Nordic Tersus
Laboratory

Test report No. sd0818

EVALUATION OF BACTERICIDAL AND YEASTICIDAL ACTIVITY ON NON-POROUS SURFACES WITH MECHANICAL ACTION EMPLOYING WIPES IN THE MEDICAL AREA (EN 16615)

Name of the product:	Bacticid AF Wipes
Batch number:	197101017
Order number:	12029
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:	White wipes
Test concentration:	Ready to use
Contact time:	1 min
Interfering substance:	0,3 g/l bovine albumin = clean conditions
Rinsing liquid:	-
Neutralizer:	Polysorbate 80 30g/l; saponin 30 g/l, lecithin 3 g/l
Test organisms:	*Candida albicans* ATCC 10231 *Pseudomonas aeruginosa* ATCC 15442 *Enterococcus hirae* ATCC 10541 *Staphylococcus aureus* ATCC 6538
Testing method:	EVS-EN 16615:2015 Quantitative test method for the evaluation of bactericidal and yeasticidal activity on non-porous surfaces with mechanical action employing wipes in the medical area (4- field test) - Test method and requirements (phase 2, step 2)
Testing date:	13.02.2018 – 15.02.2018
Results:	look appendix 1-5

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...are, MSc

Head of l ...biologist

Date of test report: 19.02.2018

Appendix 1

TEST RESULTS (suspension test)

EVS-EN 16615:2015; Phase 2, step 2;
Neutralization method;
Rinsing liquid: -;
Test organism: *Candida albicans* ATCC 10231;
Test temperature: +20° C; Incubation temperature: +30° C
Interfering substance: 0,3 g/l bovine albumin = Clean conditions;
Nordic Tersus Laboratory LLC.; Date of test: 13.02.2018 – 15.02.2018
Responsible person: Diana Kaare

Validation and controls

Validation suspension (N_{V0})			Neutralizer control (B)			Method validation (C)		
V_{C1}	41+34=75	$\bar{x}$ = 71	V_{C1}	19+27=46	$\bar{x}$ = 54	V_{C1}	30+36=66	$\bar{x}$ = 59.5
V_{C2}	28+39=67		V_{C2}	32+30=62		V_{C2}	29+24=53	
30 ≤ $\bar{x}$ N_{vo} ≤160? yes X; no □			$\bar{x}$ of *B* is ≥ 0,5 x $\bar{x}$ of N_{vo}? (or N_V/1000) yesX; no □			$\bar{x}$ of *C* is ≥ 0,5 x $\bar{x}$ of N_{vo}? yesX; no □		

Test suspension and test

Test-suspension (*N and N0*):	*N*	V_{C1}	V_{C2}	$\bar{x}_{wm} = 2.32 \times 10^8$; log*N* = 8,37 $N_0 = N/20$; logN_0 = 7.06 6,88≤ logN_{V0}≤7,40; yesX; no □
	10^{-6}	238	223	
	10^{-7}	25	25	

Drying controls

Drying control (D_{C0})	T_0	V_{C1}	V_{C2}	$\bar{x}_{wm} = 4.90 \times 10^6$; log$T_0$ = 6.69 5,88≤ logT_0≤7,40; yesX; no □
	10^{-3}	>330	>330	
	10^{-4}	46	52	

Drying controls

Drying control (D_{Ct})	T_0	V_{C1}	V_{C2}	$\bar{x}_{wm}$ 53.5 = x 10^6; logT_t = 6.73 5,88≤ logT_t≤7,40; yesX; no □
	10^{-3}	>330	>330	
	10^{-4}	50	57	

Test field 1 (reduction)

Real conc. of the product %	Dilution step	V_{C1}	V_{C2}	N_a (=$\bar{x}$ or $\bar{x}_{wm}$)	log N_a	log R (log T_t - log N_a)	Contact time (min)
Ready to use	10^0	8	5	6.5	0.81	5.88	1 min
	10^{-1}	0	0				

Test field 2 to 4 (cfu/25cm^2)

Real conc. of the product %	Dilution step	$V_C T_2$	$V_C T_3$	$V_C T_4$	V_{T2to4} (=$\bar{x}$ or $\bar{x}_{wm}$ x 5) cfu/25cm^2	Contact time (min)
Ready to use	10^0	1	1	0	<5	1 min
Ready to use	10^{-1}	0	0	0	<5	1 min

N_W Test fields 2 to 4 (cfu/25cm^2)

Real conc. of the product %	Dilution step	$V_C T_2$	$V_C T_3$	$V_C T_4$	$V_{NWT2to4}$ (=$\bar{x}$ or $\bar{x}_{wm}$ x 5) cfu/25cm^2	Contact time (min)
Ready to use	10^0	>660	>660	>660	>16500	10 min
	10^{-1}	>660	>660	>660		

Explanations:
V_C = count per ml (one plate or more)
$\bar{x}$ = average of V_{C1} and V_{C2} (1. + 2. duplicate)
$\bar{x}_{wm}$ = weighted mean of $\bar{x}$
R = reduction (logR = log N_0 – log N_a)

Appendix 2

TEST RESULTS (suspension test)

EVS-EN 16615:2015; Phase 2, step 2;
Neutralization method;
Rinsing liquid: -;
Test organism: *Staphylococcus aureus* ATCC 6538;
Test temperature: +20° C; Incubation temperature: +37° C
Interfering substance: 0,3 g/l bovine albumin = Clean conditions;
Nordic Tersus Laboratory LLC.; Date of test: 13.02.2018 – 15.02.2018
Responsible person: Diana Kaare

Validation and controls

Validation suspension (N_{V0})			Neutralizer control (B)			Method validation (C)		
V_{C1}	38+44=82	$\bar{x}$ = 91	V_{C1}	33+24=57	$\bar{x}$ = 66.5	V_{C1}	26+30=56	$\bar{x}$ = 51
V_{C2}	49+51=100		V_{C2}	37+39=76		V_{C2}	24+22=46	
30 ≤ $\bar{x}$ N_{vo} ≤160? yes X; no □			$\bar{x}$ of *B* is ≥ 0,5 x $\bar{x}$ of N_{vo}? (or N_V/1000) yesX; no □			$\bar{x}$ of *C* is ≥ 0,5 x $\bar{x}$ of N_{vo}? yesX; no □		

Test suspension and test

Test-suspension (*N and N0*):	*N*	V_{C1}	V_{C2}	$\bar{x}_{wm} = 2.29 \times 10^9$; log*N* = 9,36 $N_0 = N/20$; logN_0 = 8.06 7,88≤ logN_{V0}≤8,40; yesX; no □
	10^{-7}	242	211	
	10^{-8}	26	24	

Drying controls

Drying control (D_{C0})	T_0	V_{C1}	V_{C2}	$\bar{x}_{wm} = 4,95 \times 10^7$; log$T_0$ = 7.69 6,88≤ logT_0≤8,40; yesX; no □
	10^{-4}	>330	>330	
	10^{-5}	48	51	

Drying controls

Drying control (D_{Ct})	T_0	V_{C1}	V_{C2}	$\bar{x}_{wm}$ 5,7 = x 10^7; logT_t = 7,76 6,88≤ logT_t≤8,40; yesX; no □
	10^{-4}	>330	>330	
	10^{-5}	54	60	

