Test report number 0735176-2 according to DIN EN 1276/2002

Quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas- Test method and requirements (phase 1)

(dirty conditions)

Identification of the test laboratory: SGS-Germany GmbH
Laboratory Services Hamburg
Weidenbaumsweg 137, 21035 Hamburg

Identification of the product:

Product name:	**Bacoban ® WB**
Batch number:	2007312_WDM_konz_krei
Manufacturer:	Sarastro GmbH, 66287 Quierschied-Göttelborn
Date of delivery:	2007-06-04
Storage conditions:	room-temperature
Active ingredient(s):	not indicated

Test method and its validation:

Method:	Dilution-neutralization-method Membrane filtration: S. aureus
Neutralizer:	3% tween 80, 3% saponin, 0,1% histidin, 0,3 % lecithin, 0,5% Na-thio. dissolved in diluent
Information about sterilization:	15 min 121°C +/- 1°C

Test conditions:

Period of analysis:	28.08. - 03.09.07
Appearance of the product:	product: light yellow / dilutions: light and clear
Test concentration (vol.-%)	0,75%; 0,50%; 0,25%
Diluent of the Dilution:	water standardised hardness (pH 7,0 +/- 0,2)
contact time:	5 min.; 15 min.
Test temperature:	20°C +/-1°C
Interfering substances:	0,3% bovine albumin (dirty conditions)
Stability of the mixture during the procedure:	no visual change
Referenced strains:	***Pseudomonas aeruginosa* ATCC 15442** ***Staphylococcus aureus* ATCC 6538** ***Escherichia coli ATCC 10536*** ***Enterococcus hirae ATCC 10541***
Temperature of incubation:	36 ± 1 °C
Counting method:	pour plate
Test results:	see tables 1-4

Conclusion:

According DIN EN 1276 (may 2002) the product Bacoban®WB, when diluted at 0,75% in hard water, possesses bactericidal activity in 5 min. and when diluted at 0,5% in hard water, possesses bactericidal activity in 15 min. at 20°C under dirty conditions for above referenced strains (required reduction: 5 log).

Hamburg, 10.09.07

i.V.
Dr. Roy Hörner (Laboratory manager)
Heidrun Globisch (Leader Microbiology)

Test report number 0735176-2 according to DIN EN 1276/2002

Quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas- Test method and requirements (phase 1)

Product: Bacoban ® WB, Period of analysis: 28.08. - 03.09.07

Test strain: P. aeruginosa

Table 1a - Verifcation of the method and Validation of the dilution- neutralization method

test suspension for validation (Nv_0)			Experimental conditions (A) 5 min.			Validation of non-toxicity of the neutralizer (B)			Validation of neutralization (C) test concentration: 0,75%; 5 min.		
Vc1	53	58	Vc1	74	75,5	Vc1	74	76	Vc1	67	78
Vc2	63		Vc2	77		Vc2	78		Vc2	89	
$45 \leq \bar{x}$ Nv0 ≤ 180?			$\bar{x}$ A $\geq 0{,}5 * \bar{x}$ Nv0?			$\bar{x}$ B $\geq 0{,}5 * \bar{x}$ Nv0?			$\bar{x}$ C $\geq 0{,}5 * \bar{x}$ Nv0?		
☒ yes		☐ no	☒ yes		☐ no	☒ yes		☐ no	☒ yes		☐ no

Experimental conditions (A) 15 min.			Validation of neutralization (C) test concentration: 0,75%; 15 min.		
Vc1	80	73,5	Vc1	69	79
Vc2	67		Vc2	89	
$\bar{x}$ A $\geq 0{,}5 * \bar{x}$ Nv0?			$\bar{x}$ C $\geq 0{,}5 * \bar{x}$ Nv0?		
☒ yes		☐ no	☒ yes		☐ no

Table 1b: Test suspension

Test suspension (N and N_0)	N	Vc1	Vc2	$N\bar{x}_{wm}$ = 4,30E+08 cfu/ml	
	10^{-6}	>330	>330	$N_0 = N/10 = lg$ 7,63	
	10^{-7}	48	38	$7{,}17 \leq N_0 \leq 7{,}70$? ☒ yes	☐ no
Test suspension (N and N_0)	N	Vc1	Vc2	$N\bar{x}_{wm}$ = 3,90E+08 cfu/ml	
	10^{-6}	>330	>330	$N_0 = N/10 = lg$ 7,59	
	10^{-7}	44	33	$7{,}17 \leq N_0 \leq 7{,}70$? ☒ yes	☐ no

Table 1c: Test results

Test concentration vol.-%	N_{ao} Vc1	N_{ao} Vc2	$N_{ao}\bar{x} * 10$	lg Na	lg R (N_0=7,63)	contact time (min)
0,75	3	0	<140	<2,15	>5,37	5
0,50	202	221	2120	3,33	4,30	5
0,75	0	0	<140	<2,15	>5,37	15
0,50	6	5	<140	<2,15	>5,37	15
Test concentration vol.-%	N_{ao} Vc1	N_{ao} Vc2	$N_{ao}\bar{x} * 10$	lg Na	lg R (N_0=7,59)	contact time (min)
0,25	>330	>330	>3300	>3,52	<4,07	15

Vc1; Vc2= cfu/platte

Na is the number of cells per ml in the test mixture at the end of the contact time and before neutralization or membrane-filtration. It is tenfold higher than the Vc (Vc1 + Vc2 /2) values due to the addition of neutralizer and water or the sample volume of 0,1 ml in the membrane-filtration.

