

Test report number 0721421-3 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<sup>®</sup> WB**  
Batch number: 2007312\_WDM\_konz\_krei  
Manufacturer: Sarastro GmbH, 66287 Quierschied-Göttelborn  
Date of delivery: 2007-03-14  
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: 2007-05-22 - 2007-06-07  
Appearance of the product: product: yellow-orange / Dilutions: light yellow  
Test concentration (vol.-%) 1,00%, 1,50%  
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 optical 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<sup>®</sup>WB, when diluted at 1,00% in hard water, possesses bactericidal activity in 5 min. at 20°C under dirty conditions for above referenced strains (required reduction: 5 log).

Hamburg, 18.6.2007

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**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: 2007-05-22 - 2007-06-07

Test strain: P. aeruginosa

**Table 1a - Validation of the carrier test method for the test product as received**

test suspension for validation (Nv <sub>0</sub> )			Experimental conditions (A) 5 min.			Validation of non-toxicity of the neutralizer (B)			Validation of neutralization (C) test concentration: 1,5%; 5 min.		
Vc1	43	57,5	Vc1	52	53,5	Vc1	78	80,5	Vc1	78	75
Vc2	72		Vc2	55		Vc2	83		Vc2	72	
45 ≤ $\bar{x}$ Nv <sub>0</sub> ≤ 180?			$\bar{x}$ A ≥ 0,5 * $\bar{x}$ Nv <sub>0</sub> ?			$\bar{x}$ B ≥ 0,5 * $\bar{x}$ Nv <sub>0</sub> ?			$\bar{x}$ C ≥ 0,5 * $\bar{x}$ Nv <sub>0</sub> ?		
<input checked="" type="checkbox"/> yes <input type="checkbox"/> no			<input checked="" type="checkbox"/> yes <input type="checkbox"/> no			<input checked="" type="checkbox"/> yes <input type="checkbox"/> no			<input checked="" type="checkbox"/> yes <input type="checkbox"/> no		
			Experimental conditions (A) 15 min.						Validation of neutralization (C) test concentration: 1,5%; 15 min.		
			Vc1	70	86				Vc1	87	87
			Vc2	102		Vc2	87		Vc2	87	
			$\bar{x}$ A ≥ 0,5 * $\bar{x}$ Nv <sub>0</sub> ?						$\bar{x}$ C ≥ 0,5 * $\bar{x}$ Nv <sub>0</sub> ?		
			<input checked="" type="checkbox"/> yes <input type="checkbox"/> no						<input checked="" type="checkbox"/> yes <input type="checkbox"/> no		

**Table 1b: Test suspension**

Test suspension (N and N <sub>0</sub> )	N	Vc1	Vc2	N $\bar{x}_{wm}$ =	3,30E+08 cfu/ml
10 <sup>-6</sup>	>330	>330		N <sub>0</sub> = N/10 = lg	7,52
10 <sup>-7</sup>	42	24		7,17 ≤ N <sub>0</sub> ≤ 7,70 ?	<input checked="" type="checkbox"/> yes <input type="checkbox"/> no

**Table 1c: Test results**

Test concentration vol.-%	N <sub>ao</sub>		N <sub>a o</sub> $\bar{x}$ * 10	lg Na	lg R (N <sub>0</sub> =7,52)	contact time (min)
	Vc1	Vc2				
1,00	8	12	<140	<2,15	>5,37	5
1,50	5	6	<140	<2,15	>5,37	5
1,00	0	0	<140	<2,15	>5,37	15
1,50	0	0	<140	<2,15	>5,37	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 used in food, industrial, domestic and institutional areas- Test method and requirements (phase 1)**

Product: Bacoban ® WB, Period of analysis: 2007-05-22 - 2007-06-07

Test strain: *E. coli*

**Table 2a - Validation of the carrier test method for the test product as received**

test suspension for validation (Nv0)			Experimental conditions (A) 5 min.			Validation of non-toxicity of the neutralizer (B)			Validation of neutralization (C) test concentration: 1,5%; 5 min.		
Vc1	161	139,5	Vc1	174	162,5	Vc1	94	116	Vc1	102	104
Vc2	118		Vc2	151		Vc2	138		Vc2	106	
45 ≤ x̄ Nv0 ≤ 180? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no			x̄ A ≥ 0,5 * x̄ Nv0? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no			x̄ B ≥ 0,5 * x̄ Nv0? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no			x̄ C ≥ 0,5 * x̄ Nv0? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no		
			Experimental conditions (A) 15 min.						Validation of neutralization (C) test concentration: 1,5%; 15 min.		
			Vc1	173	162				Vc1	96	99,5
			Vc2	151		Vc2	103				
			x̄ von A ist ≥ 0,5 * x̄ von Nv0? <input checked="" type="checkbox"/> ja <input type="checkbox"/> nein						x̄ von C ist ≥ 0,5 * x̄ von Nv0? <input checked="" type="checkbox"/> ja <input type="checkbox"/> nein		

**Table 2b: Test suspension**

Test suspension (N and N0)	N	Vc1	Vc2	Nx <sub>wm</sub> = 1,50E+08 cfu/ml
	10 <sup>-6</sup>	158	142	N <sub>0</sub> = N/10 = lg 7,17
	10 <sup>-7</sup>	15	13	7,17 ≤ N <sub>0</sub> ≤ 7,70 ? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no

**Table 2c: Test results**

Test concentration vol.-%	N <sub>ao</sub>		N <sub>a</sub> x̄ * 10	lg Na	lg R (N <sub>0</sub> =7,17)	contact time (min)
	Vc1	Vc2				
1,00	0	0	<140	<2,15	>5,02	5
1,50	0	0	<140	<2,15	>5,02	5
1,00	0	0	<140	<2,15	>5,02	15
1,50	0	0	<140	<2,15	>5,02	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.

