

Test report no. 035021sd

EVALUATION OF BACTERICIDAL ACTIVITY OF DISINFECTANTS AND ANTISEPTICS USED IN THE MEDICAL AREA (EN 13727)

Name of the product: BACTICID AF

Batch number: 197020821/2
Date of test report: 19.09.2021

Client, representative: Chemi-Pharm Ltd. Tänassilma tee 11 Saku vald, 76406, ESTONIA Maris Millner



Test report no.035021sd

EVALUATION OF BACTERICIDAL ACTIVITY OF DISINFECTANTS AND ANTISEPTICS USED IN THE MEDICAL AREA (EN 13727)

Name of the product*:

BACTICID AF

Batch number*:

197020821/2

Order number:

20045

Manufacturer*:

Chemi-Pharm Ltd.

Client, representative*:

Chemi-Pharm Ltd., Tänassilma tee 11, Tänassilma küla, Saku vald,

76406, ESTONIA, Maris Millner, +372 5177090

Date of delivery:

06.08.2021

Test material conditions:

No specific features, sample in the manufacturers tare

Storage conditions:

At room temperature, darkness

Active substance - conc.*:

Ethyl alcohol 57.0% w/w, isopropyl alcohol 6.0% w/w

Appearance of the product:

Transparent, colourless

Test concentration:

80.0%, 50.0%, 10.0%

Contact time:

15s

Interfering substance:

3.0 g/l bovine albumin + 3.0 ml/l sheep blood erythrocytes (dirty

conditions)

Neutralizer:

Rinsing liquid:

Tryptone 1 g/l + NaCl, 9 g/l

Test organisms:

Pseudomonas aeruginosa ATCC 15442 Staphylococcus aureus ATCC 6538 Enterococcus hirae ATCC 10541

Testing method:

EVS-EN 13727:2012+A2:2015

Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity in the medical area - Test

> NORDIC TERSUS LABORATORY

method and requirements (phase 2, step 1)

Testing period:

10.08.2021 - 12.08.2021

Results:

look appendix 1-3

Interpretation and conclusion: look appendix 4

Melissa Ingela Bramanis Microbiologist Date of issue: 19.09.2021

* - Data provided by the customer





Appendix 1

TEST RESULTS (bactericidal suspension test)

EVS-EN 13727:2012+A2:2015; Phase 2, step 1

Membrane filtration method

Rinsing liquid: Tryptone 1 g/l + NaCl 9 g/l

Test organism: Staphylococcus aureus ATCC 6538

Test temperature: +20° C; Incubation temperature: +37°C

Interfering substance: 3.0 g/l bovine albumin + 3.0 ml/l sheep blood erythrocytes

Nordic Tersus Laboratory LLC. Date of test: 10.08.2021

Responsible person: Melissa Ingela Bramanis

Validation and controls

Dirty conditions

Validation suspension N_{vo}		suspension N_{vo} Experimental conditions (A)				Filtration	control (B)	Method validation (C)		
V _{C1}	V _{C2}	Σ̈	V _{C1}	V_{C2}	Ā	V _{C1}	V _{C2}	x	V _{C1}	V _{C2}	x
86	74	78.5	35	53	44	40	43	41.5	48	49	48.5
30 ≤ x̄ N	vo ≤160?ye	es X; no □	x̄ A is ≥ C).5 x N vo?ye	es X;no □	$\bar{\mathbf{x}} \mathbf{B} \text{ is } \geq 0$.5 x N v₀? y	esX; no □	x̄ C is ≥ 0).5 x̄ N ∞? y	'es X;no □

Test suspension and test

Test suspension:	N	V _{C1}	V _{C2}	$\bar{x}_{wm} = 3.21 \times 10^8$; $\log N = 8.50$
N and N_0	10 ⁻⁶	310	>330	$N_0 = N/10$; $\log N_0 = 7.50$
N ana No	10 ⁻⁷	44	24	7.17≤ log N ₀≤7.70; yesX; no □

Experimental results

Concentration of the product	Dilution step	V _{C1}	V _{C2}	Na (=x̄*10)	lg <i>Na</i>	<i>lg</i> R	Contact time	Conditions
% 80.0%	-	<14	<14	<140	<2.15	>5.35	15s	Dirty
50.0%	-	>165	>165	>1650	>3.22	<4.28	15s	Dirty
10.0%	_	>165	>165	>1650	>3.22	<4.28	15s	Dirty

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 test suspension

 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 ; LogR=Log N_0 - Log Na)



Appendix 2

TEST RESULTS (bactericidal suspension test)

EVS-EN 13727:2012+A2:2015; Phase 2, step 1

Membrane filtration method

Rinsing liquid: Tryptone 1 g/l + NaCl 9 g/l; Test organism: *Enterococcus hirae* ATCC 10541

Test temperature: +20° C; Incubation temperature: +37°C

Interfering substance: 3.0 g/l bovine albumin + 3.0 ml/l sheep blood erythrocytes