Test field 1 (reduction)

Real conc. of the product %	Dilution step	V_{C1}	V_{C2}	N_a (=$\bar{x}$ or $\bar{x}_{wm}$)	log N_a	log R (log T_t - log N_a)	Contact time (min)
Ready to use	10^0	54	65	59.5	1.77	5.92	1 min
	10^{-1}	5	5				

Test field 2 to 4 (cfu/25cm^2)

Real conc. of the product %	Dilution step	$V_C T_2$	$V_C T_3$	$V_C T_4$	V_{T2to4} (=$\bar{x}$ or $\bar{x}_{wm}$ x 5) cfu/25cm^2	Contact time (min)
Ready to use	10^0	20	5	3	46,67	1 min
Ready to use	10^{-1}	2	0	0	<5	1 min

N_W Test fields 2 to 4 (cfu/25cm^2)

Real conc. of the product %	Dilution step	$V_C T_2$	$V_C T_3$	$V_C T_4$	$V_{NWT2to4}$ (=$\bar{x}$ or $\bar{x}_{wm}$ x 5) cfu/25cm^2	Contact time (min)
Ready to use	10^0	>660	>660	>660	>16500	1 min
	10^{-1}	>660	>660	>660		

Explanations:
V_C = count per ml (one plate or more)
$\bar{x}$ = average of V_{C1} and V_{C2} (1. + 2. duplicate)
$\bar{x}_{wm}$ = weighted mean of $\bar{x}$
R = reduction (logR = log N_0 – log N_a)

Appendix 3

TEST RESULTS (suspension test)
EVS-EN 16615:2015; Phase 2, step 2;
Neutralization method;
Rinsing liquid: -;
Test organism: *Pseudomonas aeruginosa* ATCC 15442;
Test temperature: +20° C; Incubation temperature: +37° C
Interfering substance: 0,3 g/l bovine albumin = Clean conditions;
Nordic Tersus Laboratory LLC.; Date of test: 13.02.2018 – 15.02.2018
Responsible person: Diana Kaare

Validation and controls

Validation suspension (N_{V0})			Neutralizer control (B)			Method validation (C)		
V_{C1}	28+32=60	$\bar{x}$ = 64.5	V_{C1}	18+35=53	$\bar{x}$ = 53.5	V_{C1}	27+17=44	$\bar{x}$ = 47.5
V_{C2}	36+33=69		V_{C2}	25+29=54		V_{C2}	22+29=51	
30 ≤ $\bar{x}$ N_{V0} ≤160? yes X; no □			$\bar{x}$ of *B* is ≥ 0,5 x $\bar{x}$ of N_{V0}? (or N_V/1000) yesX; no □			$\bar{x}$ of *C* is ≥ 0,5 x $\bar{x}$ of N_{V0}? yesX; no □		

Test suspension and test

Test-suspension (*N and N0):*	*N*	V_{C1}	V_{C2}	
	10^{-7}	193	208	$\bar{x}_{wm}$ = 2,00 x 10^9; log*N* = 9,30 N_0 = *N*/20; logN_0 = 8.00 7,88≤ logN_{V0}≤8,40; yesX; no □
	10^{-8}	21	17	

Drying controls

Drying control (D_{C0})	T_0	V_{C1}	V_{C2}	
	10^{-4}	>330	>330	$\bar{x}_{wm}$ = 4,6 x 10^7; logT_0 = 7.66 6,88≤ logT_0≤8,40; yesX; no □
	10^{-5}	42	50	

Drying controls

Drying control (D_{Ct})	T_0	V_{C1}	V_{C2}	
	10^{-4}	>330	>330	$\bar{x}_{wm}$ 7,20 = x 10^7; logT_t = 7,68 6,88≤ logT_t≤8,40; yesX; no □
	10^{-5}	47	49	

Test field 1 (reduction)

Real conc. of the product %	Dilution step	V_{C1}	V_{C2}	N_a (=$\bar{x}$ or $\bar{x}_{wm}$)	log N_a	log R (log T_t - log N_a)	Contact time (min)
Ready to use	10^0	10	8	9	0.95	6.71	1 min
	10^{-1}	1	1				

Test field 2 to 4 (cfu/25cm^2)

Real conc. of the product %	Dilution step	$V_C T_2$	$V_C T_3$	$V_C T_4$	V_{T2to4} (=$\bar{x}$ or $\bar{x}_{wm}$ x 5) cfu/25cm^2	Contact time (min)
Ready to use	10^0	2	1	0	5	1 min
Ready to use	10^{-1}	0	0	0	<5	1 min

N_W Test fields 2 to 4 (cfu/25cm^2)

Real conc. of the product %	Dilution step	$V_C T_2$	$V_C T_3$	$V_C T_4$	$V_{NWT2to4}$ (=$\bar{x}$ or $\bar{x}_{wm}$ x 5) cfu/25cm^2	Contact time (min)
Ready to use	10^0	>660	>660	>660	>16500	1 min
	10^{-1}	>660	>660	>660		

Explanations:
V_C = count per ml (one plate or more)
$\bar{x}$ = average of V_{C1} and V_{C2} (1. + 2. duplicate)
$\bar{x}_{wm}$ = weighted mean of $\bar{x}$
R = reduction (logR = log N_0 – log N_a)

Appendix 4

TEST RESULTS (suspension test)
EVS-EN 16615:2015; Phase 2, step 2;
Neutralization method;
Rinsing liquid: -;
Test organism: *Enterococcus hirae* ATCC 10541;
Test temperature: +20° C; Incubation temperature: +37° C
Interfering substance: 0,3 g/l bovine albumin = Clean conditions;
Nordic Tersus Laboratory LLC.; Date of test: 13.02.2018 – 15.02.2018
Responsible person: Diana Kaare

Validation and controls

Validation suspension (N_{V0})			Neutralizer control (B)			Method validation (C)		
V_{C1}	27+34=61	$\bar{x}$ = 61.5	V_{C1}	18+23=41	$\bar{x}$ = 36.5	V_{C1}	22+27=49	$\bar{x}$ = 46.5
V_{C2}	26+36=62		V_{C2}	17+15=32		V_{C2}	19+25=44	
30 ≤ $\bar{x}$ N_{V0} ≤160 ? yes X; no □			$\bar{x}$ of *B* is ≥ 0,5 x $\bar{x}$ of N_{V0}? (or N_V/1000) yesX; no □			$\bar{x}$ of *C* is ≥ 0,5 x $\bar{x}$ of N_{V0}? yesX; no □		

Test suspension and test

Test-suspension (*N and N0):*	*N*	V_{C1}	V_{C2}	
	10^{-7}	166	181	$\bar{x}_{wm}$ = 1.75 x 10^9; log*N* = 9.24 N_0 = *N*/20; logN_0 = 7.94 7,88≤ logN_{V0}≤8,40; yesX; no □
	10^{-8}	21	16	

Drying controls

Drying control (D_{C0})	T_0	V_{C1}	V_{C2}	
	10^{-4}	>330	>330	$\bar{x}_{wm}$ = 3.95 x 10^7; logT_0 = 7,6 6,88≤ logT_0≤8,40; yesX; no □
	10^{-5}	38	41	

