4,333 ± 0,267
chloramix DT	1 tbl/1,5 l	3,0 g/l BSA + erythrocytes	1,5	n.d.	5,417 ± 0,196	3,750 ± 0,160	n.d.	1,000 ± 0,454	2,667 ± 0,392
chloramix DT	1 tbl/5 l	0,3 g/l BSA	1,5	n.d.	6,083 ± 0,184	5,417 ± 0,188	n.d.	0,333 ± 0,432	1,000 ± 0,440
chloramix DT	1 tbl/5 l	3,0 g/l BSA + erythrocytes	1,5	n.d.	6,333 ± 0,270	6,000 ± 0,184	n.d.	0,083 ± 0,508	0,417 ± 0,432
chloramix DT	1 tbl/15 l	0,3 g/l BSA	1,5	n.d.	5,583 ± 0,170	5,167 ± 0,165	n.d.	0,750 ± 0,607	1,167 ± 0,601
chloramix DT	1 tbl/15 l	3,0 g/l BSA + erythrocytes	1,5	n.d.	6,250 ± 0,170	6,250 ± 0,114	n.d.	0,167 ± 0,409	0,167 ± 0,321
Virus control	n.a.	3,0 g/l BSA + erythrocytes	n.a.	n.d.	6,417 ± 0,114	n.d.	n.d.		
Virus control	n.a.	0,3 g/l BSA	n.a.	n.d.	6,417 ± 0,114	n.d.	n.d.		
Glutaraldehyde	0,0125%	0,3 g/l BSA	1,5		4,417 ± 0,114			2,083 ± 0,432	
Virus control	n.a.	0,3 g/l BSA	n.a.		6,500 ± 0,184				

Table n.2 The results and validation of the test for product chloramix DT to Adenovirus type 5, strain Adenoid 75

Product	Concentration	Interfering substance	Level of cytotoxicity	log 10 TCID ₅₀ / ml after ... min				Reduction factor ($\Delta\log_{10}$ TCID ₅₀ / ml after ... min)	
				1	5	30	60	5	30
chloramix DT	1 tbl/1,5 l	3,0 g/l BSA + erythrocytes	1,5	n.d.	n.d.	4,083 ± 0,160	n.d.	2,833 ± 0,516	
Virus control	n.a.	3,0 g/l BSA + erythrocytes	n.a.	n.d.	n.d.	6,917 ± 0,203	n.d.		
Glutaraldehyde	0,0125%	0,3 g/l BSA	1,5		3,500 ± 0,000			3,250 ± 0,319	
Virus control	n.a.	0,3 g/l BSA	n.a.		6,750 ± 0,160				

Prepared by: Mgr. Ludmila Porubová

Table n.3 The results and validation of the test for product chloramix DT to Murine norovirus, strain S99

Product	Concentration	Interfering substance	Level of cytotoxicity ↓	log ₁₀ TCID ₅₀ / ml after ... min				Reduction factor (Δlog ₁₀ TCID ₅₀ / ml after ... min)	
				1	5	15	60	5	15
chloramix DT	1 tbi/5 l	0,3 g/l BSA	1,5	n.d.	3,583 ± 0,261	2,083 ± 0,130	n.d.	2,750 ± 0,573	4,250 ± 0,351
chloramix DT	1 tbi/5 l	3,0 g/l BSA + erythrocytes	1,5	n.d.	2,833 ± 0,189	1,667 ± 0,141	n.d.	3,500 ± 0,445	4,667 ± 0,367
chloramix DT	1 tbi/1,5 l	0,3 g/l BSA	1,5	n.d.	3,083 ± 0,196	1,750 ± 0,160	n.d.	3,250 ± 0,458	4,583 ± 0,397
chloramix DT	1 tbi/1,5 l	3,0 g/l BSA + erythrocytes	1,5	n.d.	3,083 ± 0,070	1,833 ± 0,118	n.d.	3,250 ± 0,275	4,500 ± 0,333
chloramix DT	1 tbi/15 l	0,3 g/l BSA	1,5	n.d.	n.d.	1,750 ± 0,170	n.d.	n.d.	4,583 ± 0,414
chloramix DT	1 tbi/15 l	3,0 g/l BSA + erythrocytes	1,5	n.d.	n.d.	1,750 ± 0,160	n.d.	n.d.	4,583 ± 0,397
Virus control	n.a.	0,3 g/l BSA	n.a.	n.d.	6,500 ± 0,000	n.d.	n.d.		
Virus control	n.a.	3,0 g/l BSA + erythrocytes	n.a.	n.d.	6,333 ± 0,118	n.d.	n.d.		
					5			5	
Glutaraldehyde	0,1%	0,3 g/l BSA	2,5		4,583 ± 0,070			1,917 ± 0,141	
Virus control	n.a.	0,3 g/l BSA	n.a.		6,500 ± 0,000				

