Public Health Institute Ostrava
Centre of Clinical Laboratories
Location 1 - Ostrava
Partyzánské náměstí 2633/7, Moravská Ostrava, 702 00 Ostrava
VAT: CZ71009396

L 1554

TEST REPORT n. 35/2022/V

Customer:
SCHULKE CZ s.r.o.
Lidická 445
735 81 Bohumín

Order number: not provided
Date of delivery: 26.5.2022
Reference number: ZU/13724/2022

Digitally signed by Gritaenco Andrei
Date: 2022.12.26 15:38:39 EET
Reason: MoldSign Signature
Location: Moldova

Identification of sample:

Number of sample:	**35/2022**
Name of the product [i]:	**chloramix® dt**
Batch number [i]:	614688
Expiry date [i]:	01/2025
Manufacturing date [i]:	not provided
Manufacturer [i]:	SCHULKE CZ s.r.o., Lidická 445, 735 81 Bohumín
Storage conditions [i]:	-20 °C to +30 °C, ventilated and dry area
Product diluent recommended by the manufacturer [i]:	water
Active substance(s) and concentration(s)[i]:	750 g/kg sodium dihydrate dichloroisocyanurate active chlorine content: 1.5 g act. Cl_2/tbl
Auxiliary substance(s) and concentration(s) [i]:	-
Purpose of product [i]:	PT2, PT4
Appearance of the sample:	tablets of white color, chlorine odor
Date of delivery:	26.5.2022
Test method:	**CSN EN 16777:2020**
Date(s) of tests (period of analysis):	2.6. – 16.6.2022
Location of tests:	Location 1 - Ostrava

[i] Data provided by customer.

Results - for details see annex:

According to CSN EN 16777:2020 the test sample 35/2022 of product **chloramix®dt** lot. n. 614688, designed for surface disinfection, diluted by hard water 2 tbl/1 l, after an exposure time 15 min at temperature 20°C±1°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 reduced virus titre of reference virus:

Adenovirus type 5, strain Adenoid 75 by 4,500 ± 0,537[1] lg, i.e. demonstrated activity to virus Adenovirus at least than 4 lg.*

According to CSN EN 16777:2020 the test sample 35/2022 of product **chloramix®dt** lot. n. 614688, designed for surface disinfection, diluted by hard water 3 tbl/1 l, after an exposure time 15 min at temperature 20°C±1°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 reduced virus titre of reference virus:

Adenovirus type 5, strain Adenoid 75 by 4,500 ± 0,537[1] lg, i.e. demonstrated activity to virus Adenovirus at least than 4 lg.*

All test criteria were within limits.

[1] 95% cofidence interval calculated according to CSN EN 14476 + A2 of Annex C.
*A decision rule with neglect of uncertainty was applied to the consensus statement.

Opinion and interpretation:

Due to the proven activity of the tested sample 35/2022 of the product **chloramix®dt** according to CSN EN 16777:2020 in the medical area, it is also possible to use the product for other than in medical area and the food and feed area.

Conclusion:

The test sample 35/2022 of product **chloramix®dt** lot. n. 614688, designed for surface disinfection, diluted by water 2 tbl/l and 3 tbl/1 l demonstrated virucidal activity to Adenovirus according to the criteria given by the standard CSN EN 16777 under the dirty conditions after exposure time 15 min.*

*A decision rule with neglect of uncertainty was applied to the consensus statement.

Conclusion, opinion and interpretation prepared by: Mgr. Ludmila Porubová

In Ostrava, 1.7.2022

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

Authorized by: Mgr. Ludmila Porubová
Guarantor of testing
Laboratory for testing virucidal aktivity

Annex to the protocol n.: 35/2022/V

Identification of sample:	
Number of sample:	**35/2022**
Name of the product [i]:	**chloramix®dt**
Batch number [i]:	614688
Expiry date [i]:	01/2025
Manufacturing date [i]:	not provided
Manufacturer [i]:	SCHULKE CZ s.r.o.
Date of delivery:	26.5.2022
Storage conditions [i]:	-20 °C to +30 °C, ventilated and dry area
Product diluent recommended by the manufacturer [i]:	water
Appearance of the sample:	tablets of white color, chlorine odor
Active substance(s) and concentration(s) [i]:	750 g/kg sodium dihydrate dichloroisocyanurate active chlorine content: 1.5 g act. Cl2/tbl
Auxiliary substance(s) and concentration(s) [i]:	-
Purpose of product [i]:	PT2, PT4