Drying controls

Drying control (D_{Ct})	T_0	V_{C1}	V_{C2}	
	10^{-4}	>330	>330	$\bar{x}_{wm}$ 4,50 = x 10^7; logT_t = 7,65 6,88≤ logT_t≤8,40; yesX; no □
	10^{-5}	42	48	

Test field 1 (reduction)

Real conc. of the product %	Dilution step	V_{C1}	V_{C2}	N_a (=$\bar{x}$ or $\bar{x}_{wm}$)	log N_a	log R (log T_t - log N_a)	Contact time (min)
Ready to use	10^0	68	53	60.5	1.78	5.82	10min
	10^{-1}	7	6				

Test field 2 to 4 (cfu/25cm^2)

Real conc. of the product %	Dilution step	$V_C T_2$	$V_C T_3$	$V_C T_4$	V_{T2to4} (=$\bar{x}$ or $\bar{x}_{wm}$ x 5) cfu/25cm^2	Contact time (min)
Ready to use	10^0	50	41	6	161.67	1 min
Ready to use	10^{-1}	5	1	0	10	1 min

N_W Test fields 2 to 4 (cfu/25cm^2)

Real conc. of the product %	Dilution step	$V_C T_2$	$V_C T_3$	$V_C T_4$	$V_{NWT2to4}$ (=$\bar{x}$ or $\bar{x}_{wm}$ x 5) cfu/25cm^2	Contact time (min)
Ready to use	10^0	>660	>660	>660	>16500	1 min
	10^{-1}	>660	>660	>660		

Explanations:
V_C = count per ml (one plate or more)
$\bar{x}$ = average of V_{C1} and V_{C2} (1. + 2. duplicate)
$\bar{x}_{wm}$ = weighted mean of $\bar{x}$
R = reduction (logR = log N_0 – log N_a)

Appendix 5

Interpretation

The ready-to-use product for surface disinfection **Bacticid AF Wipes** (batch no. 197101017) was tested according to the test method EVS-EN 16615:2015. The test was performed at 20 ± 1 °C, under clean conditions with the contact time of 1 min. The dilution – neutralization method was used for testing the products' effectiveness against the reference strains: *Candida albicans* ATCC 10231; *Pseudomonas aeruginosa* ATCC 15442; *Enterococcus hirae* ATCC 10541 and *Staphylococcus aureus* ATCC 6538. Under clean conditions the product was effective against all the reference strains within 1 min of contact time.

Conclusion:

The surviving count of bacterial reference strains showed at least 5 lg and yeast reference strain showed at least 4 lg reduction which means that **under clean conditions the product Bacticid AF Wipes has a bactericidal and yeasticidal effect in case of surface disinfection within 1 min.**

Diana Kaare, MSc

Head of laboratory, microbiologist

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Chemi-Pharm AS
Pollu 132
EST – TALLINN 10917

Bremen, 06/02/2018

Expert opinion

Activity of Bacticid AF against adenovirus type 5 according to EN 14476:2013+A1:2015 under clean conditions

This expert opinion is based on the test report L17/0629aA.1 dating 06/02/2018.

The virus-inactivating properties of the surface disinfectant Bacticid AF of Chemi-Pharm AS against adenovirus type 5 were investigated by a quantitative suspension test according to EN 14476 under clean conditions.

According to EN 14476, a disinfectant or a disinfectant solution at a particular concentration is considered as having virus-inactivating properties if within the recommended exposure period the titre is reduced by ≥ 4 $\log_{10}$ (inactivation ≥ 99.99 %).

Bacticid AF was examined undiluted at 20 °C. 30, 45 and 60 seconds were chosen as exposure times. In summary, a virucidal activity against adenovirus type 5 was measured as follows:

undiluted 30 seconds clean conditions (0.3 g/l BSA)

Dr.

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Chemi-Pharm AS
Pollu 132
EST – TALLINN 10917

Bremen, 06/02/2018

Expert opinion

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According to EN 14476, a disinfectant or a disinfectant solution at a particular concentration is considered as having virus-inactivating properties if within the recommended exposure period the titre is reduced by ≥ 4 $\log_{10}$ (inactivation ≥ 99.99 %).

Bacticid AF was examined undiluted at 20 °C. 30, 45 and 60 seconds were chosen as exposure times. In summary, a virucidal activity against adenovirus type 5 was measured as follows:

undiluted 30 seconds clean conditions (0.3 g/l BSA)

Dr. Joc

Nordic Tersus
Laboratory

Test report No. sd0118

EVALUATION OF FUNGICIDAL OR YEASTICIDAL ACTIVITY IN THE MEDICAL AREA (EN 13624)

Name of the product:	Bacticid AF
Batch number:	197101017
Order number:	17028
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
Interfering substance:	15 g/l bovine albumin + 15 ml/l sheep blood erythrocytes = Dirty conditions
Rinsing liquid:	Tryptone 1 g/l + NaCl 9 g/l
Neutralizer:	-
Test organisms:	*Candida albicans* ATCC 10231
Testing method:	EVS-EN 13624:2013 Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in the medical area.
Testing date:	23.01.2018 – 25.01.2018
Results:	look appendix 1-2

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ıa Kaare, MSc

Head of laboratory, microbiologist

Date of test report: 26.01.2018

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ESTONIAN ACCREDITATION CENTRE
EN ISO/IEC 17025
L263

TEST RESULTS (yeasticidal suspension test)

EVS-EN 13624:2013; Phase 2, step 1;
Membrane filtration method;
Rinsing liquid: Tryptone 1 g/l + NaCl 9 g/l;
Test organism: *Candida albicans* ATCC 10231;
Test temperature: +20° C; Incubation temperature: +30° C
Interfering substance: 15 g/l bovine albumin + 15 ml/l sheep blood erythrocytes = Dirty conditions
Nordic Tersus Laboratory LLC.; Date of test: 23.01.2018 – 25.01.2018.
Responsible person: Diana Kaare

Validation and controls

Dirty conditions

Validation suspension N_{vo}			Experimental conditions control (A)			Filtration control (B)			Method validation (C)		
V_{C1}	45	$\bar{x}$ = 43.5	V_{C1}	38	$\bar{x}$ = 35	V_{C1}	41	$\bar{x}$ = 37	V_{C1}	39	$\bar{x}$ = 41.5
V_{C2}	42		V_{C2}	32		V_{C2}	33		V_{C2}	44	
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}? yesX; no□		

Test suspension and test

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 1.71 x 10^8; log$N$ = 8.23
N and N_0	10^{-6}	172	164	N_0 = N/100; logN_0 = 6.23
	10^{-7}	19	22	6.17≤ logN_0≤6.70; yesX; no □

Experimental results

Concentration of the product	Dilution step	V_{C1}	V_{C2}	Na (=$\bar{x}$*10)	log Na	logR	Contact time	Conditions
Ready to use	-	<14	<14	<140	<2.15	>4.08	30 s	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 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; LogR=LogN_0 - Log Na)

Interpretation:

The ready to use product for surface disinfection **BACTICID AF** (batch no. 197101017) was tested according to the test method EVS-EN 13624:2013. The test was performed at 20 °C ± 1 °C, under dirty conditions with the contact time of 30 s. The membrane filtration method was used for testing the produts effectiveness against the reference strain: *Candida albicans* ATCC 10231. Under dirty conditions the tested product was effective against the reference strain within 30 s of contact time.

