Test report No. 13/2015

EVALUATION OF BACTERICIDAL ACTIVITY (EN 13727)

Name of the product:	**CHEMISEPT G**
Batch number:	LOT 69090215
Manufacturer:	Chemi-Pharm Ltd.
Client, representative:	Chemi-Pharm Ltd., Põllu 132, Tallinn, 10917, ESTONIA
	Maris Millner, +372-51-77-090
Date of delivery:	16.04.2015
Date of registration:	16.04.2015
Test material conditions:	no specific features, sample in the manufacturers tare
Storage conditions:	in room temperature, dark
Appearance of the product:	amount 1000 ml
Contact time:	15 sec, clean and dirty conditions
Interfering substance:	3,0 g/l bovine albumin and 3,0 ml/l sheep blood erythrocytes
Test neutralizer:	polysorbate 80, 30 g/l; saponine, 30 g/l; lecithin, 3 g/l
Test organisms:	*Pseudomonas aeruginosa* ATCC 15442; *Staphylococcus aureus* ATCC 6538, *Enterococcus hirae* ATCC 10541, *Escherichia coli K12* NCTC 10538.
Testing method:	**EVS-EN 13727:2012+A1:2013**
	Quantitative suspension test for the valuation of bactericidal activity in the medical area
Testing date:	06.04 – 10.04.2015
Results:	look at appendix 1-4

Raul Rauskepp, MSc

Head of Laboratory, microbiologist

Nordic Tersus Laboratory

TEST RESULTS (bactericidal suspension test)

Product: **CHEMISEPT G**
Dilution neutralization method; Spread plate;
Neutralizers: Polysorbate 80, 30 g/l; Saponine, 30 g/l; lecithin, 3 g/l
Test organism: *Pseudomonas aeruginosa* ATCC 15442;
Test temperatuure: $+20°$ C; Incubation temperatuure: $+37°$ C
Solvents: water;
Interfering substance: BSA 3,0 g/l and sheep blood erythrocytes 3,0 ml/l
Nordic Tersus Laboratory LLC.; Date of test: 06.04 – 10.04.2015
Responsible person: Raul Raudsepp

Validation and controls

Clean conditions

Validation suspension N_{vo}			Experimental conditions (A)			Neutralizer control (B)			Method validation (C)		
V_{C1}	67+54=121	$\bar{\bar{x}}=$ 123,5	V_{C1} 15 sek	48+56= 104	$\bar{\bar{x}}=$ 111	V_{C1}	97+115= 212	$\bar{\bar{x}}=$ 212	V_{C1} 15 sek	55+67= 122	$\bar{\bar{x}}=$ 124,5
V_{C2}	57+69=126		V_{C2} 15 sek	60+58= 118		V_{C2}			V_{C2} 15 sek	69+58= 127	
$30 \leq \bar{x} \, N_{vo} \leq 160$? yes X; no ☐			$\bar{x}$ **A** is $\geq 0,5 \, \bar{x} \, N_{vo}$? yes X; no ☐			$\bar{x}$ **B** is $\geq 0,5 \, \bar{x} \, N_{vo}$? yes X; no ☐			$\bar{x}$ **C** is $\geq 0,5 \, \bar{x} \, N_{vo}$? yes X; no ☐		
Validation suspension N_{VB}			V_{C1} 66+48=114			V_{C2} 50+55=105; $\bar{x}$=109,5			$30 \leq \bar{x} \, N_{VB}/1000 \leq 160$? yes X; no ☐		

Test suspensioon and test

Test suspensioon and test

Test suspension: N and N_0	N	V_{C1}	V_{C2}	$\bar{\bar{x}} = 1,87 \times 10^9$; $logN = 9,27$
	10^{-7}	164	203	$N_0 = N/100$; $logN_0 = 7,27$
	10^{-8}	19	26	$7,17 \leq logN_0 \leq 7,7$; yes X; no ☐

