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Chemical and Microbiological Laboratory, Testing Laboratory No. 1273 certified by Czech Accreditation Institute.

Copy No.: 1
Issue No.: 1

Test report No. D98-2/2014

DETERMINATION OF VIRUCIDAL (EN 14476) ACTIVITY OF THE PRODUCT **CHLORINEX-60**

Sample ID: D98/2014
Sample name: **Chlorinex-60**
Client: AS CHEMI-PHARM, Pöllu 132, 109 17 Tallinn, Estonia
Producer: AS CHEMI-PHARM, Pöllu 132, 109 17 Tallinn, Estonia
Sampling point: AS CHEMI-PHARM, Pöllu 132, 109 17 Tallinn, Estonia

Incoming date:
9.7.2014

Delivery date:
24.11.2014

Hodonín, 24.11.2014

Zuzana Matušková, Head of Laboratory

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D98/2014
Rep No: 116
Sample name: **Chlorinex-60**
Sampled: by client
Sampling point: AS CHEMI-PHARM, Pöllu 132, 109 17 Tallinn, Estonia
Client: AS CHEMI-PHARM, Pöllu 132, 109 17 Tallinn, Estonia

Sampling date: 4.7.2014
Sample delivered: 9.7.2014
Testing date: 7.11. – 20.11.2014
Delivered amount: 300 tablets
Batch No: 810414

Subject of testing:
Determination of virucidal activity of the product.

Identification of the sample:

Name of the product:	**Chlorinex-60**
Batch number:	810414
Date of manufacture:	04.04.14
Expiry date:	04.16
Manufacturer:	AS CHEMI-PHARM, Pöllu 132, 109 17 Tallinn, Estonia
Incoming date:	9.7.2014
Storage conditions:	normal
Active compounds and concentrations:	1 tab = 1.5 g active chlorine if dissolved in water, thus 1 tab/1.5 l = 1000 ppm active chlorine

Experiment conditions:

	Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method SOP-M-19-00 (EN 14476)
Period of analysis:	13.11. – 20.11.2014
Test temperature:	20 °C ± 1 °C
Method of titration:	virus titration on monolayers of cells on microtitre plates
Product diluent:	hard water
Appearance of the products:	white tablets
Test concentration:	1 tab/1.5 l (1000 ppm AC), 2 tabs/1.5 l (1000 ppm AC), 6 tabs/1.5 l (6000 ppm AC)
Contact time:	5 min, 15 min
Interfering substances:	0.3 g/l BSA (clean conditions) 3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)
Reference product:	Formaldehyde 36 – 38% solution p.a., CAS: 50-00-0, Batch No: K44006603245, expiry date: 30.11.14
Test virus:	*Adenovirus* type 5, strain Adenoid 75, ATCC VR-5 (5th passage)
Cell lines:	HeLa cells
Incubation:	36 °C ± 1 °C, 5 % CO_2, 96 h, and additional period of 24 h or 48 h or 72 hours. After incubation, the titre infectivity is calculated according to Spearman-Kärber method.

Preparation of the test

1. Determination of the number of the microorganisms CFU/ml in the product
2. Preparation of the cell culture
3. Preparation of the test virus suspension
4. Test of the viral infectivity
5. Virus titration with the interfering substance
6. Cytotoxicity of the product
7. Reference virus inactivation test
8. Test procedure for the virucidal activity of the product

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Location: Moldova
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Note:
Virucidal activity – the capability of a product to produce a reduction in the number of infectious virus particles under defined conditions by at least 4 (lg) orders.

The standard:
EN 14476 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical area – Test method and requirements (Phase 2/Step 1) August 2013

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D98/2014
Rep No: 116
Sample name: **Chlorinex-60**
Sampled: by client
Sampling point: AS CHEMI-PHARM, Pöllu 132, 109 17 Tallinn, Estonia
Client: AS CHEMI-PHARM, Pöllu 132, 109 17 Tallinn, Estonia

Sampling date: 4.7.2014
Sample delivered: 9.7.2014
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Delivered amount: 300 tablets
Batch No: 810414

The Number of CFU in the tested product: $< 10^1$ CFU/g

1. Testing the efficacy of chemical disinfectant **Chlorinex-60** on *Adenovirus* type 5, strain Adenoid 75, ATCC VR-5

Tab No. 1.1 Table of results of product **Chlorinex-60** on *Adenovirus* type 5, strain Adenoid 75, ATCC VR-5

