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

L 1273

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Test report No. D98/2014

DETERMINATION OF BACTERICIDAL (EN 13727), FUNGICIDAL (EN 13624), SPORICIDAL (EN 13704), MYCOBACTERICIDAL AND TUBERCULOCIDAL (EN 14348) 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:
25.8.2014

Hodonín, 25.8.2014

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: 29.7. – 22.8.2014
Delivered amount: 300 tablets
Batch No: 810414

Subject of testing:
Determination of bactericidal, fungicidal, sporicidal, mycobactericidal and tuberculocidal 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

Experimental conditions:

	Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method SOP-M-19-00 (EN 13727)
Period of analysis:	29.7. – 30.7.2014
Test temperature:	20 °C ± 1 °C
Test method:	dilution neutralization method
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062
Product diluent:	hard water
Appearance of the products:	white tablets
Test concentration:	1 tab/1.5 l (1000 ppm AC), 6 tabs/1.5 l (6000 ppm AC)
Contact time:	15 min
Interfering substances:	0.3 g/l BSA (clean conditions) 3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)
Test organisms:	*Pseudomonas aeruginosa* ATCC 15442 *Staphylococcus aureus* ATCC 6538 *Enterococcus hirae* ATCC 10541
Incubation conditions:	37 °C ± 1 °C, 24 hours

Test procedure:
1. Preparation of test suspension
2. Preparation of product test solutions
3. Quantitative suspension test
4. Incubation and calculation
5. Expression and interpretation of results

Note:
Bactericidal activity – the capability of a product to produce a reduction in the number of viable bacterial cells of relevant organisms under defined conditions by at least 5 orders (10^5).

The standard:
EN 13727:2012+A1:2013 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity in the medical area – Test method and requirements (phase 2, step 1) May 2012+November 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, Põllu 132, 109 17 Tallinn, Estonia
Client: AS CHEMI-PHARM, Põllu 132, 109 17 Tallinn, Estonia

Sampling date: 4.7.2014
Sample delivered: 9.7.2014
Testing date: 29.7. – 22.8.2014
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 *Pseudomonas aeruginosa* ATCC 15442

Tab No. 1.1.1 Verification of methodology, clean conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 6 tabs/1.5 l		
V_{c1} 54	V_{c2} 57	$\Phi_{Nvo} = 55.5$	V_{c1} 53	V_{c2} 47	$\Phi_A = 50$	V_{c1} 52	V_{c2} 58	$\Phi_B = 55$	V_{c1} 49	V_{c2} 58	$\Phi_C = 53.5$
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x yes		no	x yes		no	x yes		no	x yes		no

Validation of suspension (N_{VB})	V_{c1} 50	V_{c2} 56	Φ_{NVB} 53	$30 \leq \Phi_{NVB}\ (N_{VB}/1000) \leq 160$
				x yes / no

Tab No. 1.1.2 Verification of methodology, dirty conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 6 tabs/1.5 l		
V_{c1} 54	V_{c2} 57	$\Phi_{Nvo} = 55.5$	V_{c1} 51	V_{c2} 60	$\Phi_A = 55.5$	V_{c1} 52	V_{c2} 58	$\Phi_B = 55$	V_{c1} 52	V_{c2} 56	$\Phi_C = 54$
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x yes		no	x yes		no	x yes		no	x yes		no

Validation of suspension (N_{VB})	V_{c1} 50	V_{c2} 56	Φ_{NVB} 53	$30 \leq \Phi_{NVB}\ (N_{VB}/1000) \leq 160$
				x yes / no

Tab No. 1.2 Test suspensions

Test suspension N $\Phi = 50 \times 10^7 = \lg 8.70$ $8.17 \leq \lg N \leq 8.70$	N	V_{c1}	V_{c1}	Test suspension N_0 $\lg N_0 = \lg N/10 = \lg 7.70$ $7.17 \leq \lg N_0 \leq 7.70$
	10^{-6}	> 330	> 330	
	10^{-7}	56	44	
				x yes / no

Tab No. 1.3 Testing the efficacy of chemical disinfectant **Chlorinex-60** on *Pseudomonas aeruginosa* ATCC 15442

Test concentration /contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = \lg 7.70$)**
1 tab/1.5 l /15/clean	10^0	<14	<14	< 2.15	**≥ 5.55**
1 tab/1.5 l /15/dirty	10^0	<14	<14	< 2.15	**≥ 5.55**
6 tabs/1.5 l /15/clean	10^0	<14	<14	< 2.15	**≥ 5.55**
6 tabs/1.5 l /15/dirty	10^0	<14	<14	< 2.15	**≥ 5.55**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the bacterial test suspension, N_0 = the number of cfu/ml of the bacterial test suspension at the beginning of the contact time = 0, N_V = the number of cfu/ml of the bacterial test suspension for validation N_{V0} = the number of cfu/ml of the bacterial test suspension for validation in the test mixture, A, B, C at the beginning of the contact time = 0, N_{VB} = the number of cfu/ml of the bacterial test suspension for the neutralizer control, N_a = the number of survivors per ml in the test mixture, A, B, C = the number of survivors per ml in control tests (A – experimental conditions validation, B – neutralizer toxicity validation, C – method validation), $R = N_0/N_a$ = the reduction in viability, or $\lg R = \lg N_0 - \lg N_a$

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: 29.7. – 22.8.2014
Delivered amount: 300 tablets
Batch No: 810414

