

Test report No. sd2018

EVALUATION OF MYCOBACTERICIDAL ACTIVITY (EN 14348)

Name of the product: Chlorinex-60

Batch number: 032017

Order number: 17014

Manufacturer: Chemi-Pharm Ltd.

Client, representative: Chemi-Pharm Ltd., Põllu 132, Tallinn, 10917, Estonia, Maris Millner, +372-51-77-090

Date of delivery: 05.01.2018

Test material conditions: No specific features, sample in the manufacturers tare

Storage conditions: In room temperature, dark;

Active substance – conc.: 1 tab = 1.5 g active chlorine if dissolved in water, thus 1 tab/1.5 l 1000 ppm active chlorine

Appearance of the product: White tablet

Test concentration: Ready to use

Test conditions: Dirty and clean conditions

Contact time: 15 min, 60 min (obligatory)

Interfering substance: 3,0 g/l bovine albumin + 3,0 ml SBE = dirty conditions; 0,3 g/l bovine albumin = clean conditions

Test neutralizer: -

Rinsing liquid: Water

Test organisms: *Mycobacterium terrae* ATCC 15755;
Mycobacterium avium ATCC 15769

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

Testing date: 29.01.2018 – 19.02.2018

Results: look appendix 1-3



Diana Kaare, MSc
Head of laboratory, microbiologist
Date of test report: 26.02.2018

Appendix 1

TEST RESULTS (mycobactericidal suspension test)

EVS-EN 14348:2005; Phase 2, step 1;
Membrane filtration method; Spread plate;
Rinsing liquid: Water;
Test organism: *Mycobacterium terrae* ATCC 15755;
Test temperature: +20° C; Incubation temperature: +37° C
Solvents: diluent, water;
Interfering substance: 3,0 g/l bovine albumin + 3,0 ml SBE = dirty conditions; 0,3 g/l bovine albumin = clean conditions
Nordic Tersus Laboratory LLC.;
Date of test: 29.01.2018 – 19.02.2018
Responsible person: Diana Kaare

Validation and controls

Dirty and clean conditions

Validation suspension N_{vo}			Experimental conditions (A)			Filtration control (B)			Method validation (C)		
V_{C1}	80	$\bar{x} = 76$	V_{C1}	49	$\bar{x} = 49,5$	V_{C1}	62	$\bar{x} = 53,5$	V_{C1}	74	$\bar{x} = 72,5$
V_{C2}	72		V_{C2}	50		V_{C2}	45		V_{C2}	71	
30 ≤ $\bar{x} N_{vo}$ ≤ 160? yes X; no ☐			$\bar{x} A$ is ≥ 0,5 $\bar{x} N_{vo}$? yes X; no ☐			$\bar{x} B$ is ≥ 0,5 $\bar{x} N_{vo}$? yes X; no ☐			$\bar{x} C$ is ≥ 0,5 $\bar{x} N_{vo}$? yes X; no ☐		

Test suspension and test

Test suspension: N and N_0	N	V_{C1}	V_{C2}	$\bar{x}_{wm} = 2,56 \times 10^9$; $\log N = 9,41$ $N_0 = N/10$; $\log N_0 = 8,41$ $8,17 \leq \log N_0 \leq 8,7$; yes X; no ☐
	10^{-7}	262	249	
	10^{-8}	30	23	