Prepared by: Mgr. Ludmila Porubová

Table n.4 Raw data of test for product chloramix DT to Adenovirus type 5, strain Adenoid 75

Product	Concentration	Interfering substance	Contact time	Dilution (log 10)						
				-2	-3	-4	-5	-6	-7	-8
chloramix DT	1 tbl/1,5l	0,3 g/l BSA	5 min	444444	444444	444444	324222	232002	002000	000000
				444444	444444	444444	234222	230023	002000	000000
chloramix DT	1 tbl/1,5l	0,3 g/l BSA	15 min	444444	444444	444444	324222	020000	000000	000000
				444444	444444	444444	323422	022013	000020	000000
chloramix DT	1 tbl/1,5l	3,0 g/l BSA + erythrocytes	5 min	444444	444444	444444	444444	324422	000100	000000
				444444	444444	444444	444444	020000	00010	000000
chloramix DT	1 tbl/1,5l	3,0 g/l BSA + erythrocytes	15 min	444444	444444	444444	444444	023000	020000	000000
				444444	444444	444444	444444	321422	000000	000000
chloramix DT	1 tbl/5l	0,3 g/l BSA	5 min	444444	444444	444444	324222	232002	002000	000000
				444444	444444	444444	234222	230023	002000	000000
chloramix DT	1 tbl/5l	0,3 g/l BSA	15 min	444444	444444	444444	324222	020000	000000	000000
				444444	444444	444444	323422	022013	000020	000000
chloramix DT	1 tbl/5l	3,0 g/l BSA + erythrocytes	5 min	444444	444444	444444	444444	324422	000100	000000
				444444	444444	444444	444444	020000	00010	000000
chloramix DT	1 tbl/5l	3,0 g/l BSA + erythrocytes	15 min	444444	444444	444444	444444	023000	020000	000000
				444444	444444	444444	444444	321422	000000	000000
chloramix DT	1 tbl/15l	0,3 g/l BSA	15 min	444444	444444	444444	324222	232002	002000	000000
				444444	444444	444444	234222	230023	002000	000000
chloramix DT	1 tbl/15l	3,0 g/l BSA + erythrocytes	15 min	444444	444444	444444	324222	020000	000000	000000
				444444	444444	444444	323422	022013	000020	000000
Cytotoxicity chloramix DT	1 tbl/5l	0,3 g/l BSA	n.a.	000000	000000	000000	n.d.	n.d.	n.d.	n.d.
Cytotoxicity chloramix DT	1 tbl/5l	3,0 g/l BSA + erythrocytes	n.a.	000000	000000	000000	n.d.	n.d.	n.d.	n.d.
Cytotoxicity chloramix DT	1 tbl/1,5l	0,3 g/l BSA	n.a.	000000	000000	000000	n.d.	n.d.	n.d.	n.d.
Cytotoxicity chloramix DT	1 tbl/1,5l	3,0 g/l BSA + erythrocytes	n.a.	000000	000000	000000	n.d.	n.d.	n.d.	n.d.
Cytotoxicity chloramix DT	1 tbl/15l	0,3 g/l BSA	n.a.	000000	000000	000000	n.d.	n.d.	n.d.	n.d.
Cytotoxicity chloramix DT	1 tbl/15l	3,0 g/l BSA + erythrocytes	n.a.	000000	000000	000000	n.d.	n.d.	n.d.	n.d.
Virus control	n.a.	0,3 g/l BSA	5 min	444444	444444	444444	444444	231122	000000	000000
				444444	444444	444444	444444	130022	000100	000000
Virus control	n.a.	3,0 g/l BSA + erythrocyty	5 min	444444	444444	444444	444444	222313	000000	000000
				444444	444444	444444	444444	323020	000002	000000
Cytotoxicity Glutaraldehyde	0,0125%	0,3 g/l BSA	n.a.	000000	000000	000000	n.d.	n.d.	n.d.	n.d.
Glutaraldehyde	0,0125%	0,3 g/l BSA	5 min	444444	320220	020000	000000	000000	000000	000000
				444444	023240	002100	000000	000000	000000	000000
Virus control	n.a.	0,3 g/l BSA	5 min	444444	444444	444444	444444	300422	020000	000000
				444444	444444	444444	443234	323003	033000	000000

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 Raw data of test for product chloramix DT to Adenovirus type 5, strain Adenoid 75