Conclusion:

The surviving count of the reference strain showed at least 4 lg reduction meaning that **under dirty conditions the ready to use product BACTICID AF has a yeasticidal effect in case of surface disinfection within 30 s.**

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a Kaare, MSc

Head of laboratory, microbiologist

Nordic Tersus
Laboratory

Test report No. 24021sd

EVALUATION OF FUNGICIDAL OR YEASTICIDAL ACTIVITY IN THE MEDICAL AREA (EN 13624)

Name of the product*:	**BACTICID AF**
Batch number*:	197271120
Order number:	20037
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:	10.12.2020
Test material conditions:	No specific features, sample in the manufacturers tare
Storage conditions:	At room temperature, darkness
Active substance – conc.*:	Ethyl alcohol 57.0% wt, isopropyl alcohol 6.0% wt
Appearance of the product:	Transparent liquid
Test concentration:	80.0%, 50.0%, 25.0%
Contact time:	2 min
Interfering substance:	3g/l bovine albumin + 3 ml/l sheep blood erythrocytes (dirty conditions)
Neutralizer:	-
Rinsing liquid:	Tryptone 1 g/l + NaCl 9 g/l
Test organisms:	*Aspergillus brasiliensis* ATCC 16404
Testing method:	**EVS-EN 13624:2013** **Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in the medical area.**
Testing date:	17.02.2021 – 19.02.2021
Results:	Look appendix 1
Interpretation and conclusion:	Look appendix 2

ACCREDITED LABORATORY NORDIC TERSUS LABORATORY

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eht
Chief specialist
Date of issue: 26.04.2021

** - Data provided by the customer*

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EN ISO/IEC 17025
L263

TEST RESULTS (suspension test)

EVS-EN 13624:2013; Phase 2, step 1
Membrane filtration method
Rinsing liquid: Tryptone 1 g/l + NaCl 9 g/l;
Test organism: *Aspergillus brasiliensis* ATCC 16404
Test temperature: +20° C; Incubation temperature: +30 ± 1° C
Interfering substance: 3g/l bovine albumin + 3 ml/l sheep blood erythrocytes
Nordic Tersus Laboratory LLC.;
Date of test: 17.02.2021
Responsible person: Melissa Ingela Bramanis, Allar Laaneleht

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}	$\bar{x}$
43	40	41.5	36	30	33	39	42	40.5	45	49	47
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

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm} = 2.64 \times 10^7$; log$N$ = 7.42
N and N_0	10^{-5}	**247**	**275**	$N_0 = N/10$; logN_0 = 6.42
	10^{-6}	**28**	**28**	6.17≤ logN_0≤6.70; yesX; no □

Experimental results

Concentration of the product %	Dilution step	V_{C1}	V_{C2}	Na (=$\bar{x}$*10)	lgNa	lg$R$	Contact time	Conditions
80.0%	-	19	21	200	2.30	4.12	2 min	Dirty
50.0%	-	>55	>55	>550	>2.74	<3.54	2 min	Dirty
25.0%	-	>55	>55	>550	>2.74	<3.54	2 min	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/ Na; LogR=LogN_0 - Log Na)
$\bar{x}$ = average of V_{C1} and V_{C2} (1. + 2. Duplicate)
N_0 = cfu/ml at the start of the contact time (t=0)
Na = surviving microbes after the test

Appendix 2

Interpretation:

The ready to use surface disinfection product **BACTICID AF** (batch no. 197271120) was tested according to the test method EVS-EN 13624:2013. The test was performed at 20 °C ± 1 °C, under dirty conditions during contact times of 2 min. The membrane filtration method was used for testing the product's effectiveness against the reference strains *Aspergillus brasiliensis* ATCC 16404. Under dirty conditions, the 80.0% solution of the tested sample of the product was effective against *Aspergillus brasiliensis* within 2 minutes.

Conclusion:

The surviving count of the reference strain showed at least 4 lg reduction meaning that **according to EVS-EN 13624:2013 the sample of the ready to use surface disinfection product BACTICID AF is effective against *Aspergillus brasiliensis* within 2 minutes.**

This is the end of the test report

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Date of issue: 26.04.2021

Nordic Tersus
Laboratory

Test report No. sd1918

EVALUATION OF THE ANTIBACTERIAL AND – FUNGAL PROTECTION OF A DISINFECTANT ON NONPOROUS SURFACE (EN 13697)

Name of the product:	Bacticid 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
Test conditions:	Dirty conditions
Contact time:	30sec, 5 min obligatory for bacteria; 15 min obligatory for yeast
Interfering substance:	3.0 g/l bovine albumin
Test neutralizer:	Polysorbate 80: 30g/l; saponin 30g/l; lecithin, 3g/l
Test organisms:	*Staphylococcus aureus* ATCC 6538 *Pseudomonas aeruginosa* ATCC 15442 *Enterococcus hirae* ATCC 10541 *Escherichia coli* ATCC 10536 *Candida albicans* ATCC 10231
Testing method base:	EVS-EN 13697:2015 -Chemical disinfectants and antiseptics – Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas.
Testing date:	25.02.2018 – 28.02.2018
Results:	look appendix 1-2

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Kaare

Head of laboratory, microbiologist

Date of test report: 01.03.2018

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Appendix 1

TEST RESULTS
EVS-EN 13697:2015; Phase 2, step 2;
Product: BACTICID
Contact/neutralization method; surface;
Neutralizers: Polysorbate 80: 30g/l; saponin 30/l; lecithin, 3g/l;
Test organisms: *Staphylococcus aureus* ATCC 6538; *Pseudomonas aeruginosa* ATCC 15442; *Enterococcus hirae* ATCC 10541; *Escherichia coli* ATCC 10536; *Candida albicans* ATCC 10231;
Test temperature: +20° C ±1° C; Incubation temperatures: +37° C ±1° C for bacteria and +30° ±1° C for yeast;
Solvents: water;
Interfering substances: 3.0 g/l bovine albumin = dirty conditions
Nordic Tersus Laboratory LLC.; Date of test: 25.02.2018 – 28.02.2018.