2. Testing the efficacy of chemical disinfectant **Chlorinex-60** on *Staphylococcus aureus* ATCC 6538

Tab No. 2.1.1 Verification of methodology, clean conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 6 tabs/1.5 l		
V_{c1} 47	V_{c2} 51	Φ_{Nvo}= 49	V_{c1} 41	V_{c2} 52	Φ_A = 46.5	V_{c1} 44	V_{c2} 48	Φ_B = 46	V_{c1} 48	V_{c2} 47	Φ_C = 47.5
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x yes		no	x yes		no	x yes		no	x yes		no

Validation of suspension (N_{VB})	V_{c1} 48	V_{c2} 55	Φ_{NVB} 51.5	$30 \leq \Phi_{NVB}\ (N_{VB}/1000) \leq 160$
				x yes / no

Tab No. 2.1.2 Verification of methodology, dirty conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 6 tabs/1.5 l		
V_{c1} 47	V_{c2} 51	Φ_{Nvo}= 49	V_{c1} 45	V_{c2} 50	Φ_A = 47.5	V_{c1} 44	V_{c2} 48	Φ_B = 46	V_{c1} 49	V_{c2} 51	Φ_C = 50
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x yes		no	x yes		no	x yes		no	x yes		no

Validation of suspension (N_{VB})	V_{c1} 48	V_{c2} 55	Φ_{NVB} 51.5	$30 \leq \Phi_{NVB}\ (N_{VB}/1000) \leq 160$
				x yes / no

Tab No. 2.2 Test suspensions

Test suspension N $\Phi = 42 \times 10^7 = \lg 8.62$ $8.17 \leq \lg N \leq 8.70$	N	V_{c1}	V_{c1}	Test suspension N_0 $\lg N_0 = \lg N/10 = \lg 7.62$ $7.17 \leq \lg N_0 \leq 7.70$
	10^{-6}	> 330	> 330	
	10^{-7}	40	44	
				x yes / no

Tab No. 2.3 Testing the efficacy of chemical disinfectant **Chlorinex-60** on *Staphylococcus aureus* ATCC 6538

Test concentration /contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = \lg 7.62$)**
1 tab/1.5 l /15/clean	10^0	<14	<14	< 2.15	**≥ 5.47**
1 tab/1.5 l /15/dirty	10^0	<14	<14	< 2.15	**≥ 5.47**
6 tabs/1.5 l /15/clean	10^0	<14	<14	< 2.15	**≥ 5.47**
6 tabs/1.5 l /15/dirty	10^0	<14	<14	< 2.15	**≥ 5.47**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the bacterial test suspension, N_0 = the number of cfu/ml of the bacterial test suspension at the beginning of the contact time = 0, N_V = the number of cfu/ml of the bacterial test suspension for validation N_{V0} = the number of cfu/ml of the bacterial test suspension for validation in the test mixture, A, B, C at the beginning of the contact time = 0, N_{VB} = the number of cfu/ml of the bacterial test suspension for the neutralizer control, N_a = the number of survivors per ml in the test mixture, A, B, C = the number of survivors per ml in control tests (A – experimental conditions validation, B – neutralizer toxicity validation, C – method validation), $R = N_0/ N_a$ = the reduction in viability, or $\lg R = \lg N_0 - \lg N_a$

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

Sampling date: 4.7.2014
Sample delivered: 9.7.2014
Testing date: 29.7. – 22.8.2014
Delivered amount: 300 tablets
Batch No: 810414

3. Testing the efficacy of chemical disinfectant **Chlorinex-60** on *Enterococcus hirae* ATCC 10541

Tab No. 3.1.1 Verification of methodology, clean conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 6 tabs/1.5 l		
V_{c1}	52	Φ_{Nvo}= 54	V_{c1}	55	Φ_A = 55.5	V_{c1}	59	Φ_B = 50.5	V_{c1}	37	Φ_C = 46.5
V_{c2}	56		V_{c2}	56		V_{c2}	42		V_{c2}	56	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	45	V_{c2}	49	Φ_{NVB}	47	$30 \leq \Phi_{NVB}$ (N_{VB}/1000) ≤ 160		
							x	yes	no

Tab No. 3.1.2 Verification of methodology, dirty conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 6 tabs/1.5 l		
V_{c1}	52	Φ_{Nvo}= 54	V_{c1}	51	Φ_A = 52	V_{c1}	59	Φ_B = 50.5	V_{c1}	44	Φ_C = 49.5
V_{c2}	56		V_{c2}	53		V_{c2}	42		V_{c2}	55	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	45	V_{c2}	49	Φ_{NVB}	47	$30 \leq \Phi_{NVB}$ (N_{VB}/1000) ≤ 160		
							x	yes	no

Tab No. 3.2 Test suspensions

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0
$\Phi = 45.5 \times 10^7 = \lg 8.66$				$\lg N_0 = \lg N/10 = \lg 7.66$
$8.17 \leq \lg N \leq 8.70$	10^{-6}	> 330	> 330	$7.17 \leq \lg N_0 \leq 7.70$
	10^{-7}	43	48	
				x yes no

Tab No. 3.3 Testing the efficacy of chemical disinfectant **Chlorinex-60** on *Enterococcus hirae* ATCC 10541