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Experimental results

Concentration of the product.%	Dilution step	V_{C1}	V_{C2}	Na (=$\bar{x}$*10)	log Na	logR	Contact time	Conditions
RTU	-	0	0	<140	<2,15	>5,12	15''	clean
	10x	0	0					

Validation and controls

Dirty conditions

Validation suspension N_{vo}

V_{C1}	67+52=121	$\bar{x}$ = 123,5
V_{C2}	57+69=126	

$30 \le \bar{x}\ N_{wo} \le 160$? yes X; no □

Validation suspension N_{VB}	V_{C1} 78+61=139

Experimental conditions (A)

V_{C1} 15 sec	54+62=116	$\bar{x}$ = 121,5
V_{C2} 15 sec	68+59=127	

$\bar{x}$ A is $\ge 0,5\ \bar{x}\ N_{vo}$? yes X; no □

Neutralizer control (B)

V_{C1}	97+112=209	$\bar{x}$ = 209
V_{C2}		

$\bar{x}$ B is $\ge 0,5\ \bar{x}\ N_{vo}$? yesX; no □

V_{C2} 64+68=132; $\bar{x}$=135,5

Method validation (C)

V_{C1}	73+59=132	$\bar{x}$ = 139
V_{C2}	70+76=146	

$\bar{x}$ C is $\ge 0,5\ \bar{x}\ N_{vo}$? yes X; no □

$30 \le \bar{x}\ N_{VB}/1000 \le 160$? yes X; no □

Test suspension and test

Test suspensioon and test

Test suspension: N and N_0	N	V_{C1}	V_{C2}	
	10^{-7}	164	203	$\bar{x}$ = 1,87 x 10^9; logN = 9,27
	10^{-8}	19	26	N_0 = N/100; logN_0 = 7,27
				$7,17 \le \log N_0 \le 7,7$; yes X; no □

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Experimental results

Concentration of the product.%	Dilution step	V_{C1}	V_{C2}	Na ($=\bar{x}*10$)	log Na	logR	Contact time	Conditions
RTU	-	0	0	<140	<2,15	>5,12	15''	dirty
	10x	0	0					

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_{VB} = cfu/ml on the neutralizer control

Na = surviving microbes after the test

R = reduction factor (R= N_0/Na; LogR=LogN_0 - Log Na)

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TEST RESULTS (bactericidal suspension test)

Product: CHEMISEPT G
Dilution neutralization method; Spread plate;
Neutralizers: Polysorbate 80, 30 g/l; Saponine, 30 g/l; lecithin, 3 g/l;
Test organism: *Staphylococcus aureus* ATCC 6538;
Test temperatuure: +20° C; Incubation temperatuure: +37° C
Solvents: water;
Interfering substance: BSA 3,0 g/l and sheep blood erythrocytes 3,0 ml/l
Nordic Tersus Laboratory LLC.; Date of test: 06.04 – 10.04.2015
Responsible person: Raul Raudsepp

Validation and controls

Clean conditions

	Validation suspension N_{vo}		Experimental conditions (A)		Neutralizer control (B)		Method validation (C)	
V_{C1}	63+74=137	$\bar{x}=143$	65+70=135	$\bar{x}=125,5$	70+71=141	$\bar{x}=141$	51+67=118	$\bar{x}=111,5$
V_{C2}	81+68=149		48+68=116				49+56=105	
	$30 \le \bar{x}\,N_{vo} \le 160$? yes X; no □		$\bar{x}$ A is $\ge 0,5\ \bar{x}\ N_{vo}$? yes X; no □		$\bar{x}$ B is $\ge 0,5\ \bar{x}\ N_{vo}$? yes X; no □		$\bar{x}$ C is $\ge 0,5\ \bar{x}\ N_{vo}$? yes X; no □	
Validation suspension N_{VB}	V_{C1} 89+73=162				V_{C2} 82+74=156; $\bar{x}=159$		$30 \le \bar{x}\,N_{VB}/1000 \le 160$? yes X; no □	

Test suspensioon and test

Test suspension: N and N_0	N	V_{C1}	V_{C2}
	10^{-7}	**225**	**238**
	10^{-8}	**32**	**28**

