



**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., Tännassilma tee 11, Tännassilma 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  
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 $N_{vo}$			Experimental conditions (A)			Filtration control (B)			Method validation (C)		
$V_{C1}$	$V_{C2}$	$\bar{x}$	$V_{C1}$	$V_{C2}$	$\bar{x}$	$V_{C1}$	$V_{C2}$	$\bar{x}$	$V_{C1}$	$V_{C2}$	$\bar{x}$
86	74	78.5	35	53	44	40	43	41.5	48	49	48.5
$30 \leq \bar{x} N_{vo} \leq 160$ ? yes X; no <input type="checkbox"/>			$\bar{x} A$ is $\geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>			$\bar{x} B$ is $\geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>			$\bar{x} C$ is $\geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>		

**Test suspension and test**

Test suspension: $N$ and $N_0$	$N$	$V_{C1}$	$V_{C2}$	$\bar{x}_{wm} = 3.21 \times 10^8$ ; $\log N = 8.50$ $N_0 = N/10$ ; $\log N_0 = 7.50$ $7.17 \leq \log N_0 \leq 7.70$ ; yes X; no <input type="checkbox"/>
	$10^{-6}$	310	>330	
	$10^{-7}$	44	24	

**Experimental results**

Concentration of the product %	Dilution step	$V_{C1}$	$V_{C2}$	$N_a$ ( $=\bar{x} \cdot 10$ )	$\lg N_a$	$\lg R$	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)

$N$  = cfu/ml microbes in test suspension

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

$R$  = reduction factor ( $R = N_0 / N_a$ ;  $\log R = \log N_0 - \log N_a$ )

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

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

$N_a$  = surviving microbes after the test

The test results apply to the tested sample only.

All the components of this test report are recognized as a portion of a complete report. The test report shall not be reproduced except in full, without approval of the laboratory.

N-7/29-V9

**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 $N_{vo}$			Experimental conditions (A)			Filtration control (B)			Method validation (C)		
$V_{C1}$	$V_{C2}$	$\bar{x}$	$V_{C1}$	$V_{C2}$	$\bar{x}$	$V_{C1}$	$V_{C2}$	$\bar{x}$	$V_{C1}$	$V_{C2}$	$\bar{x}$
73	63	68	68	65	66.5	61	63	62	68	65	66.5
$30 \leq \bar{x} N_{vo} \leq 160$ ? yes X; no <input type="checkbox"/>			$\bar{x} A$ is $\geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>			$\bar{x} B$ is $\geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>			$\bar{x} C$ is $\geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>		

**Test suspension and test**

Test suspension:	$N$	$V_{C1}$	$V_{C2}$	$\bar{x}_{wm} = 3.13 \times 10^8$ ; $\log N = 8.50$ $N_0 = N/10$ ; $\log N_0 = 7.50$ $7.17 \leq \log N_0 \leq 7.70$ ; yes X; no <input type="checkbox"/>
$N$ and $N_0$	$10^{-6}$	>330	298	
	$10^{-7}$	29	31	

**Experimental results**

Concentration of the product %	Dilution step	$V_{C1}$	$V_{C2}$	$N_a$ ( $=\bar{x} * 10$ )	$\lg N_a$	$\lg R$	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)

$N$  = cfu/ml microbes in test suspension

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

$R$  = reduction factor ( $R = N_0 / N_a$ ;  $\log R = \log N_0 - \log N_a$ )

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

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

$N_a$  = surviving microbes after the test

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 $N_{vo}$			Experimental conditions (A)			Filtration control (B)			Method validation (C)		
$V_{C1}$	$V_{C2}$	$\bar{x}$	$V_{C1}$	$V_{C2}$	$\bar{x}$	$V_{C1}$	$V_{C2}$	$\bar{x}$	$V_{C1}$	$V_{C2}$	$\bar{x}$
65	101	83	65	67	66	73	79	76	71	64	67.5
$30 \leq \bar{x} N_{vo} \leq 160$ ? yes X; no <input type="checkbox"/>			$\bar{x} A$ is $\geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>			$\bar{x} B$ is $\geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>			$\bar{x} C$ is $\geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>		

**Test suspension and test**

Test suspension:	$N$	$V_{C1}$	$V_{C2}$	$\bar{x}_{wm} = 3.33 \times 10^8$ ; $\log N = 8.52$ $N_0 = N/10$ ; $\log N_0 = 7.52$ $7.17 \leq \log N_0 \leq 7.70$ ; yes X; no <input type="checkbox"/>
$N$ and $N_0$	$10^{-6}$	>330	>330	
	$10^{-7}$	42	30	

**Experimental results**

Concentration of the product %	Dilution step	$V_{C1}$	$V_{C2}$	$N_a$ ( $=\bar{x} \cdot 10$ )	$\lg N_a$	$\lg R$	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
10.0%	-	>165	>165	>1650	>3.22	>4.30	15s	Dirty

**Explanations:**

$V_C$  = count per ml (one plate or more)

$N$  = cfu/ml microbes in test suspension

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

$R$  = reduction factor ( $R = N_0 / N_a$ ;  $\log R = \log N_0 - \log N_a$ )

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

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

$N_a$  = surviving microbes after the test

The test results apply to the tested sample only.

All the components of this test report are recognized as a portion of a complete report. The test report shall not be reproduced except in full, without approval of the laboratory.

N-7/29-V9

**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 ± 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.

This is the end of the test report





.....  
Melissa Ingela Bramanis  
Microbiologist  
Date of issue: 19.09.2021