Test concentration /contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a$ = $\lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = \lg 7.66$)**
1 tab/1.5 l /15/clean	10^0	<14	<14	< 2.15	**≥ 5.51**
1 tab/1.5 l /15/dirty	10^0	<14	<14	< 2.15	**≥ 5.51**
6 tabs/1.5 l /15/clean	10^0	<14	<14	< 2.15	**≥ 5.51**
6 tabs/1.5 l /15/dirty	10^0	<14	<14	< 2.15	**≥ 5.51**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the bacterial test suspension, N_0 = the number of cfu/ml of the bacterial test suspension at the beginning of the contact time = 0, N_V = the number of cfu/ml of the bacterial test suspension for validation N_{V0} = the number of cfu/ml of the bacterial test suspension for validation in the test mixture, A, B, C at the beginning of the contact time = 0, N_{VB} = the number of cfu/ml of the bacterial test suspension for the neutralizer control, N_a = the number of survivors per ml in the test mixture, A, B, C = the number of survivors per ml in control tests (A – experimental conditions validation, B – neutralizer toxicity validation, C – method validation), $R = N_0/ N_a$ = the reduction in viability, or $\lg R = \lg N_0 - \lg N_a$

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: 29.7. – 22.8.2014
Delivered amount: 300 tablets
Batch No: 810414

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

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

Bactericidal activity of the product (EN 13727)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentration	Interfering substances - conditions	lg R EN 13727	**lg R**
Pseudomonas aeruginosa ATCC 15442	20	15	1 tab/1.5 l	clean	≥ 5	**> 5**
Staphylococcus aureus ATCC 6538	20	15	1 tab/1.5 l	clean	≥ 5	**> 5**
Enterococcus hirae ATCC 10541	20	15	1 tab/1.5 l	clean	≥ 5	**> 5**
Pseudomonas aeruginosa ATCC 15442	20	15	1 tab/1.5 l	dirty	≥ 5	**> 5**
Staphylococcus aureus ATCC 6538	20	15	1 tab/1.5 l	dirty	≥ 5	**> 5**
Enterococcus hirae ATCC 10541	20	15	1 tab/1.5 l	dirty	≥ 5	**> 5**
Pseudomonas aeruginosa ATCC 15442	20	15	6 tabs/1.5 l	clean	≥ 5	**> 5**
Staphylococcus aureus ATCC 6538	20	15	6 tabs/1.5 l	clean	≥ 5	**> 5**
Enterococcus hirae ATCC 10541	20	15	6 tabs/1.5 l	clean	≥ 5	**> 5**
Pseudomonas aeruginosa ATCC 15442	20	15	6 tabs/1.5 l	dirty	≥ 5	**> 5**
Staphylococcus aureus ATCC 6538	20	15	6 tabs/1.5 l	dirty	≥ 5	**> 5**
Enterococcus hirae ATCC 10541	20	15	6 tabs/1.5 l	dirty	≥ 5	**> 5**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the bacterial test suspension, N_0 = the number of cfu/ml of the bacterial test suspension at the beginning of the contact time = 0, N_V = the number of cfu/ml of the bacterial test suspension for validation N_{V0} = the number of cfu/ml of the bacterial test suspension for validation in the test mixture, A, B, C at the beginning of the contact time = 0, N_{VB} = the number of cfu/ml of the bacterial test suspension for the neutralizer control, N_a = the number of survivors per ml in the test mixture, A, B, C = the number of survivors per ml in control tests (A – experimental conditions validation, B – neutralizer toxicity validation, C – method validation), $R = N_0/ N_a$ = the reduction in viability, or $\lg R = \lg N_0 - \lg N_a$

Prepared by: Hana Konevalíková, Lab Technician

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

Sampling date: 4.7.2014
Sample delivered: 9.7.2014
Testing date: 29.7. – 22.8.2014
Delivered amount: 300 tablets
Batch No: 810414

Experimental conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 13624)
Period of analysis:	7.8. – 11.8.2014
Test temperature:	20 °C ± 1 °C
Test method:	dilution neutralization method
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062
Product diluent:	hard water
Appearance of the products:	white tablets
Test concentration:	1 tab/1.5 l (1000 ppm AC), 6 tabs/1.5 l (6000 ppm AC)
Contact time:	15 min
Interfering substances:	0.3 g/l BSA (clean conditions) 3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)
Test organisms:	*Candida albicans* ATCC 10231 *Aspergillus brasiliensis (niger)* ATCC 16404
Incubation conditions:	30 °C ± 1 °C, 48 hours and additional period of 24 or 48 hours

Test procedure:

1. Preparation of test suspension
2. Preparation of product test solutions
3. Quantitative suspension test
4. Incubation and calculation
5. Expression and interpretation of results

Note:
Presence of a high concentration (at least 75%) of *Aspergillus brasiliensis* spiny spores in the test suspension – yes.
Fungicidal activity – the capability of a product to produce a reduction in the number of viable fungi belonging to reference strains under defined conditions by at least 4 orders (10^4).

The standard:
EN 13624 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in the medical area - Test method and requirements (phase 2, step 1) September 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
Testing date: 29.7. – 22.8.2014
Delivered amount: 300 tablets
Batch No: 810414

5. Testing the efficacy of chemical disinfectant **Chlorinex 60** on *Candida albicans* ATCC 10231

Tab No. 5.1.1 Verification of methodology, clean conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 1 tab/1.5 l		
V_{c1}	40	Φ_{Nvo}= 43.5	V_{c1}	41	Φ_A = 39	V_{c1}	44	Φ_B = 39.5	V_{c1}	43	Φ_C = 42
V_{c2}	47		V_{c2}	37		V_{c2}	35		V_{c2}	41	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	43	V_{c2}	44	Φ_{NVB}	43.5	$30 \leq \Phi_{NVB}(N_{VB}/1000) \leq 160$		
							x	yes	no