Nordic Tersus Laboratory LLC. Date of test: 10.08.2021

Responsible person: Melissa Ingela Bramanis

Validation and controls

Dirty conditions

Validation suspension N _{vo}		suspension N_{vo} Experimental conditions (A)					control (B)	Method validation (C)		
V _{C1}	V _{C2}	Σ̈	V _{C1}	V _{C2}	x	V _{C1}	V _{C2}	Ā	V _{C1}	V _{C2}	χ
73	63	68	68	65	66.5	61	61 63 62		68	68 65	
30 ≤ x̄ N	l _{vo} ≤160?ye	es X; no □	x̄ A is ≥ C).5 x̄ N v₀?y	es X;no □	\bar{x} B is $\geq 0.5 \bar{x}$ N_{vo} ? yesX; no \Box		$\bar{x} \mathbf{C} \text{ is } \geq 0$).5 x N _{vo} ? y	es X;no □	

Test suspension and test

Test suspension:	N	V _{C1}	V _{C2}	$\bar{x}_{wm} = 3.13 \times 10^8$; $\log N = 8.50$
N and N_0	10 ⁻⁶	>330	298	$N_0 = N/10$; $\log N_0 = 7.50$
IV and IV0	10 ⁻⁷	29	31	7.17≤ log N ₀≤7.70; yesX; no 🗆

Experimental results

Concentration of the product %	Dilution step	V _{C1}	V _{C2}	Na (=x̄*10)	lg <i>Na</i>	<i>lg</i> R	Contact time	Conditions
80.0%	-	<14	<14	<140	<2.15	>5.35	15s	Dirty
50.0%	-	>165	>165	>1650	>3.22	<4.28	15s	Dirty
10.0%	-	>165	>165	>1650	>3.22	<4.28	15s	Dirty

Explanations:

 V_C = count per ml (one plate or more)

 \bar{x} = average of V_{C1} and V_{C2} (1. + 2. Duplicate)

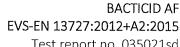
N = cfu/ml microbes in test suspension

 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 ; LogR=Log N_0 - Log Na)





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Appendix 3

TEST RESULTS (bactericidal suspension test)

EVS-EN 13727:2012+A2:2015; Phase 2, step 1

Membrane filtration method

Rinsing liquid: tryptone 1 g/l + NaCl 9 g/l

Test organism: Pseudomonas aeruginosa ATCC 15442 Test temperature: +20° C; Incubation temperature: +37°C

Interfering substance: 3.0 g/l bovine albumin + 3.0 ml/l sheep blood erythrocytes

Nordic Tersus Laboratory LLC Date of test: 10.08.2021

Responsible person: Melissa Ingela Bramanis

Validation and controls

Dirty conditions

Validation suspension N_{vo}		Experim	ental condi	tions (A)	Filtration	control (B)	Method validation (C)			
V _{C1}	V_{C2}	Ā	V _{C1}	V_{C2}	χ	V _{C1}	V_{C2}	Σ	V _{C1}	V _{C2}	x
65	101	83	65	67	66	73	73 79 76		71 64		67.5
30 ≤ x̄ Λ	<i>l_{vo}</i> ≤160?y∈	es X; no 🗆	x̄ A is ≥ C).5 x̄ Ν νο?γε	es X;no □	x̄ B is ≥ 0	\bar{x} B is $\geq 0.5 \bar{x}$ N_{vo} ? yesX; no \Box		$\bar{x} \mathbf{C} \text{ is } \geq 0$.5 x N ∞?y	res X;no □

Test suspension and test

Test suspension:	N	V _{C1}	V_{C2}	$\bar{x}_{wm} = 3.33 \times 10^8$; $\log N = 8.52$
N and N_0	10 ⁻⁶	>330	>330	$N_0 = N/10$; $\log N_0 = 7.52$
iv and ivo	10 ⁻⁷	42	30	7.17≤ log N ₀≤7.70; yesX; no □

Experimental results

Concentration of the product %	Dilution step	V _{C1}	V _{C2}	Na (=x*10)	lg <i>Na</i>	<i>lg</i> R	Contact time	Conditions
80.0%	-	<14	<14	<140	<2.15	>5.37	15s	Dirty
50.0%	-	>165	>165	>1650	>3.22	<4.30	15s	Dirty
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 V_C = count per ml (one plate or more)

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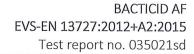
N = cfu/ml microbes in test suspension

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

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

Na = surviving microbes after the test

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





Appendix 4

Interpretation:

The ready to use product BACTICID AF (batch no. 197020821/2) was tested according to the test method EVS-EN 13727:2012+A2:2015. The test was performed at 20 °C \pm 1 °C, under dirty conditions with the contact time of 15 s. The membrane filtration method was used for testing the product's effectiveness against the reference strains: *Pseudomonas aeruginosa* ATCC 15442, *Enterococcus hirae* ATCC 10541 and *Staphylococcus aureus* ATCC 6538. Under dirty conditions the tested product was effective against all the reference strains within the contact time tested.

Conclusion:

The surviving count of bacterial reference strains showed at least 5lg reduction meaning that according to EVS-EN 13727:2012+A2:2015 under dirty conditions the sample ready to use surface disinfection product BACTICID AF has a bactericidal effect within 15 s.

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This is the end of the test report

Melissa Ingela Bramanis

Microbiologist

Date of issue: 19.09.2021