Test report number 0735176-1 according to DIN EN 1040 (march 2006)
Quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics - Test method and requirements (phase 1)

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: *P. aeruginosa*
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: 2007-08-28 - 2007-08-30
Appearance of the product: product: light yellow / dilutions: light and clear
Test concentration (vol.-%) 0,50%; 0,25%
Diluent of the Dilution: distilled water
contact time: 5 min.; 15 min.
Test temperature: 20°C +/-1°C
Stability of the mixture during the procedure: no visual change
Referenced strains: ***Staphylococcus aureus* ATCC 6538**
***Pseudomonas aeruginosa* ATCC 15442**
Temperature of incubation: 36 ± 1 °C
Counting method: pour plate

Test results: see tables 1a-c, 2a-c

Conclusion:

According DIN EN 1040 (march 2006) the product Bacoban®WB, when diluted at 0,25% in distilled water, possesses bactericidal activity in 5 min. at 20°C for referenced strains *Pseudomonas aeruginosa ATCC 15442, Staphylococcus aureus ATCC 6538* (required reduction: 5 log).

To quality the product as a chemical disinfectant and/or an antiseptic for a determined intended use, it has to be assessed by additional standard tests, which are corresponding to the intended application.

Hamburg, 18.09.07

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

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Product: Bacoban ® WB,Period of analysis: 2007-08-28 - 2007-08-30

Test strain: S. aureus

Table1a - Verifacation of the method and Validation of the dilution - neutralization method

test suspension for validation (Nv_0)			Experimental conditions (A) 5 min.			Validation of filtration (B)			Validation of neutralization (C) test concentration: 0,5%; 5 min.		
Vc1	107	100	Vc1	86	96	Vc1	108	114,5	Vc1	113	113
Vc2	93		Vc2	106		Vc2	121		Vc2	113	
$30 \leq \bar{x}$ Nv0 ≤ 160?			$\bar{x}$ 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: 0,5%;15 min.		
Vc1	95	91,5	Vc1	134	131,5
Vc2	88		Vc2	129	
$\bar{x}$ 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	$\bar{x}_{wm}$ = 3,9E+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

Table 1c: Test results

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

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Quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics - Test method and requirements (phase 1)

Product: Bacoban ® WB, Period of analysis: 2007-08-28 - 2007-08-30

Test strain: P. aeruginosa

Table 2a - Verifacation 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: 0,5%; 5 min.		
Vc1	53	58	Vc1	74	67,5	Vc1	74	76	Vc1	87	93
Vc2	63		Vc2	61		Vc2	78		Vc2	99	
$30 \leq \bar{x}$ Nv0 ≤ 160?			$\bar{x}$ 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: 0,5%; 15 min.		
Vc1	76	72	Vc1	90	91,5
Vc2	68		Vc2	93	
$\bar{x}$ 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	$\bar{x}_{wm}$ = 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

Table 2c: Test results

Test concentration vol.-%	N_{ao} Vc1	N_{ao} Vc2	$N_{a\,o}\bar{x} * 10$	lg Na	lg R (N_0= 7,63)	contact time (min.)
0,50	0	0	<140	<2,15	>5,48	5
0,25	2	0	<140	<2,15	>5,48	5
0,50	0	0	<140	<2,15	>5,48	15
0,25	0	0	<140	<2,15	>5,48	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.