Tab No. 5.1.2 Verification of methodology, dirty conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 6 tabs/1.5 l		
V_{c1}	40	Φ_{Nvo}= 43.5	V_{c1}	36	Φ_A = 38	V_{c1}	44	Φ_B = 39.5	V_{c1}	37	Φ_C = 40
V_{c2}	47		V_{c2}	40		V_{c2}	35		V_{c2}	43	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	43	V_{c2}	44	Φ_{NVB}	43.5	$30 \leq \Phi_{NVB}(N_{VB}/1000) \leq 160$		
							x	yes	no

Tab No. 5.2 Test suspensions

Test suspension N Φ = 41.5 x 10^6 = lg 7.62 $7.17 \leq \lg N \leq 7.70$	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0) $\lg N_0 = \lg N/10 = \lg 6.62$ $6.17 \leq \lg N_0 \leq 6.70$			
	10^{-5}	> 330	> 330				
	10^{-6}	45	38				
				x	yes		no

Tab No. 5.3 Testing the efficacy of chemical disinfectant **Chlorinex 60** on *Candida albicans* ATCC 10231

Test concentration /contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = \lg 6.62$)**
1 tab/1.5 l /15/clean	10^0	<14	<14	< 2.15	**≥ 4.47**
6 tabs/1.5 l /15/dirty	10^0	<14	<14	< 2.15	**≥ 4.47**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the test suspension, N_0 = the number of cfu/ml of the test suspension at the beginning of the contact time = 0, N_V = the number of cfu/ml of the test suspension for validation N_{V0} = the number of cfu/ml of the test suspension for validation in the test mixture, A, B, C at the beginning of the contact time = 0, N_{VB} = the number of cfu/ml of the test suspension for the neutralizer control, N_a = the number of survivors per ml in the test mixture, A, B, C = the number of survivors per ml in control tests (A – experimental conditions validation, B – neutralizer toxicity validation, C – method validation),
$R = N_0/ N_a$ = the reduction in viability, or $\lg R = \lg N_0 - \lg N_a$

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

Sampling date: 4.7.2014
Sample delivered: 9.7.2014
Testing date: 29.7. – 22.8.2014
Delivered amount: 300 tablets
Batch No: 810414

6. Testing the efficacy of chemical disinfectant **Chlorinex 60** on *Aspergillus brasiliensis (niger)* ATCC 16404

Tab No. 6.1.1 Verification of methodology, clean conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 1 tab/1.5 l		
V_{c1}	54	Φ_{Nvo}= 54	V_{c1}	47	Φ_A = 52	V_{c1}	53	Φ_B = 54	V_{c1}	56	Φ_C = 52
V_{c2}	54		V_{c2}	57		V_{c2}	55		V_{c2}	48	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	51	V_{c2}	58	Φ_{NVB}	54.5	$30 \leq \Phi_{NVB}(N_{VB}/1000) \leq 160$		
							x	yes	no

Tab No. 6.1.2 Verification of methodology, dirty conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 6 tabs/1.5 l		
V_{c1}	54	Φ_{Nvo}= 54	V_{c1}	49	Φ_A = 50.5	V_{c1}	53	Φ_B = 54	V_{c1}	47	Φ_C = 48
V_{c2}	54		V_{c2}	52		V_{c2}	55		V_{c2}	49	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	51	V_{c2}	58	Φ_{NVB}	54.5	$30 \leq \Phi_{NVB}(N_{VB}/1000) \leq 160$		
							x	yes	no

Tab No. 6.2 Test suspensions

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0)
Φ = 45 x 10^6 = lg 7.65	10^{-5}	> 165	> 165	lg N_0 = lg N/10 = lg 6.65
7.17 ≤ lg N ≤ 7.70	10^{-6}	48	42	6.17 ≤ lg N_0 ≤ 6.70
				x yes no

Tab No. 6.3 Testing the efficacy of chemical disinfectant **Chlorinex 60** on *Aspergillus brasiliensis (niger)* ATCC 16404

Test concentration /contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	lg N_a = lg (Φ_a x 10)	**lg R (lg N_0 = lg 6.65)**
1 tab/1.5 l /15/clean	10^0	43	38	2.61	**4.04**
6 tabs/1.5 l /15/dirty	10^0	<14	<14	< 2.15	**≥ 4.50**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the test suspension, N_0 = the number of cfu/ml of the test suspension at the beginning of the contact time = 0, N_V = the number of cfu/ml of the test suspension for validation N_{V0} = the number of cfu/ml of the test suspension for validation in the test mixture, A, B, C at the beginning of the contact time = 0, N_{VB} = the number of cfu/ml of the test suspension for the neutralizer control, N_a = the number of survivors per ml in the test mixture, A, B, C = the number of survivors per ml in control tests (A – experimental conditions validation, B – neutralizer toxicity validation, C – method validation),
R = N_0/ N_a = the reduction in viability, or lg R = lg N_0 – lg N_a

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: 29.7. – 22.8.2014
Delivered amount: 300 tablets
Batch No: 810414

7. Evaluation of fungicidal activity of the product **Chlorinex 60**

Tab No. 7.1 The efficacy of chemical disinfectant **Chlorinex 60** on test strains – fungicidal activity