Table 1. Data counted and calculated; dirty conditions; 30 sec

Test organisms	Test suspension N	Test validation		Water control Nc	Test procedure concentration
		NT	NC		Ready to use
Staphylococcus aureus ATCC 6538	10^{-6}: 204;225	10^{-3}:>330;>330	10^{-3}:>330;>330	10^{-3}: >330;>330	10^{0}: 12; 13
	10^{-7}: 22;26	10^{-4}: 193;184	10^{-4}: 166;181	10^{-4}:184;175	10^{-1}: 1; 0
	N: 6.73	10^{-5}:24;26	10^{-5}:22;19	10^{-5}: 24;28	10^{-2}: 0; 0
		NT: 7.28	NT: 7.24	Nc: 7.25	Nd: 2.10
				Nts: >100	Nts: 1
					R: 5.15
Pseudomonas aeruginosa ATCC 15442	10^{-6}: 204;215	10^{-3}:>330;>330	10^{-3}:>330;>330	10^{-3}: >330;>330	10^{0}: 4; 7
	10^{-7}: 20;24	10^{-4}: 182;154	10^{-4}:177;159	10^{-4}: 191;168	10^{-1}: 0; 1
	N: 6.72	10^{-5}:20;17	10^{-5}:14;19	10^{-5}: 22;18	10^{-2}: 0; 0
		NT: 7.23	NT: 7.23	Nc: 7.25	Nd: 1.74
				Nts: >100	Nts: 0
					R: 5.51

Enterococcus hirae ATCC 10541	10^{-6}: 186;181	10^{-3}:>330;>330	10^{-3}:>330;>330	10^{-3}: >330;>330	10^{0}: 3; 0
	10^{-7}: 20;16	10^{-4}: 162;174	10^{-4}:153;145	10^{-4}:139;148	10^{-1}: 0; 0
	N: 6.66	10^{-5}:22;19	10^{-5}:20;25	10^{-5}: 18;18	10^{-2}: 0; 0
		NT: 7.23	NT: 7.17	Nc: 7.16	Nd: 1.18
				Nts: >100	Nts: 0
					R: 5.98
Escherichia coli ATCC 10636	10^{-6}: 164;156	10^{-3}:>330;>330	10^{-3}:>330;>330	10^{-3}: >330;>330	10^{0}: 0; 0
	10^{-7}: 18;18	10^{-4}: 122;147	10^{-4}:139;127	10^{-4}:132;152	10^{-1}: 0; 0
	N: 6.61	10^{-5}:18;14	10^{-5}:16;22	10^{-5}: 18;16	10^{-2}: 0; 0
		NT: 7.13	NT: 7.12	Nc: 7.15	Nd: <0.1
				Nts: >100	Nts: 0
					R: >7.05
Candida albicans ATCC 10231	10^{-5}: 185;194	10^{-3}: 17;21	10^{-3}:18;23	10^{-3}: 21;25	10^{0}: 0; 0
	10^{-6}: 22;19	10^{-4}: 3;2	10^{-4}: 3;2	10^{-4}: 2;3	10^{-1}: 0; 0
	N: 5.68	10^{-5}: 0;0	10^{-5}: 0;1	10^{-5}: 0;0	10^{-2}: 0; 0
		NT: 5.28	NC: 5.31	Nc: 5.36	Nd: <0.1
				Nts: >100	Nts: 0
					R: >5.26

Table 2. Data counted and calculated; dirty conditions; 5 min

Test organisms	Test suspension N	Test validation		Water control Nc	Test procedure concentration
		NT	NC		Ready to use
Staphylococcus aureus ATCC 6538	10^{-6}: 204;225	10^{-3}:>330;>330	10^{-3}:>330;>330	10^{-3}: >330;>330	10^{0}: 0; 1
	10^{-7}: 22;26	10^{-4}: 193;184	10^{-4}: 166;181	10^{-4}:184;175	10^{-1}: 0; 0
	N: 6.73	10^{-5}:24;26	10^{-5}:22;19	10^{-5}: 24;28	10^{-2}: 0; 0
		NT: 7.28	NT: 7.24	Nc: 7.25	Nd: <0.1
				Nts: >100	Nts: 1
					R: >7.15
Pseudomonas aeruginosa ATCC 15442	10^{-6}: 204;215	10^{-3}:>330;>330	10^{-3}:>330;>330	10^{-3}: >330;>330	10^{0}: 1;0
	10^{-7}: 20;24	10^{-4}: 182;154	10^{-4}:177;159	10^{-4}: 191;168	10^{-1}: 0; 1
	N: 6.72	10^{-5}:20;17	10^{-5}:14;19	10^{-5}: 22;18	10^{-2}: 0; 0
		NT: 7.23	NT: 7.23	Nc: 7.25	Nd: <0.1
				Nts: >100	Nts: 0
					R: >7.15
Enterococcus hirae ATCC 10541	10^{-6}: 186;181	10^{-3}:>330;>330	10^{-3}:>330;>330	10^{-3}: >330;>330	10^{0}: 0; 0
	10^{-7}: 20;16	10^{-4}: 162;174	10^{-4}:153;145	10^{-4}:139;148	10^{-1}: 0; 0
	N: 6.66	10^{-5}:22;19	10^{-5}:20;25	10^{-5}: 18;18	10^{-2}: 0; 0
		NT: 7.23	NT: 7.17	Nc: 7.16	Nd: <0.1
				Nts: >100	Nts: 0
					R: 7.16

Escherichia coli ATCC 10636	10^{-6}: 164;156	10^{-3}:>330;>330	10^{-3}:>330;>330	10^{-3}: >330;>330	10^{0}: 0; 0
	10^{-7}: 18;18	10^{-4}: 122;147	10^{-4}:139;127	10^{-4}:132;152	10^{-1}: 0; 0
	N: 6.61	10^{-5}:18;14	10^{-5}:16;22	10^{-5}: 18;16	10^{-2}: 0; 0
		NT: 7.13	NT: 7.12	Nc: 7.15	Nd: <0.1
				Nts: >100	Nts: 0
					R: >7.05

Table 3. Data counted and calculated; dirty conditions;1 5 min

Test organisms	Test suspension N	Test validation		Water control Nc	Test procedure concentration
		NT	NC		Ready to use
Candida albicans ATCC 10231	10^{-5}: 185;194	10^{-3}: 17;21	10^{-3}:18;23	10^{-3}: 21;25	10^{0}: 0; 0
	10^{-6}: 22;19	10^{-4}: 3;2	10^{-4}: 3;2	10^{-4}: 2;3	10^{-1}: 0; 0
	N: 5.68	10^{-5}: 0;0	10^{-5}: 0;1	10^{-5}: 0;0	10^{-2}: 0; 0
		NT: 5.28	NC: 5.31	Nc: 5.36	Nd: <0.1
				Nts: >100	Nts: 0
					R: >5.26

Appendix 2

Interpretation

A ready to use product **BACTICID AF** (Batch No. 197101017) was tested according to the EN 13697 standard at 20°C ± 1°C, with the contact times 30sec, 5 min (obligatory contact time for bacteria) and 15 min (obligatory contact time for yeast) under dirty conditions. The method was used for testing the product efficacy against the microorganisms: *Staphylococcus aureus ATCC 6538*; *Pseudomonas aeruginosa ATCC* 15442*; Enterococcus hirae ATCC 10541*; *Escherichia coli ATCC 8739*; *Candida albicans ATCC 10231.* Under dirty conditions (test regime) the tested product was active against all the testorganisms within the contact times tested.

Conclusion

By the test results can be concluded that as treated by the product the surviving microorganisms count was decreasing at least four grades for bacteria and at least three grades for yeast, the tested product **BACTICID AF** is bactericidal and yeasticidal in case of surface disinfection, under dirty conditions within the contact time of 30 sec.