Produkt	Koncentrace produktu	Interferující podmínky	Doba kontaktu	Ředění (log 10)						
				-2	-3	-4	-5	-6	-7	-8
chloramix DT	1 tbl/1,5l	3,0 g/l BSA + erythrocytes	30 min	444444	333212	001200	000000	000000	000000	000000
				444444	231122	130221	000000	000000	000000	000000
Cytotoxicity chloramix DT	1 tbl/5l	3,0 g/l BSA + erythrocytes	n.a.	000000	000000	000000	n.d.	n.d.	n.d.	n.d.
Virus control	n.a.	3,0 g/l BSA + erythrocytes	30 min	444444	444444	444444	444444	323422	000220	100000
				444444	444444	444444	444444	223123	020100	000000
Cytotoxicity Glutaraldehyde	0,0125%	0,3 g/l BSA	n.a.	000000	000000	000000	n.d.	n.d.	n.d.	n.d.
Glutaraldehyde	0,0125%	0,3 g/l BSA	5 min	444444	323212	000000	000000	000000	000000	000000
				444444	231122	000000	000000	000000	000000	000000
Virus control	n.a.	0,3 g/l BSA	5 min	444444	444444	444444	444444	323422	002000	000000
				444444	444444	444444	444444	223123	020100	000000

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.6 Raw data of test for product chloramix DT to *Murine norovirus, strain S99*

Produkt	Koncentrace produktu	Interferující podmínky	Doba kontaktu	Ředění (log 10)						
				-2	-3	-4	-5	-6	-7	-8
chloramix DT	1 tbl/5l	0,3 g/l BSA	5 min	444444	444440	043000	000000	000000	000000	000000
				444444	404000	400000	000000	000000	000000	000000
chloramix DT	1 tbl/5l	0,3 g/l BSA	15 min	000000	000000	000000	000000	000000	000000	000000
				444004	404400	000000	000000	000000	000000	000000
chloramix DT	1 tbl/5l	3,0 g/l BSA + erythrocytes	5 min	444444	034000	000000	000000	000000	000000	000000
				444444	003000	300000	000000	000000	000000	000000
chloramix DT	1 tbl/5l	3,0 g/l BSA + erythrocytes	15 min	000030	000000	000000	000000	000000	000000	000000
				400000	000000	000000	000000	000000	000000	000000
chloramix DT	1 tbl/1,5l	0,3 g/l BSA	5 min	444444	440044	004400	000000	000000	000000	000000
				444444	400000	000000	000000	000000	000000	000000
chloramix DT	1 tbl/1,5l	0,3 g/l BSA	15 min	440000	000000	000000	000000	000000	000000	000000
				000040	000000	000000	000000	000000	000000	000000
chloramix DT	1 tbl/1,5l	3,0 g/l BSA + erythrocytes	5 min	444444	444444	000040	000000	000000	000000	000000
				444444	000000	000000	000000	000000	000000	000000
chloramix DT	1 tbl/1,5l	3,0 g/l BSA + erythrocytes	15 min	444000	040000	000000	000000	000000	000000	000000
				000000	000000	000000	000000	000000	000000	000000
chloramix DT	1 tbl/15l	0,3 g/l BSA	15 min	040000	000000	000000	000000	000000	000000	000000
				040000	000400	000000	000000	000000	000000	000000
chloramix DT	1 tbl/15l	3,0 g/l BSA + erythrocytes	15 min	040000	000000	000000	000000	000000	000000	000000
				044000	000000	000000	000000	000000	000000	000000
Cytotoxicity chloramix DT	1 tbl/5l	0,3 g/l BSA	n.a.	000000	000000	000000	n.d.	n.d.	n.d.	n.d.
Cytotoxicity chloramix DT	1 tbl/5l	3,0 g/l BSA + erythrocytes	n.a.	000000	000000	000000	n.d.	n.d.	n.d.	n.d.
Cytotoxicity chloramix DT	1 tbl/1,55l	0,3 g/l BSA	n.a.	000000	000000	000000	n.d.	n.d.	n.d.	n.d.
Cytotoxicity chloramix DT	1 tbl/1,5l	3,0 g/l BSA + erythrocytes	n.a.	000000	000000	000000	n.d.	n.d.	n.d.	n.d.
Cytotoxicity chloramix DT	1 tbl/15l	0,3 g/l BSA	n.a.	000000	000000	000000	n.d.	n.d.	n.d.	n.d.
Cytotoxicity chloramix DT	1 tbl/15l	3,0 g/l BSA + erythrocytes	n.a.	000000	000000	000000	n.d.	n.d.	n.d.	n.d.
Virus control	n.a.	0,3 g/l BSA	5 min	444444	444444	444444	444444	444444	000000	000000
				444444	444444	444444	444444	444444	000000	000000
Virus control	n.a.	3,0 g/l BSA + erythrocytes	5 min	444444	444444	444444	444444	444444	000000	000000
				444444	444444	444444	444444	444000	040000	000000
Cytotoxicity Glutaraldehyde	0,1%	0,3 g/l BSA	n.a.	CT	000000	000000	n.d.	n.d.	n.d.	n.d.
Glutaraldehyde	0,1%	0,3 g/l BSA	5 min	CT	444444	444444	400000	000000	000000	000000
				CT	444444	444444	000000	000000	000000	000000
Virus control	n.a.	0,3 g/l BSA	5 min	444444	444444	444444	444444	444444	000000	000000
				444444	444444	444444	444444	444444	000000	000000

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

END OF THE PROTOCOL