Fungicidal activity of the product (EN 13624)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations	Interfering substances - conditions	lg R EN 13624	**lg R**
Candida albicans ATCC 10231	20	15	1 tab/1.5 l	clean	≥ 4	**> 4**
Aspergillus brasiliensis (niger) ATCC 16404	20	15	1 tab/1.5 l	clean	≥ 4	**> 4**
Candida albicans ATCC 10231	20	15	6 tabs/1.5 l	dirty	≥ 4	**> 4**
Aspergillus brasiliensis (niger) ATCC 16404	20	15	6 tabs/1.5 l	dirty	≥ 4	**> 4**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the test suspension, N_0 = the number of cfu/ml of the test suspension at the beginning of the contact time = 0, N_V = the number of cfu/ml of the test suspension for validation N_{V0} = the number of cfu/ml of the test suspension for validation in the test mixture, A, B, C at the beginning of the contact time = 0, N_{VB} = the number of cfu/ml of the test suspension for the neutralizer control, N_a = the number of survivors per ml in the test mixture, A, B, C = the number of survivors per ml in control tests (A – experimental conditions validation, B – neutralizer toxicity validation, C – method validation),
$R = N_0 / N_a$ = the reduction in viability, or $\lg R = \lg N_0 - \lg N_a$

Prepared by: Hana Konevalíková, Lab Technician

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

Sampling date: 4.7.2014
Sample delivered: 9.7.2014
Testing date: 29.7. – 22.8.2014
Delivered amount: 300 tablets
Batch No: 810414

Experimental conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 13704)
Period of analysis:	8.8. – 11.8.2014
Test temperature:	20 °C ± 1 °C
Test method:	dilution neutralization method
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062
Product diluent:	hard water
Appearance of the products:	white tablets
Test concentration:	10 tabs/1.5 l (10000 ppm AC)
Contact time:	15 min
Interfering substances:	3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)**
Test organisms:	*Bacillus subtilis* ATCC 6633
Incubation conditions:	30 °C ± 1 °C, minimum 3 and maximum 7 days

Test procedure:

1. Preparation of the test suspension
2. Preparation of the product test solutions
3. Quantitative suspension test
4. Incubation and calculation
5. Expression and interpretation of the results

Note:
Sporicidal activity – the capability of a product to produce a reduction in the number of bacterial spores belonging to reference strain of *Bacillus subtilis* under defined conditions by at least 3 orders (10^3).
$\lg R = \lg [(N \times 10^{-1})/N_a]$
** According to the client's request dirty conditions were used.

The standard:
EN 13704 Chemical disinfectants – Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1) February 2002

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: 29.7. – 22.8.2014
Delivered amount: 300 tablets
Batch No: 810414

8. Testing the efficacy of chemical disinfectant **Chlorinex-60** on *Bacillus subtilis* ATCC 6633

Tab No. 8.1 Verification of methodology

Validation of suspension (N_v) x 10^1			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 10 tabs/1.5 l		
V_{c1}	80	$\Phi_{Nv} = 86$	V_{c1}	82	$\Phi_A = 83.5$	V_{c1}	84	$\Phi_B = 77$	V_{c1}	82	$\Phi_C = 76.5$
V_{c2}	92		V_{c2}	85		V_{c2}	70		V_{c2}	71	
$60 \leq \Phi_{Nv} \leq 300$			$\Phi_A \geq 0.05\ \Phi_{Nv}$			$\Phi_B \geq 0.05\ \Phi_{Nv}$			$\Phi_C \geq 0.5\ \Phi_B$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 8.2 Test suspension

Test suspension (N)	N	V_{c1}	V_{c1}	$\Phi = 36.5 \times 10^5 = \lg 6.56$ $6.17 \leq \lg N \leq 6.70$
	10^{-4}	>300	>300	
	10^{-5}	33	40	
				x yes no

Tab No. 8.3 Testing the efficacy of chemical disinfectant **Chlorinex-60** on *Bacillus subtilis* ATCC 6633

Test concentration /contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R (lg N = lg 6.56)**
10 tabs/1.5 l / 15 / dirty**	10^0	<15	<15	< 2.18	**≥ 3.38**

9. Evaluation of sporicidal activity of the product **Chlorinex-60**

Tab No. 9.1 The efficacy of chemical disinfectant **Chlorinex-60** on test strains – sporicidal activity

Sporicidal activity of the product (EN 13704)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations	Interfering substances - conditions	lg R EN 13704	**lg R**
Bacillus subtilis ATCC 6633	20	15	10 tabs/1.5 l	dirty**	≥ 3	**> 3**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the spore test suspension, N_a = the number of survivors per ml in the test mixture at the end of the contact time, N_v = the number of cfu/ml of the spore validation test suspension, A,B,C = the number of cfu/ml in control tests (A – experimental conditions validation, B – neutralizer toxicity validation, C – method validation), lg $R = \lg [(N \times 10^{-1})/N_a]$ = the reduction in viability
** According to the client's request dirty conditions were used.

Prepared by: Hana Konevalíková, Lab Technician

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

Sampling date: 4.7.2014
Sample delivered: 9.7.2014
Testing date: 29.7. – 22.8.2014
Delivered amount: 300 tablets
Batch No: 810414
Page: 13

Experimental conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 14348)
Period of analysis:	24.7. – 14.8.2014
Test temperature:	20 °C ± 1 °C
Test method:	dilution neutralization method
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062
Product diluent:	hard water
Appearance of the products:	white tablets
Test concentration:	2 tabs/1.5 l (2000 ppm AC), 6 tabs/1.5 l (6000 ppm AC)
Contact time:	15 min
Interfering substances:	0.3 g/l BSA (clean conditions) 3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)
Test organisms:	*Mycobacterium avium* ATCC 15769 *Mycobacterium terrae* ATCC 15755
Incubation conditions:	37 °C ± 1 °C, 21 days

Test procedure:

1. Preparation of test suspension
2. Preparation of product test solutions
3. Quantitative suspension test
4. Incubation and calculation
5. Expression and interpretation of results