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Head of laboratory, microbiologist

Date of test report: 01.03.2018

Nordic Tersus
Laboratory

Test report No. sd1817

EVALUATION OF THE ANTIBACTERIAL AND – FUNGAL PROTECTION OF A DISINFECTANT ON NONPOROUS SURFACE (EN 13697)

Name of the product:	Bacticid 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
Test conditions:	Dirty conditions
Contact time:	3 min; 15 min obligatory
Interfering substance:	3.0 g/l bovine albumin
Test neutralizer:	Polysorbate 80: 30g/l; saponin 30g/l; lecithin, 3g/l
Test organisms:	*Aspergillus brasiliensis* ATCC 16404
Testing method base:	EVS-EN 13697:2015 -Chemical disinfectants and antiseptics – Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas.
Testing date:	25.02.2018 – 28.02.2018
Results:	look appendix 1-2

Head of laboratory, microbiologist

Date of test report: 01.03.2018

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Appendix 1

TEST RESULTS
EVS-EN 13697:2015; Phase 2, step 2;
Product: Bacticid AF
Contact/neutralization method; surface;
Neutralizers: Polysorbate 80: 30g/l; saponin 30/l; lecithin, 3g/l;
Test organisms: *Aspergillus brasiliensis* ATCC 16404
Test temperature: +20° C ± 1° C; Incubation temperature: +30° C ± 1° C;
Solvents: water;
Interfering substances: 3.0 g/l bovine albumin = dirty conditions
Nordic Tersus Laboratory LLC.; Date of test: 25.02.2018 – 28.02.2018.

Table 1. Data counted and calculated; dirty conditions; 3 min

Test organisms	Test suspension N	Test validation		Water control Nc	Test procedure concentration
		NT	NC		Ready to use
Aspergillus brasiliensis ATCC 16404	10^{-5}: 163;188	10^{-3}:22;16	10^{-3}: 26;30	10^{-3}: 31;36	10^{0}: 3; 1
	10^{-6}: 14;16	10^{-4}: 4;1	10^{-4}: 2;1	10^{-4}: 3;4	10^{-1}: 0; 0
	N: 6.64	10^{-5}: 0;0	10^{-5}: 0;0	10^{-5}: 0;0	10^{-2}: 0; 0
		NT: 5.28	NT: 5.45	Nc: 5.53	Nd: 1.30
				Nts: >100	Nts: 1
					R: 4.23

Table 2. Data counted and calculated; dirty conditions; 15 min

Test organisms	Test suspension N	Test validation		Water control Nc	Test procedure concentration
		NT	NC		Ready to use
Aspergillus brasiliensis ATCC 16404	10^{-5}: 163;188	10^{-3}:22;16	10^{-3}: 26;30	10^{-3}: 31;36	10^{0}: 0; 0
	10^{-6}: 14;16	10^{-4}: 4;1	10^{-4}: 2;1	10^{-4}: 3;4	10^{-1}: 0; 0
	N: 6.64	10^{-5}: 0;0	10^{-5}: 0;0	10^{-5}: 0;0	10^{-2}: 0; 0
		NT: 5.28	NT: 5.45	Nc: 5.53	Nd: <0.1
				Nts: >100	Nts: 1
					R: > 5.43

Appendix 2

Interpretation

A ready to use product **BACTICID AF** (Batch No. 197101017) was tested according to the EN 13697 standard at 20°C ± 1°C, with the contact times 3 min, 15 min (obligatory contact time) under dirty conditions. The method was used for testing the product efficacy against the microorganism: *Aspergillus brasiliensis* ATCC 16404. Under dirty conditions (test regime) the tested product was active against the testorganism within the contact times tested.

Conclusion

By the test results can be concluded that as treated by the product the surviving microorganisms count was at least lg3 reduction, the tested product **BACTICID AF** is fungicidal in case of surface disinfection, under dirty conditions within the contact time of 3 min.

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a Kaare

Head of laboratory, microbiologist

Date of test report: 01.03.2018

Nordic Tersus
Laboratory

Test report No. sd0918

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

Name of the product:	Bacticid AF
Batch number:	197101017
Order number:	17028
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
Interfering substance:	15 g/l bovine albumin + 15 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 *Staphylococcus aureus* MRSA ATCC 33592 *Enterococcus faecium* VRE ATCC 700221
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 date:	17.02.2018 – 19.02.2018
Results:	look appendix 1-6

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e, MSc
Head of laboratory, microbiologist
Date of test report: 21.02.2018

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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: 15 g/l bovine albumin + 15 ml/l sheep blood erythrocytes = Dirty conditions
Nordic Tersus Laboratory LLC.;
Date of test: 17.02.2018 – 19.02.2018
Responsible person: Diana Kaare

Validation and controls

Dirty conditions

Validation suspension N_{vo}			Experimental conditions control (A)			Filtration control (B)			Method validation (C)		
V_{C1}	83	$\bar{x}$ = 86.5	V_{C1}	72	$\bar{x}$ = 68.5	V_{C1}	68	$\bar{x}$ = 71.5	V_{C1}	81	$\bar{x}$ = 77
V_{C2}	90		V_{C2}	65		V_{C2}	75		V_{C2}	73	
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

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 2.45 x 10^9; log$N$ = 9.39
	10^{-7}	257	224	N_0 = N/100; logN_0 = 7,39
N and N_0	10^{-8}	28	30	7,17≤ logN_0≤7,70; yesX; no □

Experimental results

Concentration of the product %	Dilution step	V_{C1}	V_{C2}	Na (=$\bar{x}$*10)	log Na	logR	Contact time	Conditions
Ready to use	-	<14	<14	<140	<2.15	>5.24	30 sec	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 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; LogR=LogN_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: 15 g/l bovine albumin + 15 ml/l sheep blood erythrocytes = Dirty conditions
Nordic Tersus Laboratory LLC.;
Date of test: 17.02.2018 – 19.02.2018
Responsible person: Diana Kaare

Validation and controls

Dirty conditions

Validation suspension N_{vo}			Experimental conditions control (A)			Filtration control (B)			Method validation (C)		
V_{C1}	62	$\bar{x}$ = 66	V_{C1}	47	$\bar{x}$ = 46	V_{C1}	52	$\bar{x}$ = 53.5	V_{C1}	59	$\bar{x}$ = 61
V_{C2}	70		V_{C2}	45		V_{C2}	55		V_{C2}	53	
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

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 2.15 x 10^9; log$N$ = 9.33
	10^{-7}	203	224	N_0 = N/100; logN_0 = 7,33
N and N_0	10^{-8}	22	25	7,17≤ logN_0≤7,70; yesX; no □

Experimental results

Concentration of the product %	Dilution step	V_{C1}	V_{C2}	Na (=x̄*10)	log Na	logR	Contact time	Conditions
Ready to use	-	<14	<14	<140	<2.15	>5.18	30 sec	dirty

Explanations:

V_C = count per ml (one plate or more)

x̄ = 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; LogR=LogN_0 - Log Na)

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: 15 g/l bovine albumin + 15 ml/l sheep blood erythrocytes = Dirty conditions
Nordic Tersus Laboratory LLC.;
Date of test: 17.02.2018 – 19.02.2018
Responsible person: Diana Kaare

Validation and controls

Dirty conditions

Validation suspension N_{vo}			Experimental conditions control (A)			Filtration control (B)			Method validation (C)		
V_{C1}	94	$\bar{x}$ = 87	V_{C1}	71	$\bar{x}$ = 72.5	V_{C1}	66	$\bar{x}$ = 68	V_{C1}	75	$\bar{x}$ = 78.5
V_{C2}	80		V_{C2}	74		V_{C2}	70		V_{C2}	82	
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