Experimental conditions:	
Test method:	Chemical disinfectants and antiseptics - Quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity of chemical disinfectants used in the medical area - Test method and requirements (phase 2, step 2) according to CSN EN 16777:2020 (SOP n. 11002)
Date(s) of tests (period of analysis):	2.6. – 16.6.2022
Diluent:	hard water
Testing concentration [i]:	2 tbl/1l, 2 tbl/1,5 l, 3 tbl/1l
Other testing concentration:	-
Appearance of dilution of the sample:	clear colourless liquid
Contact times [i]:	15 minut
Testing temperature [i]:	20 °C±1 °C
Interfering substance [i]:	dirty conditions – 3,0 g/l bovine serum albumin + 3,0 ml/l sheep erythrocytes
Stability of mixture during testing:	stable
Incubation temperature:	37 °C±1 °C
Method of filtration:	-
Test virus:	*Adenovirus type 5, strain Adenoid 75* (ATCC-VR-5), 5. passage, EMEM + 2% FBS
Cell line:	HeLa cells (ATCC-CCL-2), EMEM +10% FBS
Process to stop action of sample:	virucidal activity of sample 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 (ThermoFisher GmbH, Lot.n.. 10204382)
Titers calculated by:	Spaerman – Kärber's method

[i] Data provided by customer.

Table n. 1: Test results for the test sample 35/2022 of product chloramix®dt for *Adenovirus type 5*, strain Adenoid 75 — dirty conditions

	Concentration	Interfering substance	Level of cytotoxicity	Contact time	lg $TCID_{50}$/ml ±Sm^2
chloramix®dt	2 tbl/l	3 g/l BSA + erythrocytes	2,5	15 min	≤2,801 ± 0,000
chloramix®dt	2 tbl/1,5l	3 g/l BSA + erythrocytes	2,5	15 min	4,468 ± 0,218
chloramix®dt	3 tbl/l	3 g/l BSA + erythrocytes	2,5	15 min	≤2,801± 0,000
Virus control	n.a.	3 g/l BSA + erythrocytes	n.a.	15 min	7,301 ± 0,269
Reference test	Concentration	Interfering substance	Level of cytotoxicity	Contact time	lg $TCID_{50}$/ml ±Sm^2
Glutaraldehyde	0,0125% (m/V)	0,3 g/l BSA	1,5	5 min	4,218 ± 0,189
Virus control	n.a.	0,3 g/l BSA	n.a.	5 min	7,634 ± 0,223
Interference control – control of cell susceptibility	Concentration	Interfering substance	Level of cytotoxicity	Contact time	lg $TCID_{50}$/ml ±Sm^2
chloramix®dt	0,003 tbl/l	3 g/l BSA + erythrocytes	n.a.	60 min	6,801 ± 0,000
PBS	n.a.	3 g/l BSA + erythrocytes	n.a.	60 min	7,301 ± 0,224
Control of efficiency of suppression of sample activity	Concentration	Interfering substance	Level of cytotoxicity	Contact time	lg $TCID_{50}$/ml ±Sm^2
chloramix®dt	3 tbl/l	3 g/l BSA + erythrocytes	n.a.	30 min	7,468 ± 0,211
virus control without carrier	n.a.	3 g/l BSA + erythrocytes	n.a.	30 min	7,801 ± 0,000

[2] Standard deviation of the logarithmic titre calculated according to ČSN EN 16777 Annex C.
n.a. not relevant
n.d. not tested
BSA bovine serum albumin
PBS polyphosphate buffer

Prepared by: Mgr. Ludmila Porubová

Table n. 2: The reduction for the test sample 35/2022 of product chloramix®dt to *Adenovirus type 5*, Adenoid strain 75 – dirty conditions