Note:
Mycobactericidal activity – the capability of a product to produce a reduction in the number of viable cells of *Mycobacterium terrae* and *Mycobacterium avium* under defined conditions by at least 4 orders (10^4).
Tuberculocidal activity - the capability of a product to produce a reduction in the number of viable cells of *Mycobacterium terrae* under defined conditions by at least 4 orders (10^4).
$R = N_0 / N_a$ nebo $\lg R = \lg N_0 - \lg N_a$ the reduction in viability

The standard:
EN 14348 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants in the medical area including instrument disinfectants - Test method and requirements (phase 2, step 1) January 2005

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

Sampling date: 4.7.2014
Sample delivered: 9.7.2014
Testing date: 29.7. – 22.8.2014
Delivered amount: 300 tablets
Batch No: 810414

10. Testing the efficacy of chemical disinfectant **Chlorinex-60** on *Mycobacterium avium* ATCC 15769

Tab No. 10.1.1 Verification of methodology, clean conditions

Validation of suspension (N_{vo})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 2 tab/1.5 l		
V_{c1}	28	$\Phi_{Nvo} = 34.5$	V_{c1}	33	$\Phi_A = 31.5$	V_{c1}	26	$\Phi_B = 30$	V_{c1}	28	$\Phi_C = 31$
V_{c2}	41		V_{c2}	30		V_{c2}	34		V_{c2}	34	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 10.1.2 Verification of methodology, dirty conditions

Validation of suspension (N_{vo})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 6 tab/1.5 l		
V_{c1}	28	$\Phi_{Nvo} = 34.5$	V_{c1}	34	$\Phi_A = 33$	V_{c1}	26	$\Phi_B = 30$	V_{c1}	36	$\Phi_C = 31$
V_{c2}	41		V_{c2}	32		V_{c2}	34		V_{c2}	26	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 10.2 Test suspensions

Test suspension N $\Phi = 36 \times 10^8 = \lg 9.56$ $9.17 \leq \lg N \leq 9.70$	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0) $\lg N_0 = \lg N/10 = \lg 8.56$ $8.17 \leq \lg N_0 \leq 8.70$
	10^{-7}	> 330	> 330	
	10^{-8}	39	33	
				x yes no

Tab No. 10.3 Testing the efficacy of chemical disinfectant **Chlorinex-60** on *Mycobacterium avium* ATCC 15769

Test concentration /contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**$\lg R$ ($\lg N_0 = \lg 8.56$)**
2 tabs/1.5 l /15/clean	10^{-1}	<14	<14	< 3.15	**≥ 5.41**
6 tabs/1.5 l /15/dirty	10^{-1}	<14	<14	< 3.15	**≥ 5.41**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the test suspension, N_0 = the number of cfu/ml of the test suspension at the beginning of the contact time (time „0"), N_a = the number of survivors per ml in the test mixture at the end of the contact time and before the dilution neutralization method, N_v = the number of cfu/ml of the test suspension for validation, N_{v0}= the number of cfu/ml of the test suspension in the mixture A,B,C at the beginning of the contact time (time „0"), A,B,C = the number of survivors per ml in control tests (A – experimental conditions control, B – neutralization validation, C – method validation)
$R = N_0 / N_a$ nebo $\lg R = \lg N_0 - \lg N_a$ the reduction in viability

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: 29.7. – 22.8.2014
Delivered amount: 300 tablets
Batch No: 810414

11. Testing the efficacy of chemical disinfectant **Chlorinex-60** on *Mycobacterium terrae* ATCC 15755

Tab No. 11.1.1 Verification of methodology, clean conditions

Validation of suspension (N_{vo})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 2 tab/1.5 l		
V_{c1}	55	$\Phi_{Nvo} = 52$	V_{c1}	45	$\Phi_A = 48$	V_{c1}	61	$\Phi_B = 54.5$	V_{c1}	31	$\Phi_C = 35.5$
V_{c2}	49		V_{c2}	51		V_{c2}	48		V_{c2}	40	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 11.1.2 Verification of methodology, dirty conditions

Validation of suspension (N_{vo})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 6 tab/1.5 l		
V_{c1}	55	$\Phi_{Nvo} = 52$	V_{c1}	48	$\Phi_A = 48.5$	V_{c1}	61	$\Phi_B = 54.5$	V_{c1}	43	$\Phi_C = 46.5$
V_{c2}	49		V_{c2}	49		V_{c2}	48		V_{c2}	50	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 11.2 Test suspensions

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0)
$\Phi = 48 \times 10^8 = \lg 9.68$	10^{-7}	> 330	> 330	$\lg N_0 = \lg N/10 = \lg 8.68$
$9.17 \leq \lg N \leq 9.70$	10^{-8}	45	51	$8.17 \leq \lg N_0 \leq 8.70$
				x yes no

Tab No. 11.3 Testing the efficacy of chemical disinfectant **Chlorinex-60** on *Mycobacterium terrae* ATCC 15755

Test concentration /contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**$\lg R$ ($\lg N_0 = \lg 8.68$)**
2 tabs/1.5 l /15/clean	10^{-1}	<14	<14	< 3.15	**≥ 5.53**
6 tabs/1.5 l /15/dirty	10^{-1}	<14	<14	< 3.15	**≥ 5.53**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the test suspension, N_0 = the number of cfu/ml of the test suspension at the beginning of the contact time (time „0"), N_a = the number of survivors per ml in the test mixture at the end of the contact time and before the dilution neutralization method, N_v = the number of cfu/ml of the test suspension for validation, N_{v0}= the number of cfu/ml of the test suspension in the mixture A,B,C at the beginning of the contact time (time „0"), A,B,C = the number of survivors per ml in control tests (A – experimental conditions control, B – neutralization validation, C – method validation)
$R = N_0 / N_a$ nebo $\lg R = \lg N_0 - \lg N_a$ the reduction in viability