Experimental results

Concentration of the product. %	Dilution step	V _{C1}	V _{C2}	Na (=x*10)	log Na	logR	Contact time	Conditions
1 tab/ 25l	10 ⁰	>165	>165	1280000	6,11	2,30	15 min	dirty
	10 ⁻¹	>165	>165					
	10 ⁻²	>165	>165					
	10 ⁻³	132	124					
1 tab/ 25l	10 ⁰	>165	>165	1070000	6,03	2,38	60 min	dirty
	10 ⁻¹	>165	>165					
	10 ⁻²	>165	>165					
	10 ⁻³	104	110					
1 tab/ 25l	10 ⁰	>165	>165	905000	5,96	2,45	15 min	clean
	10 ⁻¹	>165	>165					
	10 ⁻²	>165	>165					
	10 ⁻³	93	88					
1 tab/ 25l	10 ⁰	>165	>165	795000	5,90	2,51	60 min	clean
	10 ⁻¹	>165	>165					
	10 ⁻²	>165	>165					
	10 ⁻³	82	77					
1 tab / 1.5l	10 ⁰	<14	<14	<140	<2,15	>6,26	15 min	dirty
	10 ⁻¹	<14	<14					
	10 ⁻²	<14	<14					
	10 ⁻³	<14	<14					
1 tab / 1.5l	10 ⁰	<14	<14	<140	<2,15	>6,26	60 min	dirty
	10 ⁻¹	<14	<14					
	10 ⁻²	<14	<14					
	10 ⁻³	<14	<14					
1 tab / 1.5l	10 ⁰	<14	<14	<140	<2,15	>6,26	15 min	clean
	10 ⁻¹	<14	<14					
	10 ⁻²	<14	<14					
	10 ⁻³	<14	<14					
1 tab / 1.5l	10 ⁰	<14	<14	<140	<2,15	>6,26	60 min	clean
	10 ⁻¹	<14	<14					
	10 ⁻²	<14	<14					
	10 ⁻³	<14	<14					
5 tab / 1.5l	10 ⁰	<14	<14	<140	<2,15	>6,26	15 min	dirty
	10 ⁻¹	<14	<14					
	10 ⁻²	<14	<14					
	10 ⁻³	<14	<14					
5 tab / 1.5l	10 ⁰	<14	<14	<140	<2,15	>6,26	60 min	dirty
	10 ⁻¹	<14	<14					
	10 ⁻²	<14	<14					
	10 ⁻³	<14	<14					

5 tab / 1.5l	10^0	<14	<14	<140	<2,15	>6,26	15 min	clean
	10^{-1}	<14	<14					
	10^{-2}	<14	<14					
	10^{-3}	<14	<14					
5 tab / 1.5l	10^0	<14	<14	<140	<2,15	>6,26	60 min	clean
	10^{-1}	<14	<14					
	10^{-2}	<14	<14					
	10^{-3}	<14	<14					

Explanations:

V_c = count per ml (one plate or more)

$\bar{x}$ = average of V_{c1} and V_{c2} (1. + 2. duplicate)

N = cfu/ml microbes in testsuspension

N_0 = cfu/ml at the start of the contact time (t=0)

N_{vo} = cfu/ml in the validation suspension (t=0)

Na = surviving microbes after the test

R = reduction factor ($R = N_0 / Na$; $\text{Log}R = \text{Log}N_0 - \text{Log}Na$)

Appendix 2

TEST RESULTS (mycobactericidal suspension test)

EVS-EN 14348:2005; Phase 2, step 1;
Membrane filtration method; Spread plate;
Rinsing liquid: Water;
Test organism: *Mycobacterium avium* ATCC 15769;
Test temperature: +20° C; Incubation temperature: +37° C
Solvents: diluent, water;
Interfering substance: 3,0 g/l bovine albumin + 3,0 ml SBE = dirty conditions; 0,3 g/l bovine albumin = clean conditions
Nordic Tersus Laboratory LLC.;
Date of test: 29.01.2018 – 19.02.2018
Responsible person: Diana Kaare

Validation and controls

Dirty and clean conditions

Validation suspension N_{vo}			Experimental conditions (A)			Filtration control (B)			Method validation (C)		
V_{C1}	94	$\bar{x} = 93$	V_{C1}	80	$\bar{x} = 74,5$	V_{C1}	65	$\bar{x} = 62$	V_{C1}	93	$\bar{x} = 94,5$
V_{C2}	92		V_{C2}	69		V_{C2}	59		V_{C2}	96	
30 ≤ $\bar{x} N_{vo}$ ≤ 160 ? yes X; no ☐			$\bar{x}$ A is ≥ 0,5 $\bar{x} N_{vo}$? yes X; no ☐			$\bar{x}$ B is ≥ 0,5 $\bar{x} N_{vo}$? yesX; no ☐			$\bar{x}$ C is ≥ 0,5 $\bar{x} N_{vo}$? yes X; no ☐		

Test suspension and test

Test suspension: N and N_0	N	V_{C1}	V_{C2}	$\bar{x}_{wm} = 2,86 \times 10^9$; $\log N = 9,46$ $N_0 = N/10$; $\log N_0 = 8,46$ $8,17 \leq \log N_0 \leq 8,7$; yes X; no ☐
	10^{-7}	283	291	
	10^{-8}	31	25	