**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: 2007-05-22 - 2007-06-07

Test strain: *S. aureus*

**Table 3a - Validation of the carrier test method for the test product as received**

test suspension for validation ( $N_{v0}$ )			Experimental conditions (A) 5 min.			Validation of the filtration (B)			Validation of neutralization (C) test concentration: 1,5%; 5 min.		
Vc1	107	107,5	Vc1	112	97	Vc1	64	68	Vc1	126	146
Vc2	108		Vc2	82		Vc2	72		Vc2	166	
$45 \leq \bar{x} N_{v0} \leq 180$ ? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no			$\bar{x} A \geq 0,5 * \bar{x} N_{v0}$ ? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no			$\bar{x} B \geq 0,5 * \bar{x} N_{v0}$ ? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no			$\bar{x} C \geq 0,5 * \bar{x} N_{v0}$ ? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no		
			Experimental conditions (A) 15 min.						Validation of neutralization (C) test concentration: 1,5%; 15 min.		
			Vc1	113	110				Vc1	123	107,5
			Vc2	107		Vc2	92				
			$\bar{x} A \geq 0,5 * \bar{x} N_{v0}$ ? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no						$\bar{x} C \geq 0,5 * \bar{x} N_{v0}$ ? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no		

**Table 3b: Test suspension**

Test suspension (N and $N_0$ )	N	Vc1	Vc2	$N \bar{x}_{wm} =$	1,61E+08 cfu/ml
$10^{-6}$		182	140	$N_0 = N/10 = \lg$	7,21
$10^{-7}$		10	12	$7,17 \leq N_0 \leq 7,70$ ?	<input checked="" type="checkbox"/> yes <input type="checkbox"/> no

**Table 3c: Test results**

Test concentration vol.-%	$N_{a0}$		$N_{a0} \bar{x} * 10$	$\lg Na$	$\lg R (N_0=7,21)$	contact time (min)
	Vc1	Vc2				
1,00	0	0	<140	<2,15	>5,06	5
1,50	0	0	<140	<2,15	>5,06	5
1,00	0	0	<140	<2,15	>5,06	15
1,50	0	0	<140	<2,15	>5,06	15

Vc1; Vc2= cfu/platte

$N_a$  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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Product: Bacoban ® WB, Period of analysis: 2007-05-22 - 2007-06-07

Test strain: *E. hirae*

**Table 4a - Validation of the carrier test method for the test product as received**

test suspension for validation (Nv <sub>0</sub> )			Experimental conditions (A) 5 min.			Validation of non-toxicity of the neutralizer (B)			Validation of neutralization (C) test concentration: 1,5%; 5 min.		
Vc1	105	98,5	Vc1	121	114	Vc1	94	97	Vc1	75	83
Vc2	92		Vc2	107		Vc2	100		Vc2	91	
45 ≤ $\bar{x}$ Nv <sub>0</sub> ≤ 180?			$\bar{x}$ A ≥ 0,5 * $\bar{x}$ Nv <sub>0</sub> ?			$\bar{x}$ B ≥ 0,5 * $\bar{x}$ Nv <sub>0</sub> ?			$\bar{x}$ C ≥ 0,5 * $\bar{x}$ Nv <sub>0</sub> ?		
<input checked="" type="checkbox"/> yes <input type="checkbox"/> no			<input checked="" type="checkbox"/> yes <input type="checkbox"/> no			<input checked="" type="checkbox"/> yes <input type="checkbox"/> no			<input checked="" type="checkbox"/> yes <input type="checkbox"/> no		
			Experimental conditions (A) 15 min.						Validation of neutralization (C) test concentration: 1,5%; 15 min.		
			Vc1	131	111,5				Vc1	64	72
			Vc2	92		Vc2	80				
			$\bar{x}$ A ≥ 0,5 * $\bar{x}$ Nv <sub>0</sub> ?						$\bar{x}$ C ≥ 0,5 * $\bar{x}$ Nv <sub>0</sub> ?		
			<input checked="" type="checkbox"/> yes <input type="checkbox"/> no						<input checked="" type="checkbox"/> yes <input type="checkbox"/> no		

**Table 4b: Test suspension**

Test suspension (N and N <sub>0</sub> )	N	Vc1	Vc2	N $\bar{x}_{wm}$ =	lg R (N <sub>0</sub> =7,24)
				1,74E+08 cfu/ml	
	10 <sup>-6</sup>	168	180	N <sub>0</sub> = N/10 = lg	7,24
	10 <sup>-7</sup>	16	18	7,17 ≤ N <sub>0</sub> ≤ 7,70 ?	<input checked="" type="checkbox"/> yes <input type="checkbox"/> no

**Table 4c: Test results**

Test concentration vol.-%	N <sub>ao</sub>		N <sub>a</sub> $\bar{x}$ * 10	lg Na	lg R (N <sub>0</sub> =7,24)	contact time (min)
	Vc1	Vc2				
1,00	0	0	<140	<2,15	>5,09	5
1,50	0	0	<140	<2,15	>5,09	5
1,00	0	0	<140	<2,15	>5,09	15
1,50	0	0	<140	<2,15	>5,09	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.