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ROPIMEX R. OPEL GmbH
Bildstocker Straße 12
DE - 66538 Neunkirchen

Hamburg, 27 January 2022

Expert opinion

Bactericidal Activity of **Bacoban WBUS** in the quantitative surface test according to DIN EN 14349:2013 (Phase 2, Step 2)

The disinfectant **Bacoban WBUS** was tested and evaluated according to DIN EN 14349:2013 "Chemical disinfectants and antiseptics - Quantitative surface test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary area on non-porous surfaces without mechanical action - Test method and requirements (phase 2, step 2)".

According to the test report no. L21/01041.6 dated 27/01/2022 of Dr. Brill + Partner GmbH the preparation showed bactericidal activity under clean conditions at a test temperature of 10°C ± 1°C.

Bacoban WBUS complies with the requirements of DIN EN 14349:2013 (phase 2, step 2) with the following concentration-time relationship:

Bactericidal:	clean conditions	1.0 %	30 minutes
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Dr. Florian H. H. Brill



Test report no L21/01041.6

Quantitative surface test for the evaluation of bactericidal activity of **Bacoban WBUS** in the veterinary area (DIN EN 14349:2013; Phase 2, Step 2*)

In accordance with your order, we tested the preparation **Bacoban WBUS** for its activity in the quantitative surface test according to DIN EN 14349:2013* under clean conditions.

1 General Information and Material

1.1 Client

Client: ROPILEX R. OPEL GmbH, Mrs Jennifer Sahl, Bildstocker Straße 12,
DE - 66538 Neunkirchen, Germany
Date of order: 23/12/2021
Confirmation no.: 226415

1.2 Identification of Test Laboratory

Location: Dr. Brill + Partner GmbH · Institute for Hygiene and Microbiology,
Stiegstück 34, DE-22339 Hamburg, Germany
Study manager: Dipl.-Biol. Dr. rer. nat. Jan-Hendrik Klock
Scientific assistant: Dipl.-Biol. Henrik Gabriel
Laboratory technicians: Elahe Saroukhani

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1.4 Identification of Sample

Name of product: **Bacoban WBUS**
Batch no.: 20210920_Bacoban WBUS_imi

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Internal no.:	21/01202
Manufacturer:	ROPIMEX R. OPEL GmbH, DE - 66538 Neunkirchen, Germany
Date of delivery:	21/09/2021
Storage conditions:	room temperature and darkness
Appearance of product:	clear liquid
Odour:	characteristic
Product type:	surface disinfectant
Recommended diluent:	Tap water
Diluent used:	water of standardised hardness (WSH, pH 7.0)
pH value, concentrate:	4.3
pH value, 1.0 % (measured in WSH):	6.3
pH value, 0.5 % (measured in WSH):	6.9
pH value, 0.1 % (measured in WSH):	7.4
Active agents (Manufacturer's data):	33.31 g benzalkoniumchloride

1.5 Test Conditions

Test period:	26/10/ - 28/10/2021, 30/12/2021 - 03/01/2022	
Lab task no.:	L21/01041.2+.6	
Test pieces:	stainless steel	
Drying time:	<60 minutes (22.5 min ± 2.5 min)	
Product test concentrations:	0.1 + 0.5 + 1.0 %	
Exposure time:	30 minutes	
Test temperature:	10°C ± 1°C	
Relative humidity:	45 % ± 5 %	
Incubation temperature:	36°C ± 1°C	
Organic load:	clean conditions (3.0 g/L bovine albumin)	
Neutraliser:	60 g/L polysorbate 80, 60 g/L saponine, 8 g/L lecithin, 1 g/L cysteine, 2.5 g/L SDS (TLSC-SDS)	
Test organisms:	<i>Staphylococcus aureus</i>	ATCC 6538
	<i>Enterococcus hirae</i>	ATCC 10541
	<i>Proteus hauseri (vulgaris)</i>	ATCC 13315
	<i>Pseudomonas aeruginosa</i>	ATCC 15442

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2 Methods

The tests were carried out according to DIN EN 14349:2013 "Chemical disinfectants and antiseptics - Quantitative surface test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary area on non-porous surfaces without mechanical action - Test method and requirements (phase 2, step 2)".

3 Results

The test results based on DIN EN 14349: 2013 are summarised in tables 1.

The test bacteria were sufficiently (RF >4) inactivated with the following concentration-time relationship:

Bactericidal: clean conditions 1.0 % 30 minutes

Hamburg, 27/01/2022

Dipl.-Biol. Dr. rer. nat. Jan-Hendrik Klock
Study Manager

Dipl.-Biol. Henrik Gabriel
Head of Laboratory



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Table 1.1: Validation, Controls and Evaluation

Product name: **Bacoban WBUS** Batch: 20210920_Bacoban
WBUS_imi
Test organism: *Staphylococcus aureus* Temperature: 10°C ± 1°C
Organic load: clean conditions Neutraliser: TLSC-SDS
Contact time: **30 minutes** Test surface: stainless steel

Test and validation suspension (<i>N</i>)				Control concerning toxicity of neutraliser (<i>B</i>)				Control of neutralisation (<i>C</i>) At concentration: 1,0 %			
Dilution	V _{c1}	V _{c2}	Σ	Dilution	V _{c1}	V _{c2}	Σ	Dilution	V _{c1}	V _{c2}	Σ
1,00E-07	>330	>330	92	1,00E-04	>330	>330	60	1,00E-04	>330	>330	78
1,00E-08	42	50		1,00E-05	28	32		1,00E-05	41	37	
$\bar{x}_{xm} = 1,15E+08 = 8,061$				$\bar{x} = 3,00E+07 = 7,477$				$\bar{x} = 3,90E+07 = 7,591$			
7,57 ≤ lg <i>N</i> ≤ 8,10 ?			Yes	x̄ of <i>B</i> ≥ 0,5 x x̄ von <i>N_w</i> ?			Yes	x̄ of <i>C</i> ≥ 0,5 x x̄ von <i>N_w</i> ?			Yes