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: 29.7. – 22.8.2014
Delivered amount: 300 tablets
Batch No: 810414

12. Evaluation of mycobactericidal and tuberculocidal activity of the product **Chlorinex-60**

Tab No. 12.1 The efficacy of chemical disinfectant **Chlorinex-60** on test strain – mycobactericidal and tuberculocidal activity

Mycobactericidal activity of the product (EN 14348)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations	Interfering substances - conditions	lg R EN 14348	**lg R**
Mycobacterium avium ATCC 15769	20	15	2 tabs/1.5 l	clean	≥ 4	**> 4**
Mycobacterium terrae ATCC 15755	20	15	2 tabs/1.5 l	clean	≥ 4	**> 4**
Mycobacterium avium ATCC 15769	20	15	6 tabs/1.5 l	dirty	≥ 4	**> 4**
Mycobacterium terrae ATCC 15755	20	15	6 tabs/1.5 l	dirty	≥ 4	**> 4**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the test suspension, N_0 = the number of cfu/ml of the test suspension at the beginning of the contact time (time „0"), N_a = the number of survivors per ml in the test mixture at the end of the contact time and before the dilution neutralization method, N_v = the number of cfu/ml of the test suspension for validation, N_{v0}= the number of cfu/ml of the test suspension in the mixture A,B,C at the beginning of the contact time (time „0"), A,B,C = the number of survivors per ml in control tests (A – experimental conditions control, B – neutralization validation, C – method validation)
$R = N_0 / N_a$ nebo $\lg R = \lg N_0 - \lg N_a$ the reduction in viability

Prepared by: Mgr. Mirka Horáková, Ph.D., Lab Technician

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: 29.7. – 22.8.2014
Delivered amount: 300 tablets
Batch No: 810414

Experimental conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 13704)
Period of analysis:	18.8. – 22.8.2014
Test temperature:	20 °C ± 1 °C
Test method:	dilution neutralization method
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062
Product diluent:	hard water
Appearance of the products:	white tablets
Test concentration:	6 tabs/1.5 l (6000 ppm AC)
Contact time:	15 min
Interfering substances:	3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)***
Test organisms:	*Clostridium difficile* ATCC 9689***
Incubation conditions:	37 °C ± 1 °C, minimum 4 and maximum 7 days

Test procedure:

1. Preparation of the test suspension
2. Preparation of the product test solutions
3. Quantitative suspension test
4. Incubation and calculation
5. Expression and interpretation of the results

Note:
Sporicidal activity – the capability of a product to produce a reduction in the number of bacterial spores belonging to reference strain of *Bacillus subtilis* under defined conditions by at least 3 orders (10^3).
$\lg R = \lg [(N \times 10^{-1})/N_a]$
*** According to the client's request the strain and dirty conditions were used.

The standard:
EN 13704 Chemical disinfectants – Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1) February 2002

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: 29.7. – 22.8.2014
Delivered amount: 300 tablets
Batch No: 810414

13. Testing the efficacy of chemical disinfectant **Chlorinex-60** on *Clostridium difficile* ATCC 9689***

Tab No. 13.1 Verification of methodology

Validation of suspension (N_v) x 10^1			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 6 tabs/1.5 l		
V_{c1}	89	$\Phi_{Nv} = 94.5$	V_{c1}	90	$\Phi_A = 94$	V_{c1}	92	$\Phi_B = 93.5$	V_{c1}	88	$\Phi_C = 90.5$
V_{c2}	100		V_{c2}	98		V_{c2}	95		V_{c2}	93	
$60 \leq \Phi_{Nv} \leq 300$			$\Phi_A \geq 0.05\ \Phi_{Nv}$			$\Phi_B \geq 0.05\ \Phi_{Nv}$			$\Phi_C \geq 0.5\ \Phi_B$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 13.2 Test suspension

Test suspension (N)	N	V_{c1}	V_{c1}	$\Phi = 189 \times 10^4 = \lg 6.27$ $6.17 \leq \lg N \leq 6.70$
	10^{-4}	168	209	
	10^{-5}	21	18	
				x yes no

Tab No. 13.3 Testing the efficacy of chemical disinfectant **Chlorinex-60** on *Clostridium difficile* ATCC 9689***

Test concentration /contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R (lg N = lg 6.27)**
6 tabs/1.5 l / 15 / dirty***	10^0	<15	<15	< 2.18	**≥ 3.12**

14. Evaluation of sporicidal activity of the product **Chlorinex-60**

Tab No. 14.1 The efficacy of chemical disinfectant **Chlorinex-60** on test strains – sporicidal activity

Sporicidal activity of the product (EN 13704)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations	Interfering substances - conditions	lg R EN 13704	**lg R**
Clostridium difficile ATCC 9689***	20	15	6 tabs/1.5 l	dirty***	≥ 3	**> 3**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the spore test suspension, N_a = the number of survivors per ml in the test mixture at the end of the contact time, N_v = the number of cfu/ml of the spore validation test suspension, A,B,C = the number of cfu/ml in control tests (A – experimental conditions validation, B – neutralizer toxicity validation, C – method validation), lg $R = \lg [(N \times 10^{-1})/N_a]$ = the reduction in viability
*** According to the client's request the strain and dirty conditions were used.