Product	Concentration	Interfering substances	Level of cytoxicity	- $\log_{10}$ $TCID_{50}$ after 5 min	- $\log_{10}$ $TCID_{50}$ after 15 min	- $\log_{10}$ $TCID_{50}$ after 30 min	- $\log_{10}$ $TCID_{50}$ after 60 min
Chlorinex-60	1 tab/1.5 l	clean	-	4.17	-	-	-
Chlorinex-60	2 tab/1.5 l	clean	-	-	3.50	-	-
Chlorinex-60	2 tab/1.5 l	dirty	-	-	3.83	-	-
Chlorinex-60	6 tab/1.5 l	dirty	2.50	-	3.50	-	-
Formaldehyde	0.7 % (w/v)	PBS	3.50	-	-	5.50	4.50
			Virus titration, time = 0				
Virus control	-	PBS	9.00	-	-	9.00	8.83
Virus control	-	clean	9.00	8.83	8.83	-	-
Virus control	-	dirty	8.83	-	8.83	-	-

Tab No. 1.2 Testing the efficacy of chemical disinfectant **Chlorinex-60** on *Adenovirus* type 5, strain Adenoid 75, ATCC VR-5

Test concentration	Titre of the virus suspension - $\log_{10}$ $TCID_{50}$	Interfering substances	Contact time	- $\log_{10}$ $TCID_{50}$ after test procedure	$\Delta\log_{10}$ $TCID_{50}$
1 tab/1.5 l	9.00	clean	5 min	4.17	**4.83**
2 tab/1.5 l	9.00	clean	15 min	3.50	**5.50**
2 tab/1.5 l	8.83	dirty	15 min	3.83	**5.00**
6 tab/1.5 l	8.83	dirty	15 min	3.50	**5.33**

2. Evaluation of virucidal activity of the product **Chlorinex-60**

Tab No. 2.1 The efficacy of chemical disinfectant **Chlorinex-60** on test viruses – virucidal activity

Virucidal activity of the product (EN 14476)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations	Interfering substances - conditions	$\Delta\log_{10}$ $TCID_{50}$ EN 14476	$\Delta\log_{10}$ $TCID_{50}$
Adenovirus type 5, strain Adenoid 75, ATCC VR-5	20	5	1 tab/1.5 l	clean	≥ 4	**> 4**
Adenovirus type 5, strain Adenoid 75, ATCC VR-5	20	15	2 tab/1.5 l	clean	≥ 4	**> 4**
Adenovirus type 5, strain Adenoid 75, ATCC VR-5	20	15	2 tab/1.5 l	dirty	≥ 4	**> 4**
Adenovirus type 5, strain Adenoid 75, ATCC VR-5	20	15	6 tab/1.5 l	dirty	≥ 4	**> 4**

Note:
$TCID_{50}$- 50% infecting dose of a virus suspension or that dilution of the virus suspension that induce a CPE in 50% of cell culture units

Prepared by: Bc. Iva Čížová, Lab Technician

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D98/2014
Rep No: 116
Sample name: **Chlorinex-60**
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Sampling date: 4.7.2014
Sample delivered: 9.7.2014
Testing date: 7.11. – 20.11.2014
Delivered amount: 300 tablets
Batch No: 810414

Experiment conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 14476)
Period of analysis:	7.11. – 13.11.2014
Test temperature:	20 °C ± 1 °C
Method of titration:	virus titration on monolayers of cells on microtitre plates
Product diluent:	hard water
Appearance of the products:	white tablets
Test concentration:	1 tab/1.5 l (1000 ppm AC), 2 tabs/1.5 l (1000 ppm AC), 6 tabs/1.5 l (6000 ppm AC)
Contact time:	5 min, 15 min
Interfering substances:	0.3 g/l BSA (clean conditions) 3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)
Reference product:	Formaldehyde 36 – 38% solution p.a., CAS: 50-00-0, Batch No: K44006603245, expiry date: 30.11.14
Test virus:	*Poliovirus* type 1, LSc-2ab (5th passage)
Cell lines:	HeLa cells
Incubation:	36 °C ± 1 °C, 5 % CO_2, 96 h, and additional period of 24 h or 48 h

hours. After incubation, the titre infectivity is calculated according to Spearman-Kärber method.

Preparation of the test

1. Determination of the number of the microorganisms CFU/ml in the product
2. Preparation of the cell culture
3. Preparation of the test virus suspension
4. Test of the viral infectivity
5. Virus titration with the interfering substance
6. Cytotoxicity of the product
7. Reference virus inactivation test
8. Test procedure for the virucidal activity of the product

Note:
Virucidal activity – the capability of a product to produce a reduction in the number of infectious virus particles under defined conditions by at least 4 (lg) orders.

