Nordic Tersus
Laboratory

Test report No 22021-1sd

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*:	227251120
Order number:	20042
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:	15.01.2021
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 wipe, 20x20 cm
Test concentration:	Ready to use
Contact time:	1 min
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:	*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) (Phase 2, step 2)**
Testing date:	22.03.2021 – 25.03.2021
Results:	look appendix 1-5

ACCREDITED LABORATORY No L263 ISO/IEC 17025 REPUBLIC OF ESTONIA NORDIC TERSUS LABORATORY OÜ

Melissa Ingela Bramanis
Microbiologist
Date of issue: 20.07.2021

** - Data provided by the customer*

Nordic Tersus Laboratory OÜ
Harjumaa, Tallinn, Serva tn 44a, 11618
Registrikood: 12561442
Tel: +372 503 0197
E-mail: info@ntl.ee

TEST RESULTS (yeasticidal 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: 3 g/l bovine albumin + 3 ml/l sheep blood erythrocytes
Nordic Tersus Laboratory LLC.; Date of test: 22.03.2021
Responsible person: Allar Laaneleht, Melissa Ingela Bramanis

Validation and controls

Validation suspension (N_{V0})			Neutralizer control (B)			Method validation (C)		
V_{C1}	75	$\bar{x}$ = 67.5	V_{C1}	58	$\bar{x}$ = 54.5	V_{C1}	50	$\bar{x}$ = 50
V_{C2}	60		V_{C2}	51		V_{C2}	50	
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}	$\bar{x}_{wm} = 2.13 \times 10^8$; log*N* = 8.33 $N_0 = N/20$; logN_0 = 7.03 6.88≤ logN_{V0}≤7.40; yesX; no □
	10^{-6}	204	216	
	10^{-7}	22	26	

Drying controls

Drying control (D_{C0})	T_0	V_{C1}	V_{C2}	$\bar{x}_{wm} = 5.25 \times 10^6$; log$T_0$ = 6.72 5.88≤ logT_0≤7.40; yesX; no □	Dirty conditions 1 min
	10^{-3}	>330	>330		
	10^{-4}	55	50		

Drying controls

Drying control (D_{Ct})	T_0	V_{C1}	V_{C2}	$\bar{x}_{wm} = 5.45 \times 10^6$; log$T_t$ = 6.74 5.88≤ logT_t≤7.40; yesX; no □	Dirty conditions 1 min
	10^{-3}	>330	>330		
	10^{-4}	64	45		

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)	Conditions
Ready to use	10^0	2	2	<14	<1.15	>5.59	1 min	Dirty
	10^{-1}	0	0					

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.

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)	Conditions
Ready to use	10^0	0	1	1	<5	1 min	Dirty
	10^{-1}	0	0	0	<5		

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)	Conditions
Ready to use	10^0	>330	>330	>330	2383.33	1 min	Dirty
	10^{-1}	71	52	19			

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)

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 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: 3 g/l bovine albumin + 3 ml/l sheep blood erythrocytes
Nordic Tersus Laboratory LLC.; Date of test: 22.03.2021
Responsible person: Allar Laaneleht, Melissa Ingela Bramanis

Validation and controls

Validation suspension (N_{V0})			Neutralizer control (B)			Method validation (C)		
V_{C1}	93	$\bar{x}$ = 96	V_{C1}	56	$\bar{x}$ = 60	V_{C1}	58	$\bar{x}$ = 56
V_{C2}	99		V_{C2}	64		V_{C2}	54	
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}	$\bar{x}_{wm}$ = 2.60 x 10^9; log*N* = 9.41 N_0 = *N*/20; logN_0 = 8.11 6.88≤ logN_{V0}≤7.40; yesX; no □
	10^{-7}	244	272	
	10^{-8}	26	31	

Drying controls

Drying control (D_{C0})	T_0	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 5.80 x 10^7; logT_0 = 7.76 6.88≤ logT_0≤8.40; yesX; no □	Dirty conditions 1 min
	10^{-4}	>330	>330		
	10^{-5}	50	66		

Drying controls

Drying control (D_{Ct})	T_t	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 7.20 x 10^7; logT_t = 7.86 6.88≤ logT_t≤8.40; yesX; no □	Dirty conditions 1 min
	10^{-4}	>330	>330		
	10^{-5}	61	83		

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.

