

Test report No. id0418

EVALUATION OF BACTERICIDAL ACTIVITY FOR INSTRUMENTS USED IN MEDICAL AREA (EN 14561)

Name of the product: Bactacid AF
Batch number: 197101017
Order number: 17029
Manufacturer: Chemi-Pharm Ltd.
Client, representative: Chemi-Pharm Ltd., Põllu 132, Tallinn, 10917, ESTONIA
Maris Millner, +372-51-77-090
Date of delivery: 15.12.2017
Test material conditions: No specific features, sample in the manufacturers tare
Storage conditions: In room temperature, dark
Active substance – conc.: Ethyl alcohol 57.0% wt, isopropyl alcohol 6.0% wt
Appearance of the product: Transparent liquid
Test concentration: Ready to use
Contact time: 30 sec, 60 min (obligatory contact time)
Interfering substance: 3 g/l bovine albumin + 3 ml/l sheep blood erythrocytes= dirty conditions
Rinsing liquid: -
Neutralizer: Polysorbate 80 30g/l; saponin 30 g/l, lecithin 3 g/l
Test organisms: *Pseudomonas aeruginosa* ATCC 15442
Enterococcus hirae ATCC 10541
Staphylococcus aureus ATCC 6538
Testing method: EVS-EN 14561:2006
Quantitative carrier test for the evaluation of bactericidal activity for instruments used in medical area (phase 2, step 2)
Testing date: 10.02.2018 – 12.02.2018
Results: look appendix 1-4



Diana Kaare, MSc

Head of laboratory, microbiologist

Date of test report: 14.02.2018

Appendix 1

TEST RESULTS (bactericidal carrier test)

EVS-EN 14561; Phase 2, step 2;
Dilution – neutralization method;
Rinsing liquid: -;
Test organism: *Pseudomonas aeruginosa* ATCC 15442;
Test temperature: +20° C; Incubation temperature: +37° C
Interfering substance: 3 g/l bovine albumin + 3 ml/l sheep blood erythrocytes= dirty conditions;
Nordic Tersus Laboratory LLC.;
Date of test: 10.02.2018 – 12.02.2018;
Responsible person: Diana Kaare

Validation and controls

Validation suspension (N_{vo})				Experimental Conditions control (A)				Neutralizer control (B)				Method validation (C)			
Counts per plate		V_{C1}	V_{C2}	Counts per plate		V_{C1}	V_{C2}	Counts per plate		V_{C1}	V_{C2}	Counts per plate		V_{C1}	V_{C2}
27+22	25+28	49	53	18+23	19+23	41	42	15+21	18+18	36	36	24+20	22+16	44	38
$\bar{x} = 51$				$\bar{x} = 41.5$				$\bar{x} = 36$				$\bar{x} = 41$			
$30 \leq \bar{x} \text{ of } N_{vo} \leq 160?$ yes <input checked="" type="checkbox"/> ; no <input type="checkbox"/>				$\bar{x} \text{ A is } \geq 0,5 \bar{x} \text{ of } N_{vo}?$ yes <input checked="" type="checkbox"/> ; no <input type="checkbox"/>				$\bar{x} \text{ B is } \geq 0,5 \bar{x} \text{ of } N_{vo}?$ yes <input checked="" type="checkbox"/> ; no <input type="checkbox"/>				$\bar{x} \text{ C is } \geq 0,5 \bar{x} \text{ of } N_{vo}?$ yes <input checked="" type="checkbox"/> ; no <input type="checkbox"/>			

Test suspension

Test suspension (N):	N	V_{C1}	V_{C2}	$\bar{x} \text{ wm} = 1.92 \times 10^9; \lg N = 9.28$ $9.17 \leq \lg N \leq 9.7$ yes <input checked="" type="checkbox"/> ; no <input type="checkbox"/>
	10^{-7}	184	199	
	10^{-8}	23	17	

Water control

Water control (N_w):	N_w	Counts per plate		V_{C1}	V_{C2}	$\bar{x} \times 10 = 1.70 \times 10^7$ $7.15 \leq \lg N_w = 7.23 \leq (\lg N - 1.3)?$ yes <input checked="" type="checkbox"/> ; no <input type="checkbox"/>
	10^{-5}	6+6	9+13	12	22	