$\bar{x} = 2.38 \times 10^9$; $\log N = 9,37$
$N_0 = N/100$; $\log N_0 = 7,37$
$7,17 \le \log N_0 \le 7,7$; yes X; no □

Experimental results

Concentration of the product.%	Dilution step	V_{C1}	V_{C2}	Na (=$\bar{x}$*10)	log Na	logR	Contact time	Conditions
RTU	-	0	0	<140	<2,15	>5,22	15''	clean
	10x	0	0					

Validation and controls

Dirty conditions

Validation suspension N_{vo}

V_{C1}	63+72=137	$\bar{x}$ = 143
V_{C2}	81+68=149	

30 ≤ $\bar{x}$ N_{vo} ≤160 ? yes X; no □

Validation suspension N_{VB}	V_{C1} 78+61=139

Experimental conditions (A)

V_{C1} 15 sek	81+76=157	$\bar{x}$ =
V_{C2} 15 sek	59+82=141	

$\bar{x}$ A is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no □

Neutralizer control (B)

V_{C1}	70+71=141	$\bar{x}$ = 141
V_{C2}		

$\bar{x}$ B is ≥ 0,5 $\bar{x}$ N_{vo}? yesX; no □

V_{C2} 64+68=132; $\bar{x}$=135,5

Method validation (C)

V_{C1} 30 sek	66+69=135	$\bar{x}$ = 140
V_{C2} 30 sek	75+70=145	

$\bar{x}$ C is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no □

30 ≤ $\bar{x}$ N_{VB}/1000 ≤160 ? yes X; no □

Test suspensioon and test

Test suspension: N and N_0	N	V_{C1}	V_{C2}
	10^{-7}	225	238
	10^{-8}	32	28

$\bar{x}$ = 2.38 x 10^9; logN = 9,37

N_0 = N/100; logN_0 = 7,37

7,17≤ logN_0≤7,7; yes X; no □

Experimental results

Concentration of the product.%	Dilution step	V_{C1}	V_{C2}	Na ($=\bar{x}*10$)	log Na	logR	Contact time	Conditions
RTU	-	0	0	<140	<2,15	>5,22	15''	dirty
	10x	0	0					

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_{VB} = cfu/ml on the neutralizer control

Na = surviving microbes after the test

R = reduction factor ($R= N_0/Na$; $LogR=LogN_0 - Log Na$)

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TEST RESULTS (bactericidal suspension test)

Product: CHEMISEPT G
Dilution neutralization method; Spread plate;
Neutralizers: Polysorbate 80, 30 g/l; Saponine, 30 g/l; lecithin, 3 g/l;
Test organism: *Enterococcus hirae* ATCC 10541;
Test temperatuure: +20° C; Incubation temperatuure: +37° C
Solvents: water;
Interfering substance: BSA 3,0 g/l and sheep blood erythrocytes 3,0 ml/l
Nordic Tersus Laboratory LLC.; Date of test: 06.04 – 10.04.2015
Responsible person: Raul Raudsepp

Validation and controls

Clean conditions

Validation suspension N_{vo}		Experimental conditions (A)		Neutralizer control (B)		Method validation (C)	
V_{C1}	72+86=158	V_{C1}	80+8=167	V_{C1}	131+103=234	V_{C1}	76+84=160
	$\bar{x}=154,5$		$\bar{x}=168$		$\bar{x}=234$		$\bar{x}=155,5$
V_{C2}	81+70=151	V_{C2}	79+90=169	V_{C2}		V_{C2}	83+68=151
$30 \leq \bar{x}\,N_{vo} \leq160$? yes X; no □		$\bar{x}$ A is $\geq$ 0,5 $\bar{x}\,N_{vo}$? yes X; no □		$\bar{x}$ B is $\geq$ 0,5 $\bar{x}\,N_{vo}$? yesX; no □		$\bar{x}$ C is $\geq$ 0,5 $\bar{x}\,N_{vo}$? yes X; no □	
Validation suspension N_{VB}	V_{C1} 78+61=139			V_{C2} 64+68=132; $\bar{x}$=135,5		$30 \leq \bar{x}\,N_{VB}/1000 \leq160$? yes X; no □	