	Concentration	Interfering substance	Contact time	Reduktion (*R*) Δlg $TCID_{50}$/ ml $\pm K_R$[1]	Condition fulfilled $\Delta \geq 4$[3]
chloramix®dt	2 tbl/l	3 g/l BSA + erythrocytes	15 min	≥4,500 ± 0,537	YES
chloramix®dt	2 tbl/1,5l	3 g/l BSA + erythrocytes	15 min	2,833 ± 0,692	NO
chloramix®dt	3 tbl/l	3 g/l BSA + erythrocytes	15 min	≥4,500 ± 0,537	YES
Reference test	Concentration	Interfering substance	Contact time	Reduktion (*R*) Δlg $TCID_{50}$/ ml $\pm K_R$[1]	5 min: Δ 2,0 - 3,5
Glutaraldehyde	0,0125% (m/V)	0,3 g/l BSA	5 min	3,417 ± 0,585	YES
Interference control – control of cell susceptibility	Concentration	Interfering substance	Contact time	Reduktion (*R*) Δlg $TCID_{50}$/ ml	<1
chloramix®dt	0,003 tbl/l	3 g/l BSA + erythrocytes	60 min	0,500	YES
Control of efficiency of suppression of samples activity	Concentration	Interfering substance	Contact time	Reduktion (*R*) Δlg $TCID_{50}$/ ml	≤0,5
chloramix®dt	3 tbl/l	3 g/l BSA + erythrocytes	30 min	0,333	YES

[1] 95% reduction confidence interval calculated according to ČSN EN 16777 Annex C

[3] If the cytotoxicity is so great that the residual infectivity titre is less than the required 4 lg TCID50 and the reduction of 4 lg cannot be determined, the result is chosen for evaluation using the MicroSpin box.

n.a. not relevant

n.d. not tested

BSA bovine serum albumin

Prepared by: Mgr. Ludmila Porubová

Table n.3 Raw date for the test sample 35/2022 of product chloramix®dt to *Adenovirus type 5, strain Adenoid 75* – dirty condition

	Concentration	Interfering substance	Contact time	Dilution (lg)						
				-1	-2	-3	-4	-5	-6	-7
chloramix®dt	2 tbl/l	3 g/l BSA + erythrocytes	15 min	CT	000000	000000	000000	000000	000000	000000
				CT	000000	000000	000000	000000	000000	000000
chloramix®dt	2 tbl/1,5l	3 g/l BSA + erythrocytes	15 min	CT	230221	100300	000000	000000	000000	000000
				CT	444444	321221	020000	000000	000000	000000
chloramix®dt	3 tbl/l	3 g/l BSA + erythrocytes	15 min	CT	000000	000000	000000	000000	000000	000000
				CT	000000	000000	000000	000000	000000	000000
cytotoxicity chloramix®dt	2 tbl/l	3 g/l BSA + erythrocytes	n.a.	CT	000000	000000	n.d.	n.d.	n.d.	n.d.
cytotoxicity chloramix®dt	2 tbl/1,5l	3 g/l BSA + erythrocytes	n.a.	CT	000000	000000	n.d.	n.d.	n.d.	n.d.
cytotoxicity chloramix®dt	3 tbl/l	3 g/l BSA + erythrocytes	n.a.	CT	000000	000000	n.d.	n.d.	n.d.	n.d.
Virus control	n.a.	3 g/l BSA + erythrocytes	15 min	444444	444444	444444	444444	324223	023000	002000
				444444	444444	444444	444444	232344	020020	100000
Cytotoxicity Glutaraldehyde	0,0125% (m/V)	0,3 g/l BSA	n.a.	000000	000000	000000	n.d.	n.d.	n.d.	n.d.
Glutaraldehyde	0,0125% (m/V)	0,3 g/l BSA	5 min	444444	324222	120023	000000	000000	000000	000000
				444444	322233	000200	000000	000000	000000	000000
Virus control	n.a.	3 g/l BSA + erythrocytes	5 min	444444	444444	444444	434423	234112	123023	000020
				444444	444444	444444	324442	321223	020312	000000
Interference control – control of cell susceptibility - PBS	n.a.	3 g/l BSA + erythrocytes	60 min	444444	444444	444444	444444	332222	120002	000000
Interference control – control of cell susceptibility – chloramix®dt	0,003 tbl/l	3 g/l BSA + erythrocytes	60 min	444444	444444	444444	444444	324223	000000	000000
Control of efficiency of suppression of sample activity – chloramix®dt	3 tbl/l	3 g/l BSA + erythrocytes	30 min	444444	444444	444444	444444	342244	321002	000000
Virus control without carrier	n.a.	3 g/l BSA + erythrocytes	30 min	444444	444444	444444	444444	423442	231223	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
BSA bovine serum albumin, PBS polyphosphate buffer
CT Cytotoxicologic effect, CPE Cytopathogenic effect

Prepared by: Mgr. Ludmila Porubová

END OF THE PROTOCOL