Test report number 0735176-2 according to DIN EN 1276/2002

Quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas- Test method and requirements (phase 1)

Product: Bacoban ® WB, Period of analysis: 28.08. - 03.09.07

Test strain: E. coli

Table 2a - Verifcation of the method and Validation of the dilution- neutralization method

test suspension for validation (Nv_0)			Experimental conditions (A) 5 min.			Validation of non-toxicity of the neutralizer (B)			Validation of neutralization (C) test concentration: 0,75%; 5 min.		
Vc1	131	133	Vc1	122	120	Vc1	132	124,5	Vc1	132	121,5
Vc2	135		Vc2	118		Vc2	117		Vc2	111	
$45 \leq \bar{x}$ Nv0 ≤ 180?			$\bar{x}$ A $\geq 0{,}5 * \bar{x}$ Nv0?			$\bar{x}$ B $\geq 0{,}5 * \bar{x}$ Nv0?			$\bar{x}$ C $\geq 0{,}5 * \bar{x}$ Nv0?		
☒ yes		☐ no	☒ yes		☐ no	☒ yes		☐ no	☒ yes		☐ no

Experimental conditions (A) 15 min.			Validation of neutralization (C) test concentration: 0,75%; 15 min.		
Vc1	126	121	Vc1	103	117,5
Vc2	116		Vc2	132	
$\bar{x}$ A $\geq 0{,}5 * \bar{x}$ Nv0?			$\bar{x}$ C $\geq 0{,}5 * \bar{x}$ Nv0?		
☒ yes		☐ no	☒ yes		☐ no

Table 2b: Test suspension

Test suspension (N and N_0)	N	Vc1	Vc2	$N\bar{x}_{wm}$ = 4,70E+08 cfu/ml	
	10^{-6}	>330	>330	$N_0 = N/10 = \lg$ 7,67	
	10^{-7}	50	44	$7{,}17 \leq N_0 \leq 7{,}70$? ☒ yes	☐ no
Test suspension (N and N_0)	N	Vc1	Vc2	$N\bar{x}_{wm}$ = 3,10E+08 cfu/ml	
	10^{-6}	>330	>330	$N_0 = N/10 = \lg$ 7,49	
	10^{-7}	27	35	$7{,}17 \leq N_0 \leq 7{,}70$? ☒ yes	☐ no

Table 2c: Test results

Test concentration vol.-%	N_{ao} Vc1	N_{ao} Vc2	$N_a \bar{x}_o * 10$	lg Na	lg R (N_0=7,67)	contact time (min)
0,75	0	0	<140	<2,15	>5,52	5
0,50	0	0	<140	<2,15	>5,52	5
0,75	0	0	<140	<2,15	>5,52	15
0,50	1	0	<140	<2,15	>5,52	15
Test concentration vol.-%	N_{ao} Vc1	N_{ao} Vc2	$N_a \bar{x}_o * 10$	lg Na	lg R (N_0=7,49)	contact time (min)
0,25	0	0	<140	<2,15	>5,34	15

Vc1; Vc2= cfu/platte

Na is the number of cells per ml in the test mixture at the end of the contact time and before neutralization or membrane-filtration. It is tenfold higher than the Vc (Vc1 + Vc2 /2) values due to the addition of neutralizer and water or the sample volume of 0,1 ml in the membrane-filtration.

Test report number 0735176-2 according to DIN EN 1276/2002

Quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas- Test method and requirements (phase 1)

Product: Bacoban ® WB, Period of analysis: 28.08. - 03.09.07

Test strain: S. aureus

Table 3a - Verifcation of the method and Validation of the dilution- neutralization method

test suspension for validation (Nv_0)			Experimental conditions (A) 5 min.			Validation of non-toxicity of the neutralizer (B)			Validation of neutralization (C) test concentration: 0,75%; 5 min.		
Vc1	107	100	Vc1	105	108,5	Vc1	108	114,5	Vc1	122	108
Vc2	93		Vc2	112		Vc2	121		Vc2	94	
$45 \leq \bar{x}$ Nv0 ≤ 180?			$\bar{x}$ A $\geq 0{,}5 * \bar{x}$ Nv0?			$\bar{x}$ B $\geq 0{,}5 * \bar{x}$ Nv0?			$\bar{x}$ C $\geq 0{,}5 * \bar{x}$ Nv0?		
☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no		
			Experimental conditions (A) 15 min.						Validation of neutralization (C) test concentration: 0,75%; 15 min.		
			Vc1	119	114,5				Vc1	115	127
			Vc2	110					Vc2	139	
			$\bar{x}$ A $\geq 0{,}5 * \bar{x}$ Nv0?						$\bar{x}$ C $\geq 0{,}5 * \bar{x}$ Nv0?		
			☒ yes ☐ no						☒ yes ☐ no		