Test suspension and test

Test suspension: N and N_0	N	V_{C1}	V_{C2}	
	10^{-7}	271	325	$\bar{x}$ = 2.98 x 10^9; log$N$ = 9,47
	10^{-8}	25	35	N_0 = N/100; logN_0 = 7,47
				7,17≤ logN_0≤7,7; yes X; no □

Experimental results

Concentration of the product.%	Dilution step	V_{C1}	V_{C2}	Na (=x̄*10)	log Na	logR	Contact time	Conditions
RTU	-	0	0	<140	<2,15	>5,32	15"	clean
	10x	0	0					

Validation and controls

Dirty conditions

Validation suspension N_{vo}			Experimental conditions (A)			Neutralizer control (B)			Method validation (C)		
V_{C1}	72+86=158	x̄ = 154,5	V_{C1} 15 sek	101+97=198	x̄ = 191	V_{C1}	131+103=234	x̄ = 234	V_{C1}	88+93=181	x̄ = 177
V_{C2}	81+67=15 1		V_{C2} 15 sek	95+89=184		V_{C2}			V_{C2}	79+94=173	
30 ≤ x̄ N_{vo} ≤160 ? yes X; no □			x̄ A is ≥ 0,5 x̄ N_{vo}? yes X; no □			x̄ B is ≥ 0,5 x̄ N_{vo}? yesX; no □			x̄ C is ≥ 0,5 x̄ N_{vo}? yes X; no □		
Validation suspension N_{VB}			V_{C1} 78+61=139			V_{C2} 64+68=132; x̄=135,5			30 ≤ x̄ N_{VB}/1000 ≤160 ? yes X; no □		

Test suspensioon and test

Test suspension: N and N_0	N	V_{C1}	V_{C2}	
	10^{-7}	271	325	x̄ = 2.98 x 10^9; logN = 9,47
	10^{-8}	25	35	N_0 = N/100; logN_0 = 7,47
				7,17≤ logN_0≤7,7; yes X; no □

Experimental results

Concentration of the product.%	Dilution step	V_{C1}	V_{C2}	Na (=x̄*10)	log Na	logR	Contact time	Conditions
RTU	-	0	0	<140	<2,15	>5,32	15"	dirty
	10x	0	0					

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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)

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N_{VB} = cfu/ml on the neutralizer control

Na = surviving microbes after the test

R = reduction factor ($R = N_0/Na$; $LogR = LogN_0 - Log\,Na$)

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TEST RESULTS (bactericidal suspension test)

Product: CHEMISEPT G

Dilution neutralization method; Spread plate;
Neutralizers: Polysorbate 80, 30 g/l; Saponine, 30 g/l; lecithin, 3 g/l;
Test organism: *Escherichia coli* K12 NCTC 10538;
Test temperatuure: +20° C; Incubation temperatuure: +37° C
Solvents: water;
Interfering substance: BSA 3,0 g/l and sheep blood erythrocytes 3,0 ml/l
Nordic Tersus Laboratory LLC.; Date of test: 06.04 – 10.04.2015
Responsible person: Raul Raudsepp