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)	Conditions
Ready to use	10^0	10	10	<14	<1.15	>6.71	1 min	Dirty
	10^{-1}	0	0					

Test field 2 to 4 (cfu/25cm²)

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/25cm²	Contact time (min)	Conditions
Ready to use	10^0	1	1	1	<5	1 min	Dirty
	10^{-1}	0	0	0	<5		

N_W Test fields 2 to 4 (cfu/25cm²)

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/25cm²	Contact time (min)	Conditions
Ready to use	10^0	>330	>330	>330	5083.33	1 min	Dirty
	10^{-1}	162	93	50			

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)

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

Appendix 3

TEST RESULTS (bactericidal 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: 3 g/l bovine albumin + 3 ml/l sheep blood erythrocytes
Nordic Tersus Laboratory LLC.; Date of test: 22.03.2021
Responsible person: Allar Laaneleht, Melissa Ingela Bramanis

Validation and controls

Validation suspension (N_{V0})			Neutralizer control (B)			Method validation (C)		
V_{C1}	107	$\bar{x}$ = 104	V_{C1}	85	$\bar{x}$ = 82.5	V_{C1}	83	$\bar{x}$ = 81
V_{C2}	101		V_{C2}	80		V_{C2}	79	
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}	$\bar{x}_{wm}$ = 2.79 x 10^9; log*N* = 9.45 N_0 = *N*/20; logN_0 = 8.14 6.88≤ logN_{V0}≤7.40; yesX; no □
	10^{-7}	262	297	
	10^{-8}	25	30	

Drying controls

Drying control (D_{C0})	T_0	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 4.60 x 10^7; logT_0 = 7.66 6.88≤ logT_0≤8.40; yesX; no □	Dirty conditions 1 min
	10^{-4}	>330	>330		
	10^{-5}	45	47		

Drying controls

Drying control (D_{Ct})	T_t	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 5.25 x 10^7; logT_t = 7.72 6.88≤ logT_t≤8.40; yesX; no □	Dirty conditions 1 min
	10^{-4}	>330	>330		
	10^{-5}	42	62		

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.

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)	Conditions
Ready to use	10^0	11	7	<14	<1.15	>6.57	1 min	Dirty
	10^{-1}	0	0					

Test field 2 to 4 (cfu/25cm²)

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/25cm²	Contact time (min)	Conditions
Ready to use	10^0	2	1	1	<5	1 min	Dirty
	10^{-1}	0	0	0	<5		

N_W Test fields 2 to 4 (cfu/25cm²)

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/25cm²	Contact time (min)	Conditions
Ready to use	10^0	>330	>330	>330	1633.33	1 min	Dirty
	10^{-1}	49	32	17			

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)

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 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: 3 g/l bovine albumin + 3 ml/l sheep blood erythrocytes;
Nordic Tersus Laboratory LLC.; Date of test: 22.03.2021
Responsible person: Allar Laaneleht, Melissa Ingela Bramanis

Validation and controls

Validation suspension (N_{V0})			Neutralizer control (B)			Method validation (C)		
V_{C1}	49	$\bar{x}$ = 45.5	V_{C1}	36	$\bar{x}$ = 37.5	V_{C1}	35	$\bar{x}$ = 40
V_{C2}	42		V_{C2}	39		V_{C2}	45	
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}	162	173	$\bar{x}_{wm}$ = 1.68 x 10^9; log*N* = 9.23 N_0 = *N*/20; logN_0 = 7.92 6.88≤ logN_{V0}≤7.40; yesX; no □
	10^{-8}	19	16	

Drying controls

Drying control (D_{C0})	T_0	V_{C1}	V_{C2}		
	10^{-4}	>330	>330	$\bar{x}_{wm}$ = 3.70 x 10^7; logT_0 = 7.57 6.88≤ logT_0≤8.40; yesX; no □	Dirty conditions 1 min
	10^{-5}	36	38		

Drying controls

Drying control (D_{Ct})	T_t	V_{C1}	V_{C2}		
	10^{-4}	>330	>330	$\bar{x}_{wm}$ = 4.10 x 10^7; logT_t = 7.61 6.88≤ logT_t≤8.40; yesX; no □	Dirty conditions 1 min
	10^{-5}	40	42		

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 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)	Conditions
Ready to use	10^0	8	8	<14	<1.15	>6.46	1 min	Dirty
	10^{-1}	0	0					

Test field 2 to 4 (cfu/25cm²)

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/25cm²	Contact time (min)	Conditions
Ready to use	10^0	3	0	0	<5	1 min	Dirty
	10^{-1}	0	0	0	<5		

N_W Test fields 2 to 4 (cfu/25cm²)

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/25cm²	Contact time (min)	Conditions
Ready to use	10^0	>330	>330	>330	>16500	1 min	Dirty
	10^{-1}	>330	>330	>330			

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)

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.

Appendix 5

Interpretation

The ready to use product for surface disinfection **BACTICID AF WIPES** (batch no. 227251120) was tested according to the test method EVS-EN 16615:2015. The ready to use wipes were presoaked by the manufacturer. The test was performed at 20 ± 1 °C, under dirty conditions during the contact time of 1 min. The dilution – neutralization method was used for testing the product's effectiveness against the reference strains: *Candida albicans* ATCC 10231; *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 1 min.

Conclusion

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

This is the end of the test report.

This is a corrected version of test report no 22021sd and supersedes the previous version of the test report.

ACCREDITED LABORATORY. No L263. ISO/IEC 17025. NORDIC TERSUS LABORATORY OÜ REPUBLIC OF ESTONIA

Melissa Ingela Bramanis
Microbiologist
Date of issue: 20.07.2021

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