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 2.74 x 10^9; log$N$ = 9.44
N and N_0	10^{-7}	284	266	N_0 = N/100; logN_0 = 7,44
	10^{-8}	24	29	7,17≤ logN_0≤7,70; yesX; no □

Experimental results

Concentration of the product %	Dilution step	V_{C1}	V_{C2}	Na (=$\bar{x}$*10)	log Na	logR	Contact time	Conditions
Ready to use	-	<14	<14	<140	<2.15	>5.29	30 sec	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 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; LogR=LogN_0 - Log Na)

Appendix 4

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* MRSA ATCC 33592
Test temperature: +20° C; Incubation temperature: +37 °C
Interfering substance: 15 g/l bovine albumin + 15 ml/l sheep blood erythrocytes = Dirty conditions
Nordic Tersus Laboratory LLC.;
Date of test: 17.02.2018 – 19.02.2018
Responsible person: Diana Kaare

Validation and controls

Dirty conditions

Validation suspension N_{vo}			Experimental conditions control (A)			Filtration control (B)			Method validation (C)		
V_{C1}	66	$\bar{x}$ = 61.5	V_{C1}	42	$\bar{x}$ = 45.5	V_{C1}	49	$\bar{x}$ = 47.5	V_{C1}	52	$\bar{x}$ = 53
V_{C2}	57		V_{C2}	49		V_{C2}	46		V_{C2}	54	
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

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 1.99 x 10^9; log$N$ = 9.30
	10^{-7}	204	191	N_0 = N/100; logN_0 = 7.30
N and N_0	10^{-8}	18	24	7,17≤ logN_0≤7,70; yesX; no □

Experimental results

Concentration of the product %	Dilution step	V_{C1}	V_{C2}	Na (=x̄*10)	log Na	logR	Contact time	Conditions
Ready to use	-	<14	<14	<140	<2.15	>5.15	30 sec	dirty

Explanations:

V_C = count per ml (one plate or more)

x̄ = 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; LogR=LogN_0 - Log Na)

Appendix 5

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 faecium* VRE ATCC 700221
Test temperature: +20° C; Incubation temperature: +37 °C
Interfering substance: 15 g/l bovine albumin + 15 ml/l sheep blood erythrocytes = Dirty conditions
Nordic Tersus Laboratory LLC.;
Date of test: 17.02.2018 – 19.02.2018
Responsible person: Diana Kaare

Validation and controls

Dirty conditions

Validation suspension N_{vo}			Experimental conditions control (A)			Filtration control (B)			Method validation (C)		
V_{C1}	62	$\bar{x}$ = 64	V_{C1}	52	$\bar{x}$ = 54.5	V_{C1}	43	$\bar{x}$ = 47	V_{C1}	58	$\bar{x}$ = 59
V_{C2}	66		V_{C2}	57		V_{C2}	51		V_{C2}	60	
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

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 1.88 x 10^9; log$N$ = 9.27
	10^{-7}	181	195	$N_0 = N/100$; logN_0 = 7.27
N and N_0	10^{-8}	22	18	7,17≤ logN_0≤7,70; yesX; no □

Experimental results

Concentration of the product %	Dilution step	V_{C1}	V_{C2}	Na (=$\bar{x}$*10)	log Na	logR	Contact time	Conditions
Ready to use	-	<14	<14	<140	<2.15	>5.12	30 sec	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 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; LogR=LogN_0 - Log Na)

Appendix 6

Interpretation:

The product for surface disinfection **Bacticid AF** (batch no. 197101017) 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 30 sec. The membrane filtration method was used for testing the products' effectiveness against the reference strains: *Pseudomonas aeruginosa* ATCC 15442, *Enterococcus hirae* ATCC 10541, *Staphylococcus aureus* ATCC 6538, Staphylococcus aureus MRSA ATCC 33592 and *Enterococcus faecium* VRE ATCC 700221. Under dirty conditions the tested 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 5lg reduction meaning that **under dirty conditions the ready to use product Bacticid AF has a bectericidal effect in case of surface disinfection within 30 sec.**

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Head of laboratory, microbiologist

Nordic Tersus
Laboratory

Test report No. sd1418

EVALUATION OF MYCOBACTERICIDAL ACTIVITY (EN 14348)

Name of the product:	Bacticid AF
Batch number:	197101017
Order number:	17028
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
Test conditions:	Dirty and clean conditions
Contact time:	30 sec, 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:	Distilled 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

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

Nordic Tersus Laboratory OÜ
Harjumaa, Tallinn, Serva tn 44a, 11618
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Appendix 1

TEST RESULTS (mycobactericidal suspension test)

EVS-EN 14348:2005; Phase 2, step 1;
Membrane filtration method; Spread plate;
Rinsing liquid: distilled 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}? yesX; no □			$\bar{x}$ C is ≥ 0,5 $\bar{x}$ N_{vo}? yes X;no □		

Test suspension and test

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 2,56 x 10^9; log$N$ = 9,41
	10^{-7}	262	249	$N_0 = N/10$; logN_0 = 8,41
N and N_0	10^{-8}	30	23	8,17≤ logN_0≤8,7; 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	10^0	<14	<14	<140	<2,15	>6,26	30 sec	dirty
	10^{-1}	<14	<14					
	10^{-2}	<14	<14					
	10^{-3}	<14	<14					
RTU	10^0	<14	<14	<140	<2,15	>6,26	60 min	dirty
	10^{-1}	<14	<14					
	10^{-2}	<14	<14					
	10^{-3}	<14	<14					
RTU	10^0	<14	<14	<140	<2,15	>6,26	30 sec	clean
	10^{-1}	<14	<14					
	10^{-2}	<14	<14					
	10^{-3}	<14	<14					
RTU	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)

x̄ = 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; LogR=LogN_0 - Log Na)

Appendix 2

TEST RESULTS (mycobactericidal suspension test)

EVS-EN 14348:2005; Phase 2, step 1;
Membrane filtration method; Spread plate;
Rinsing liquid: distilled 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 $N_0 = N/10$; logN_0 = 8,46 8,17≤ logN_0≤8,7; yes X; no □
	10^{-7}	283	291	
	10^{-8}	31	25	

Experimental results

Concent-ration of the product.%	Dilu-tion step	V_{C1}	V_{C2}	Na (=x̄*10)	log Na	logR	Contact time	Condi-tions
RTU	10^0	<14	<14	<140	<2,15	>6,31	30 sec	dirty
	10^{-1}	<14	<14					
	10^{-2}	<14	<14					
	10^{-3}	<14	<14					
RTU	10^0	<14	<14	<140	<2,15	>6,31	60 min	dirty
	10^{-1}	<14	<14					
	10^{-2}	<14	<14					
	10^{-3}	<14	<14					
RTU	10^0	<14	<14	<140	<2,15	>6,31	30 sec	clean
	10^{-1}	<14	<14					
	10^{-2}	<14	<14					
	10^{-3}	<14	<14					
RTU	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)

x̄ = 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; LogR=LogN_0 - Log Na)

Appendix 3

Interpretation

Using the EN 14348 standard, there was tested ready to use product – **Bacticid AF**- (No. 197101017), at 20 °C ± 1 °C, with the contact time 30 sec 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 was decreasing at least four grades that under clean and dirty conditions the ready to use product **Bacticid AF** is mycobactericidal in case of surface disinfection, during conctact time of 30 sec.