Test

Conc. of the product %	Dilution step	Counts per plate		V _{c1}	V _{c2}	Lg N _a = Lg (\bar{x} or \bar{x}_{wm}) + 1	Lg R (lg N _w = 7.23)	Contact time
Ready to use	10 ⁰	8+8	9+8	16	17	2.22	5.01	30 sec
	10 ⁻¹	0+2	1+1	<14	<14			
	10 ⁻²	0+0	0+0	<14	<14			
	10 ⁻³	0+0	0+0	<14	<14			
Ready to use	10 ⁰	0+2	1+0	<14	<14	<2.15	>5.08	60 min
	10 ⁻¹	0+0	0+0	<14	<14			
	10 ⁻²	0+0	0+0	<14	<14			
	10 ⁻³	0+0	0+0	<14	<14			

Explanations

V_c = count per ml (one plate or more)
 \bar{x} = average of V_{c1} and V_{c2}

R = reduction (lg R = lg N_w - lg N_a)
 If N_a < 140, lg R = > [lg N_w - 2,15]

TEST RESULTS (bactericidal carrier test)

EVS-EN 14561; Phase 2, step 2;
Dilution – neutralization method;
Rinsing liquid: -;
Test organism: *Enterococcus hirae* ATCC 10541;
Test temperature: +20° C; Incubation temperature: +37° C
Interfering substance: 3 g/l bovine albumin + 3 ml/l sheep blood erythrocytes= dirty conditions;
Nordic Tersus Laboratory LLC.;
Date of test: 10.02.2018 – 12.02.2018;
Responsible person: Diana Kaare

Validation and controls

Validation suspension (N_{vo})				Experimental Conditions control (A)				Neutralizer control (B)				Method validation (C)			
Counts per plate		V_{c1}	V_{c2}	Counts per plate		V_{c1}	V_{c2}	Counts per plate		V_{c1}	V_{c2}	Counts per plate		V_{c1}	V_{c2}
42+39	46+49	81	95	37+39	31+44	76	75	35+32	41+39	67	80	29+33	27+30	62	57
$\bar{x} = 88$				$\bar{x} = 75.5$				$\bar{x} = 65.5$				$\bar{x} = 44.5$			
$30 \leq \bar{x} \text{ of } N_{vo} \leq 160?$ yes <input checked="" type="checkbox"/> ; no <input type="checkbox"/>				$\bar{x} A \text{ is } \geq 0,5 \bar{x} \text{ of } N_{vo}?$ yes <input checked="" type="checkbox"/> ; no <input type="checkbox"/>				$\bar{x} B \text{ is } \geq 0,5 \bar{x} \text{ of } N_{vo}?$ yes <input checked="" type="checkbox"/> ; no <input type="checkbox"/>				$\bar{x} C \text{ is } \geq 0,5 \bar{x} \text{ of } N_{vo}?$ yes <input checked="" type="checkbox"/> ; no <input type="checkbox"/>			

Test suspension

Test suspension (N):	N	V_{c1}	V_{c2}	$\bar{x} \text{ wm} = 2.52 \times 10^9$; $\lg N = 9.40$ $9.17 \leq \lg N \leq 9.7$ yes <input checked="" type="checkbox"/> ; no <input type="checkbox"/>
	10^{-7}	241	263	
	10^{-8}	30	21	

Water control

Water control (N_w):	N_w	Counts per plate		V_{c1}	V_{c2}	$\bar{x} \times 10 = 2.60 \times 10^7$ $7.15 \leq \lg N_w = 7.41 \leq (\lg N - 1.3)?$ yes <input checked="" type="checkbox"/> ; no <input type="checkbox"/>
	10^{-5}	13+15	9+15	28	24	

Test

Conc. of the product %	Dilution step	Counts per plate		V _{c1}	V _{c2}	Lg N _a = Lg (\bar{x} or \bar{x}_{wm}) + 1	Lg R (lg N _w = 7.41)	Contact time
Ready to use	10 ⁰	13+9	11+15	22	26	2.38	5.03	30 sec
	10 ⁻¹	0+2	1+1	<14	<14			
	10 ⁻²	0+0	0+0	<14	<14			
	10 ⁻³	0+0	0+0	<14	<14			
Ready to use	10 ⁰	4+6	5+8	<14	<14	<2.15	>5.26	60 min
	10 ⁻¹	0+1	0+1	<14	<14			
	10 ⁻²	0+0	0+0	<14	<14			
	10 ⁻³	0+0	0+0	<14	<14			

Explanations

V_c = count per ml (one plate or more)

\bar{x} = average of V_{c1} and V_{c2}

R = reduction (lg R = lg N_w - lg N_a)