Table 3b: Test suspension

Test suspension (N and N_0)	N	Vc1	Vc2	$N\bar{x}_{wm}$ = 3,90E+08 cfu/ml	
	10^{-6}	>330	>330	$N_0 = N/10 = lg$ 7,59	
	10^{-7}	35	43	$7{,}17 \leq N_0 \leq 7{,}70$? ☒ yes	☐ no
Test suspension (N and N_0)	N	Vc1	Vc2	$N\bar{x}_{wm}$ = 2,80E+08 cfu/ml	
	10^{-6}	>330	>330	$N_0 = N/10 = lg$ 7,45	
	10^{-7}	27	28	$7{,}17 \leq N_0 \leq 7{,}70$? ☒ yes	☐ no

Table 3c: Test results

Test concentration vol.-%	N_{ao} Vc1	N_{ao} Vc2	$N_{ao}\bar{x} * 10$	lg Na	lg R (N_0=7,59)	contact time (min)
0,75	0	0	<140	<2,15	>5,44	5
0,50	0	0	<140	<2,15	>5,44	5
0,75	0	0	<140	<2,15	>5,44	15
0,50	0	0	<140	<2,15	>5,44	15
Test concentration vol.-%	N_{ao} Vc1	N_{ao} Vc2	$N_{ao}\bar{x} * 10$	lg Na	lg R (N_0=7,45)	contact time (min)
0,25	0	0	<140	<2,15	>5,30	15

Vc1; Vc2= cfu/platte

Na is the number of cells per ml in the test mixture at the end of the contact time and before neutralization or membrane-filtration. It is tenfold higher than the Vc (Vc1 + Vc2 /2) values due to the addition of neutralizer and water or the sample volume of 0,1 ml in the membrane-filtration.

Test report number 0735176-2 according to DIN EN 1276/2002

Quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas- Test method and requirements (phase 1)

Product: Bacoban ® WB, Period of analysis: 28.08. - 03.09.07

Test strain: E. hirae

Table 4a - Verifcation of the method and Validation of the dilution- neutralization method

test suspension for validation (Nv_0)			Experimental conditions (A) 5 min.			Validation of non-toxicity of the neutralizer (B)			Validation of neutralization (C) test concentration: 0,75%; 5 min.		
Vc1	96	98,5	Vc1	80	79	Vc1	89	80	Vc1	55	54,5
Vc2	101		Vc2	78		Vc2	71		Vc2	54	
$45 \leq \bar{x}$ Nv0 ≤ 180?			$\bar{x}$ A ≥ 0,5 * $\bar{x}$ Nv0?			$\bar{x}$ B ≥ 0,5 * $\bar{x}$ Nv0?			$\bar{x}$ C ≥ 0,5 * $\bar{x}$ Nv0?		
☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no		
			Experimental conditions (A) 15 min.						Validation of neutralization (C) test concentration: 0,75%; 15 min.		
			Vc1	91	87,5				Vc1	74	71
			Vc2	84					Vc2	68	
			$\bar{x}$ A ≥ 0,5 * $\bar{x}$ Nv0?						$\bar{x}$ C ≥ 0,5 * $\bar{x}$ Nv0?		
			☒ yes ☐ no						☒ yes ☐ no		

Table 4b: Test suspension

Test suspension (N and N_0)	N	Vc1	Vc2	$N\bar{x}_{wm}$ = 4,40E+08 cfu/ml	
	10^{-6}	>330	>330	N_0 = N/10 = lg 7,64	
	10^{-7}	40	47	$7,17 \leq N_0 \leq 7,70$? ☒ yes	☐ no
Test suspension (N and N_0)	N	Vc1	Vc2	$N\bar{x}_{wm}$ = 2,73E+08 cfu/ml	
	10^{-6}	284	>330	N_0 = N/10 = lg 7,45	
	10^{-7}	24	20	$7,17 \leq N_0 \leq 7,70$? ☒ yes	☐ no

Table 4c: Test results

Test concentration vol.-%	N_{ao} Vc1	N_{ao} Vc2	$N_a{}_o\bar{x}$ * 10	lg Na	lg R (N_0=7,64)	contact time (min)
0,75	0	0	<140	<2,15	>5,49	5
0,50	0	0	<140	<2,15	>5,49	5
0,75	0	0	<140	<2,15	>5,49	15
0,50	0	0	<140	<2,15	>5,49	15
Test concentration vol.-%	N_{ao} Vc1	N_{ao} Vc2	$N_a{}_o\bar{x}$ * 10	lg Na	lg R (N_0=7,45)	contact time (min)
0,25	0	0	<140	<2,15	>5,30	15

Vc1; Vc2= cfu/platte

Na is the number of cells per ml in the test mixture at the end of the contact time and before neutralization or membrane-filtration. It is tenfold higher than the Vc (Vc1 + Vc2 /2) values due to the addition of neutralizer and water or the sample volume of 0,1 ml in the membrane-filtration.