The standard:
EN 14476 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical area – Test method and requirements (Phase 2/Step 1) August 2013

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

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3. Testing the efficacy of chemical disinfectant **Chlorinex-60** on *Poliovirus* type 1, LSc-2ab

Tab No. 3.1 Table of results of product **Chlorinex-60** on *Poliovirus* type 1, LSc-2ab

Product	Concentration	Interfering substances	Level of cytoxicity	- $\log_{10}$ $TCID_{50}$ after 5 min	- $\log_{10}$ $TCID_{50}$ after 15 min	- $\log_{10}$ $TCID_{50}$ after 30 min	- $\log_{10}$ $TCID_{50}$ after 60 min
Chlorinex-60	1 tab/1.5 l	clean	-	4.00	-	-	-
Chlorinex-60	2 tab/1.5 l	clean	-	-	3.50	-	-
Chlorinex-60	2 tab/1.5 l	dirty	-	-	3.67	-	-
Chlorinex-60	6 tab/1.5 l	dirty	2.50	-	3.50	-	-
Formaldehyde	0.7 % (w/v)	PBS	3.50	-	-	7.00	5.83
			Virus titration, time = 0				
Virus control	-	PBS	8.33	-	-	8.50	8.50
Virus control	-	clean	8.50	8.50	8.33		
Virus control	-	dirty	8.50	-	8.67		

Tab No. 3.2 Testing the efficacy of chemical disinfectant **Chlorinex-60** on *Poliovirus* type 1, LSc-2ab

Test concentration	Titre of the virus suspension - $\log_{10}$ $TCID_{50}$	Interfering substances	Contact time	- $\log_{10}$ $TCID_{50}$ after test procedure	$\Delta\log_{10}$ $TCID_{50}$
1 tab/1.5 l	8.50	clean	5 min	4.00	**4.50**
2 tab/1.5 l	8.50	clean	15 min	3.50	**5.00**
2 tab/1.5 l	8.50	dirty	15 min	3.67	**4.83**
6 tab/1.5 l	8.50	dirty	15 min	3.50	**5.00**

4. Evaluation of virucidal activity of the product **Chlorinex-60**

Tab No. 4.1 The efficacy of chemical disinfectant **Chlorinex-60** on test viruses – virucidal activity

Virucidal activity of the product (EN 14476)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations	Interfering substances - conditions	$\Delta\log_{10}$ $TCID_{50}$ EN 14476	$\Delta\log_{10}$ $TCID_{50}$
Poliovirus type 1, LSc-2ab	20	5	1 tab/1.5 l	clean	≥ 4	**> 4**
Poliovirus type 1, LSc-2ab	20	15	2 tab/1.5 l	clean	≥ 4	**> 4**
Poliovirus type 1, LSc-2ab	20	15	2 tab/1.5 l	dirty	≥ 4	**> 4**
Poliovirus type 1, LSc-2ab	20	15	6 tab/1.5 l	dirty	≥ 4	**> 4**

Note:
$TCID_{50}$- 50% infecting dose of a virus suspension or that dilution of the virus suspension that induce a CPE in 50% of cell culture units

Prepared by: Bc. Iva Čížová, Lab Technician

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

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Client: AS CHEMI-PHARM, Pöllu 132, 109 17 Tallinn, Estonia

Sampling date: 4.7.2014
Sample delivered: 9.7.2014
Testing date: 7.11. – 20.11.2014
Delivered amount: 300 tablets
Batch No: 810414
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Interpretation:
Results of tests are in Tabs.
According to EN 14476 the tested product **Chlorinex-60**, batch No: 810414, in concentration 1 tab/1.5 l, diluted in hard water, and in the contact time 5 min under clean conditions and in concentration 2 tabs/1.5 l, diluted in hard water, and in the contact time 15 min under clean and dirty conditions and in concentration 6 tabs/1.5 l, diluted in hard water, and in the contact time 15 min under dirty conditions at temperature 20 °C ± 1 °C **proved** by the method of virus titration on monolayers of cells on microtitre plates to reduce the number of infectious *Adenovirus* type 5, strain Adenoid 75, ATCC VR-5 particles under defined conditions by at least 4 (lg) orders.

According to EN 14476 the tested product **Chlorinex-60**, batch No: 810414, in concentration 1 tab/1.5 l, diluted in hard water, and in the contact time 5 min under clean conditions and in concentration 2 tabs/1.5 l, diluted in hard water, and in the contact time 15 min under clean and dirty conditions and in concentration 6 tabs/1.5 l, diluted in hard water, and in the contact time 15 min under dirty conditions at temperature 20 °C ± 1 °C **proved** by the method of virus titration on monolayers of cells on microtitre plates to reduce the number of infectious *Poliovirus* type 1, LSc-2ab particles under defined conditions by at least 4 (lg) orders.

Conclusion:
The product **Chlorinex-60** is capable of reducing the number of infectious *Adenovirus* and *Poliovirus* particles under defined conditions to the declared values, and consequently, may be called virucidal on *Adenovirus* and *Poliovirus*.

24.11.2014, Hodonín

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