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Diana Kaare

Head of laboratory, microbiologist

DR. BRILL + DR. STEINMANN
INSTITUTE FOR HYGIENE AND MICROBIOLOGY

ilac-MRA DAkkS Deutsche Akkreditierungsstelle D-PL-13412-01-01 D-PL-13412-01-02
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ZLG-AP-216.11.02

06/02/2018

Test report L17/0629aA.1

Evaluation of the effectiveness of

Bacticid AF

Test virus: adenovirus type 5

Method: EN 14476:2013+A1:2015 (clean conditions)

quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in human medicine

Sponsor:
Chemi-Pharm AS
Pollu 132
EST – TALLINN 10917

TEST REPORT

Norderoog 2, DE - 28259 Bremen
Tel.: +49 40-557631-0, Fax: +49 40-557631-11
info@brillhygiene.com, http://www.brillhygiene.com

1. Identification of test laboratory

Dr. Brill + Partner GmbH Institute for Hygiene and Microbiology, Norderoog 2, DE - 28259 Bremen

2. Identification of sample

Manufacturer	Chemi-Pharm AS
Name of product	Bacticid AF
Confirmation no.	203850
Product diluent recommended by the manufacturer	-
Batch number	197101017
Application	surface disinfection
Production date	10/10/2017
Expiry date	10/10/2020
Active compound (s) (100 g)	57 g ethanol 6 g IPA
Appearance, odour	clear, colorless liquid product specific
pH-values	undiluted: 7.49 (20 °C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	13/10/2017

3. Materials

3.1 Culture medium and reagents

- Eagle`s Minimum Essential Medium with Earle`s BSS (EMEM, Biozym Scientific GmbH, catalogue no. 880121)
- fetal calf serum (Biochrom AG, article no. S 0115)
- 1.4 % formaldehyde solution (dilution of Roti®-Histofix 4 %, Carl Roth GmbH)
- Aqua bidest. (SG ultrapure water system, type Ultra Clear; serial no. 86996-1)
- PBS (Invitrogen, article no. 18912-014)
- BSA (Sigma-Aldrich-Chemie GmbH, article no. CA-2153).

3.2 Virus and cells

The adenovirus type 5 strain adenoid 75 was obtained from PD Dr. A. Heim, Institute of Medical Virology, Hannover Medical School, Hannover, Germany. Before the inactivation assays, the virus had been passaged 3 times in *A549 cells* (human lung epithelial carcinoma cells).

The *A549 cells* (passage 111) originated from Vircell, S.L., Spain, 18320 Santa Fe (now BIOTRIN International GmbH, DE - 69126 Heidelberg).

The cells were inspected regularly for morphological alterations and for contamination by mycoplasmas. No morphological alterations of cells and no contamination by mycoplasmas could be detected.

3.3 Apparatus, glassware and small items of equipment

- CO_2 incubator, Nunc GmbH & Co. KG, model QWJ 350
- Agitator (Vortex Genie Mixer, type G 560E)
- pH measurement 315i (WTW, article no. 2A10-100)
- Centrifuge (Sigma-Aldrich-Chemie GmbH, type 113)
- Microscope (Olympus, type CK 30)
- Centrifuge 5804 R (Eppendorf AG)
- Water bath (JULABO, Julabo U 3)
- Adjustable and fixed-volume pipettes (Eppendorf AG)
- Polysterol 96-well microtitre plate (Nunc GmbH & Co. KG, Wiesbaden)
- Cell culture flask (Nunc GmbH & Co. KG, Wiesbaden)
- Sealed test tubes (Sarstedt AG & Co., Nümbrecht).

4. Experimental conditions

Test temperature	20 °C ± 1.0 °C
Concentration of test product	undiluted (80.0 %) and as 50.0 % and 10.0 % (demonstration of non-active range) solutions
Appearance of product dilutions	no precipitation
Contact times	30, 45 and 60 seconds and 30 minutes
Interfering substance	0.3 g/l bovine serum albumin (clean conditions, EN 14476)
Procedure to stop action of disinfectant	immediate dilution
Diluent	water
Stability of product in the mix with virus and interfering substance (80.0 % solution)	no clouding, no precipitation
Virus strain	adenovirus type 5 strain adenoid 75 (ATCC VR-5)
Date of testing	19/12/2017 – 06/02/2018
End of testing	06/02/2018

5. Methods

5.1 Preparation of test virus suspension

For preparation of test virus suspension according to EN 5.4.1 *A549 cells* were infected with a multiplicity of infection of 0.1 at 37 °C. After cells showed a cytopathic effect, they were subjected to a threefold freeze/thaw procedure followed by a low speed centrifugation in order to sediment cell debris. After aliquotation of the supernatant, test virus suspension was stored at -80 °C.

5.2 Preparation of disinfectant (dilutions)

The test product was tested undiluted. Due to the addition of interfering substance and test virus suspension an 80.0 % solution resulted.

Furthermore, the product was evaluated as 50.0 % and 10.0 % solutions (demonstrating of non-active range). These solutions were prepared with water immediately before the inactivation tests.

5.3 Infectivity assay

Infectivity was determined as endpoint titration according to EN 5.5 transferring 0.1 ml of each dilution into eight wells of a microtitre plate, beginning with the highest dilution. This was followed by the addition of 0.1 ml of freshly trypsinized *A549 cells*. This cell suspension was adjusted to reach 10-15 x 10^3 cells per well. Microtitre plates were incubated at 37 °C in a 5 % CO_2-atmosphere. The cytopathic effect was read by using an inverted microscope after ten days. Calculation of the infective dose $TCID_{50}$/ml was calculated with the method of Spearman (2) and Kärber (3) with the following formula:

$$- \log_{10} TCID_{50} = X_0 - 0.5 + \sum r/n$$

meaning

X_0 = $\log_{10}$ of the lowest dilution with 100 % positive reaction

r = number of pos. determinations of lowest dilution step with 100 % positive and all higher positive dilution steps

n = number of determinations for each dilution step.

5.4 Calculation and verification of virucidal activity

The virucidal activity of the test disinfectant was evaluated by calculating the decrease in titre in comparison with the control titration without disinfectant. The difference is given as reduction factor (RF).

According to the EN 14476, a disinfectant or a disinfectant solution at a particular concentration is having virus-inactivating efficacy if the titre is reduced at least by four $\log_{10}$ steps within the recommended exposure period. This corresponds to an inactivation of ≥ 99.99 %.

5.5 Inactivation assay

Determination of virucidal activity has been carried out in accordance to EN 5.5. The test product was examined undiluted (80.0 %) and as 50.0 % and 10.0 % (demonstration of non-active range) solutions in water at 20 °C according to EN 14476. 30, 45 and 60 seconds and 30 minutes were chosen as contact times.

Immediately at the end of a chosen contact time, activity of the disinfectant was stopped by dilution to 10^{-8}.