Validation and controls

Clean conditions

Validation suspension N_{vo}			Experimental conditions (A)				Neutralizer control (B)				Method validation (C)			
V_{C1}	67+80=147	$\bar{x} =$ 133,5	V_{C1} 15 sek	95+99= 194	$\bar{x} =$ 179,5		V_{C1}	98+108= 206	$\bar{x} =$ 206		V_{C1}	86+90= 176	$\bar{x} =$ 169,5	
V_{C2}	62+58=120		V_{C2} 15 sek	79+86= 165			V_{C2}				V_{C2}	78+85= 163		
$30 \leq \bar{x}\ N_{vo} \leq 160$? yes X; no ☐			$\bar{x}$ A is $\geq 0,5\ \bar{x}\ N_{vo}$? yes X; no ☐				$\bar{x}$ B is $\geq 0,5\ \bar{x}\ N_{vo}$? yes X; no ☐				$\bar{x}$ C is $\geq 0,5\ \bar{x}\ N_{vo}$? yes X; no ☐			
Validation suspension N_{VB}			V_{C1} 55+67=122				V_{C2} 71+68=139; $\bar{x}$=130,5				$30 \leq \bar{x}\ N_{VB}/1000 \leq 160$? yes X; no ☐			

Test suspension and test

Test suspension: N and N_0	N	V_{C1}	V_{C2}	$\bar{x} = 3,04 \times 10^9$; $\log N = 9,48$
	10^{-7}	316	298	$N_0 = N/100$; $\log N_0 = 7,48$
	10^{-8}	31	24	$7,17 \leq \log N_0 \leq 7,7$; yes X; no ☐

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Experimental results

Concentration of the product.%	Dilution step	V_{C1}	V_{C2}	Na (=x̄*10)	log Na	logR	Contact time	Conditions
RTU	-	0	0	<140	<2,15	>5,33	15''	clean
	10x	0	0					

Validation and controls

Dirty conditions

Validation suspension N_{vo}			Experimental conditions (A)			Neutralizer control (B)			Method validation (C)		
V_{C1}	72+83=155	x̄ = 152,5	V_{C1} 15 sek	103+97= 200	x̄ = 191,5	V_{C1}	131+103= 234	x̄ = 234	V_{C1}	98+90= 188	x̄ = 188,5
V_{C2}	81+69=150		V_{C2} 15 sek	89+94= 183		V_{C2}			V_{C2}	88+101= 189	
30 ≤ x̄ N_{vo} ≤160 ? yes X; no □			x̄ A is ≥ 0,5 x̄ N_{vo}? yes X; no □			x̄ B is ≥ 0,5 x̄ N_{vo}? yesX; no □			x̄ C is ≥ 0,5 x̄ N_{vo}? yes X; no □		
Validation suspension N_{VB}			V_{C1} 78+61=139			V_{C2} 64+68=132; x̄=135,5			30 ≤ x̄ $N_{VB}/1000$ ≤160 ? yes X; no □		

Test suspension and test

Test suspension: N and N_0	N	V_{C1}	V_{C2}	
	10^{-7}	316	298	x̄ = 3,04 x 10^9; logN = 9,48
	10^{-8}	31	24	N_0 = N/100; logN_0 = 7,48
				7,17≤ logN_0≤7,7; yes X; no □

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Experimental results

Concentration of the product.%	Dilution step	V_{C1}	V_{C2}	Na ($=\bar{x}*10$)	log Na	logR	Contact time	Conditions
RTU	-	0	0	<140	<2,15	>5,33	15''	dirty
	10x	0	0					

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_{VB} = cfu/ml on the neutralizer control

Na = surviving microbes after the test

R = reduction factor (R= N_0/ Na; LogR=LogN_0 - Log Na)

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Interpretation

Using **EN 13727** methodics, there was tested ready-to-use product for hand rubbing - **CHEMISEPT G,** in temperature at 20 °C ± 1 °C, with the contact times: 15 seconds and the conditions: clean and dirty. The dilution neutralization method was been used for the testing of product effect onto the microorganisms: *Pseudomonas aeruginosa* ATCC 15442; *Staphylococcus aureus* ATCC 6538, *Enterococcus hirae* ATCC 10541, *Escherichia coli* K12 NCTC 10538. In clean and dirty conditions tested product was active against all the testorganims at the all tested contact times.

Conclusions

By the test results it can be made conclusion, that tested product **CHEMISEPT G has bactericidal effect** in case of hand rubbing at the conditions tested, as treated by the product the surviving microorganisms count was decreasing at least five grades.

Raul Raudsepp

Head of laboratory, microbiologist