If N_a < 140, lg R = > [lg N_w - 2,15]

TEST RESULTS (bactericidal carrier test)

EVS-EN 14561; Phase 2, step 2;
Dilution – neutralization method;
Rinsing liquid: -;
Test organism: *Staphylococcus aureus* ATCC 6538;
Test temperature: +20° C; Incubation temperature: +37° C
Interfering substance: 3 g/l bovine albumin + 3 ml/l sheep blood erythrocytes= dirty conditions;
Nordic Tersus Laboratory LLC.;
Date of test: 10.02.2018 – 12.02.2018;
Responsible person: Diana Kaare

Validation and controls

Validation suspension (N_{vo})				Experimental Conditions control (A)				Neutralizer control (B)				Method validation (C)			
Counts per plate		V_{C1}	V_{C2}	Counts per plate		V_{C1}	V_{C2}	Counts per plate		V_{C1}	V_{C2}	Counts per plate		V_{C1}	V_{C2}
32+29	40+36	61	76	25+31	30+33	56	63	26+34	39+32	60	71	22+19	24+24	41	48
$\bar{x} = 68.5$				$\bar{x} = 59.5$				$\bar{x} = 65.5$				$\bar{x} = 44.5$			
30 $\leq \bar{x}$ of $N_{vo} \leq 160$? yes <input checked="" type="checkbox"/> ; no <input type="checkbox"/>				\bar{x} A is $\geq 0,5 \bar{x}$ of N_{vo} ? yes <input checked="" type="checkbox"/> ; no <input type="checkbox"/>				\bar{x} B is $\geq 0,5 \bar{x}$ of N_{vo} ? yes <input checked="" type="checkbox"/> ; no <input type="checkbox"/>				\bar{x} C is $\geq 0,5 \bar{x}$ of N_{vo} ? yes <input checked="" type="checkbox"/> ; no <input type="checkbox"/>			

Test suspension

Test suspension (N):	N	V_{C1}	V_{C2}	\bar{x} wm = 2.11×10^9 ; lg N = 9.32 $9.17 \leq \lg N \leq 9.7$ yes <input checked="" type="checkbox"/> ; no <input type="checkbox"/>
	10^{-7}	204	218	
	10^{-8}	18	25	

Water control

Water control (N_w):	N_w	Counts per plate		V_{C1}	V_{C2}	$\bar{x} \times 10 = 2.55 \times 10^7$ $7.15 \leq \lg N_w = 7.41 \leq (\lg N - 1.3)$? yes <input checked="" type="checkbox"/> ; no <input type="checkbox"/>
	10^{-5}	11+14	10+16	25	26	

Test

Conc. of the product %	Dilution step	Counts per plate		V _{c1}	V _{c2}	Lg N _a = Lg (\bar{x} or \bar{x}_{wm}) + 1	Lg R (lg N _w = 7.41)	Contact time
Ready to use	10 ⁰	10+7	11+5	17	16	2.38	5.19	30 sec
	10 ⁻¹	1+1	1+1	<14	<14			
	10 ⁻²	0+0	0+0	<14	<14			
	10 ⁻³	0+0	0+0	<14	<14			
Ready to use	10 ⁰	3+2	1+1	<14	<14	<2.15	>5.26	60 min
	10 ⁻¹	0+1	1+0	<14	<14			
	10 ⁻²	0+0	0+0	<14	<14			
	10 ⁻³	0+0	0+0	<14	<14			

Explanations

V_c = count per ml (one plate or more)
 \bar{x} = average of V_{c1} and V_{c2}

R = reduction (lg R = lg N_w - lg N_a)
 If N_a < 140, lg R = > [lg N_w - 2,15]

Appendix 4

Interpretation

The product for instrument disinfection Bactacid AF (batch no. 197101017) was tested according to the test method EVS-EN 14561:2006. The test was performed at 20 ± 1 °C, under dirty conditions with the contact time of 30 sec. The dilution – neutralization method was used for testing the products' effectiveness against the reference strains: *Pseudomonas aeruginosa* ATCC 15442; *Enterococcus hirae* ATCC 10541 and *Staphylococcus aureus* ATCC 6538. Under dirty conditions the product was effective against all the reference strains within 30 sec of contact time.

Conclusion

The surviving count of bacterial reference strains showed at least 5 lg reduction meaning that under dirty conditions the ready to use product Bactacid AF has a bactericidal effect in case of instrument disinfection within 30 sec.



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Diana Kaare, MSc

Head of laboratory, microbiologist