Prepared by: Hana Konevalíková, Lab Technician

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: 29.7. – 22.8.2014
Delivered amount: 300 tablets
Batch No: 810414

Interpretation:
Results of tests are in Tabs.
According to EN 13727 the tested product **Chlorinex-60**, batch No: 810414, in concentrations 1 tab/1.5 l and 6 tabs/1.5 l, diluted in hard water, and in the contact time 15 min under clean and dirty conditions at temperature 20 °C ± 1 °C by the dilution neutralization method **decreased** the number of alive microbes *Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538, *Enterococcus hirae* ATCC 10541 by at least 5 (lg) orders.

According to EN 13624 the tested product **Chlorinex 60**, batch No: 810414, in the concentration 1 tab/1.5 l, diluted in hard water, in the contact time 15 min under clean conditions and in the concentration 6 tabs/1.5 l, diluted in hard water, in the contact time 15 min under dirty conditions at temperature 20 °C ± 1 °C by the dilution neutralization method **decreased** the number of alive microbes *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis (niger)* ATCC 16404 by at least 4 (lg) orders.

The tested product **Chlorinex 60**, batch No: 810414, in the concentration 10 tabs/1.5 l, diluted in hard water, in the contact time 15 min under dirty** conditions at temperature 20 °C ± 1 °C by the dilution neutralization method **decreased** the number of bacterial spores *Bacillus subtilis* ATCC 6633 by at least 3 (lg) orders (EN 13704).
** According to the client's request dirty conditions were used.

The tested product **Chlorinex 60**, batch No: 810414, in the concentration 2 tabs/1.5 l, diluted in hard water, in the contact time 15 min under clean conditions and in the concentration 6 tabs/1.5 l, diluted in hard water, in the contact time 15 min under dirty conditions at temperature 20 °C ± 1 °C by the dilution neutralization method **decreased** the number of alive microbes *Mycobacterium avium* ATCC 15769, *Mycobacterium terrae* ATCC 15755 by at least 4 (lg) orders (EN 14348).

The tested product **Chlorinex 60**, batch No: 810414, in the concentration 6 tabs/1.5 l, diluted in hard water, in the contact time 15 min under dirty*** conditions at temperature 20 °C ± 1 °C by the dilution neutralization method **decreased** the number of bacterial spores *Clostridium difficile* ATCC 9689*** by at least 3 (lg) orders (EN 13704).
*** According to the client's request the strain and dirty conditions were used.

Conclusion:
The product **Chlorinex 60** is capable of reducing the number of viable bacterial and vegetative yeast cells and mould spores of the relevant organism under defined conditions to the declared values, and consequently, may be called bactericidal and fungicidal.
The product **Chlorinex 60** is capable of reducing the number of bacterial spores of the relevant organisms under defined conditions to the declared values, and consequently, may be called sporicidal.
The product **Chlorinex 60** is capable of reducing the number of viable mycobacterial cells of the relevant organisms under defined conditions to the declared values and, consequently, may be called mycobactericidal and tuberculocidal.

25.8.2014, Hodonín

Ing. Jana Šitrov... ...f Studý

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

Copy No.: 2
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 Sampling date: 4.7.2014
Rep No: 116 Sample delivered: 9.7.2014
Sample name: **Chlorinex-60** Testing date: 7.11. – 20.11.2014
Sampled: by client Delivered amount: 300 tablets
Sampling point: AS CHEMI-PHARM, Pöllu 132, 109 17 Tallinn, Estonia Batch No: 810414
Client: AS CHEMI-PHARM, Pöllu 132, 109 17 Tallinn, Estonia Page: 2

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

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 Sampling date: 4.7.2014
Rep No: 116 Sample delivered: 9.7.2014
Sample name: **Chlorinex-60** Testing date: 7.11. – 20.11.2014
Sampled: by client Delivered amount: 300 tablets
Sampling point: AS CHEMI-PHARM, Pöllu 132, 109 17 Tallinn, Estonia Batch No: 810414
Client: AS CHEMI-PHARM, Pöllu 132, 109 17 Tallinn, Estonia Page: 3

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 Sampling date: 4.7.2014
Rep No: 116 Sample delivered: 9.7.2014
Sample name: **Chlorinex-60** Testing date: 7.11. – 20.11.2014
Sampled: by client Delivered amount: 300 tablets
Sampling point: AS CHEMI-PHARM, Pöllu 132, 109 17 Tallinn, Estonia Batch No: 810414
Client: AS CHEMI-PHARM, Pöllu 132, 109 17 Tallinn, Estonia Page: 4

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*

Sample ID: D98/2014 Sampling date: 4.7.2014
Rep No: 116 Sample delivered: 9.7.2014
Sample name: **Chlorinex-60** Testing date: 7.11. – 20.11.2014
Sampled: by client Delivered amount: 300 tablets
Sampling point: AS CHEMI-PHARM, Pöllu 132, 109 17 Tallinn, Estonia Batch No: 810414
Client: AS CHEMI-PHARM, Pöllu 132, 109 17 Tallinn, Estonia

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*

Sample ID: D98/2014 Sampling date: 4.7.2014
Rep No: 116 Sample delivered: 9.7.2014
Sample name: **Chlorinex-60** Testing date: 7.11. – 20.11.2014
Sampled: by client Delivered amount: 300 tablets
Sampling point: AS CHEMI-PHARM, Pöllu 132, 109 17 Tallinn, Estonia Batch No: 810414
Client: AS CHEMI-PHARM, Pöllu 132, 109 17 Tallinn, Estonia Page: 6

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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Ing. Jana Šlitrová, Leader of Study

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

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

Ing. Jana Slitrová, Leader of Study

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

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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

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Zuzana Matuškova, Head of Laboratory