Experimental results

Concentration of the product. %	Dilution step	V _{C1}	V _{C2}	Na (=x*10)	log Na	logR	Contact time	Conditions
1 tab/ 25l	10 ⁰	>165	>165	1120000	6,05	2,41	15 min	dirty
	10 ⁻¹	>165	>165					
	10 ⁻²	>165	>165					
	10 ⁻³	103	121					
1 tab/ 25l	10 ⁰	>165	>165	905000	5,96	2,50	60 min	dirty
	10 ⁻¹	>165	>165					
	10 ⁻²	>165	>165					
	10 ⁻³	95	86					
1 tab/ 25l	10 ⁰	>165	>165	810000	5,91	2,55	15 min	clean
	10 ⁻¹	>165	>165					
	10 ⁻²	>165	>165					
	10 ⁻³	79	83					
1 tab/ 25l	10 ⁰	>165	>165	745000	5,87	2,59	60 min	clean
	10 ⁻¹	>165	>165					
	10 ⁻²	>165	>165					
	10 ⁻³	72	77					
1 tab / 1.5l	10 ⁰	<14	<14	<140	<2,15	>6,31	15 min	dirty
	10 ⁻¹	<14	<14					
	10 ⁻²	<14	<14					
	10 ⁻³	<14	<14					
1 tab / 1.5l	10 ⁰	<14	<14	<140	<2,15	>6,31	60 min	dirty
	10 ⁻¹	<14	<14					
	10 ⁻²	<14	<14					
	10 ⁻³	<14	<14					
1 tab / 1.5l	10 ⁰	<14	<14	<140	<2,15	>6,31	15 min	clean
	10 ⁻¹	<14	<14					
	10 ⁻²	<14	<14					
	10 ⁻³	<14	<14					
1 tab / 1.5l	10 ⁰	<14	<14	<140	<2,15	>6,31	60 min	clean
	10 ⁻¹	<14	<14					
	10 ⁻²	<14	<14					
	10 ⁻³	<14	<14					
5 tab / 1.5l	10 ⁰	<14	<14	<140	<2,15	>6,31	15 min	dirty
	10 ⁻¹	<14	<14					
	10 ⁻²	<14	<14					
	10 ⁻³	<14	<14					
5 tab / 1.5l	10 ⁰	<14	<14	<140	<2,15	>6,31	60 min	dirty
	10 ⁻¹	<14	<14					
	10 ⁻²	<14	<14					
	10 ⁻³	<14	<14					

5 tab / 1.5l	10^0	<14	<14	<140	<2,15	>6,31	15 min	clean
	10^{-1}	<14	<14					
	10^{-2}	<14	<14					
	10^{-3}	<14	<14					
5 tab / 1.5l	10^0	<14	<14	<140	<2,15	>6,31	60 min	clean
	10^{-1}	<14	<14					
	10^{-2}	<14	<14					
	10^{-3}	<14	<14					

Explanations:

V_c = count per ml (one plate or more)

$\bar{x}$ = average of V_{c1} and V_{c2} (1. + 2. duplicate)

N = cfu/ml microbes in testsuspension

N_0 = cfu/ml at the start of the contact time (t=0)

N_{vo} = cfu/ml in the validation suspension (t=0)

N_a = surviving microbes after the test

R = reduction factor ($R = N_0 / N_a$; $\text{Log}R = \text{Log}N_0 - \text{Log}N_a$)

Appendix 3

Interpretation

Using the EN 14348 standard, there was tested a product – Chlorinex-60 - (Batch No. 032017), at 20 °C $\pm$ 1 °C, with the contact time 15 min and 60 min under dirty and clean conditions. The membrane filtration method was used for testing products' effectiveness against the reference strains: *Mycobacterium terrae* ATCC 15755, *Mycobacterium avium* ATCC 15769. Under dirty and clean conditions the tested product was active against all the testorganisms at contact times tested.

Conclusion

By the test results can be concluded that as treated by the product the surviving microorganisms count showed at least lg 4 reduction, meaning that under clean and dirty conditions the 1 tablet in 1.5l water solution of Chlorinex-60 is mycobactericidal in case of surface disinfection, during contact time of 15 min.



Diana Kaare

Head of laboratory, microbiologist