Water control (<i>N_w</i>)	<i>N_w</i>	Microbial count				V _{c1}	V _{c2}	\bar{x}	lg <i>N_w</i>	lg <i>N_w</i> ≥ lg 6,2 ?
	1,00E-04	>330		>330		>330	>330	3,00E+07	7,48	Yes
	1,00E-05	30		30		30	30			

Product-concentration [%]	Dilution	Microbial count				V _{c1}	V _{c2}	<i>N_a</i>	lg <i>N_a</i> = lg (x̄ oder x̄ _{wm})	lg <i>R</i> = (lg <i>N_w</i> = 7,48)
0,1	1,00E+00	>330		>330		>330	>330	1,47E+04	4,17	3,31
	1,00E-01	130		162		130	162			
	1,00E-02	16		16		16	16			
		<i>N_{ts}</i> =	58		<i>N_{ts}</i> < 1 ?	No	5 ≤ Quotient of x̄ _{wm} ≤ 15 ?		Yes	
0,5	1,00E+00	34		35		34	35	3,45E+02	2,54	4,94
	1,00E-01	6		7		<14	<14			
	1,00E-02	0		0		<14	<14			
		<i>N_{ts}</i> =	3		<i>N_{ts}</i> < 1 ?	No	5 ≤ Quotient of x̄ _{wm} ≤ 15 ?		Na	
1,0	1,00E+00	2		9		<14	<14	<1,40E+02	< 2,15	≥ 5,33
	1,00E-01	0		0		<14	<14			
	1,00E-02	0		0		<14	<14			
		<i>N_{ts}</i> =	0		<i>N_{ts}</i> < 1 ?	Yes	5 ≤ Quotient of x̄ _{wm} ≤ 15 ?		Na	

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Table 1.2: Validation, Controls and Evaluation

Product name: **Bacoban WBUS** Batch: 20210920_Bacoban
 Test organism: *Enterococcus hirae* WBUS_imi
 Organic load: clean conditions Temperature: 10°C ± 1°C
 Contact time: **30 minutes** Neutraliser: TLSC-SDS
 Test surface: stainless steel

Test and validation suspension (<i>N</i>)				Control concerning toxicity of neutraliser (<i>B</i>)				Control of neutralisation (<i>C</i>) At concentration: 1,0 %			
Dilution	V _{c1}	V _{c2}	Σ	Dilution	V _{c1}	V _{c2}	Σ	Dilution	V _{c1}	V _{c2}	Σ
1,00E-07	>330	>330	84	1,00E-04	>330	>330	47	1,00E-04	>330	>330	45
1,00E-08	42	42		1,00E-05	23	24		1,00E-05	20	25	
$\bar{x}_{xm} = 1,05E+08 = 8,021$				$\bar{x} = 2,35E+07 = 7,371$				$\bar{x} = 2,25E+07 = 7,352$			
7,57 ≤ lg <i>N</i> ≤ 8,10 ?			Yes	x̄ of <i>B</i> ≥ 0,5 x x̄ von <i>N_w</i> ?			Yes	x̄ of <i>C</i> ≥ 0,5 x x̄ von <i>N_w</i> ?			Yes

Water control (<i>N_w</i>)	<i>N_w</i>	Microbial count				V _{c1}	V _{c2}	\bar{x}	lg <i>N_w</i>	lg <i>N_w</i> ≥ lg 6,2 ?
		1,00E-04	>330		>330		>330	>330	4,20E+07	7,62
	1,00E-05	37		47		37	47			

Product-concentration [%]	Dilution	Microbial count				V _{c1}	V _{c2}	<i>N_a</i>	lg <i>N_a</i> = lg (x̄ oder x̄ _{wm})	lg <i>R</i> =
										(lg <i>N_w</i> = 7,62)
0,1	1,00E+00	>330		>330		>330	>330	1,14E+04	4,06	3,57
	1,00E-01	114		114		114	114			
	1,00E-02	11		16		<14	16			
	<i>N_{ts}</i> =	50		<i>N_{ts}</i> < 1 ?	No	5 ≤ Quotient of x̄ _{wm} ≤ 15 ?		Yes		
0,5	1,00E+00	23		29		23	29	2,60E+02	2,41	5,21
	1,00E-01	3		5		<14	<14			
	1,00E-02	0		0		<14	<14			
	<i>N_{ts}</i> =	1		<i>N_{ts}</i> < 1 ?	No	5 ≤ Quotient of x̄ _{wm} ≤ 15 ?		Na		
1,0	1,00E+00	0		0		<14	<14	<1,40E+02	< 2,15	≥ 5,48
	1,00E-01	0		0		<14	<14			
	1,00E-02	0		0		<14	<14			
	<i>N_{ts}</i> =	0		<i>N_{ts}</i> < 1 ?	Yes	5 ≤ Quotient of x̄ _{wm} ≤ 15 ?		Na		

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Table 1.3: Validation, Controls and Evaluation

Product name: **Bacoban WBUS** Batch: 20210920_Bacoban
WBUS_imi
Test organism: *Proteus hauseri* Temperature: 10°C ± 1°C
Organic load: clean conditions Neutraliser: TLSC-SDS
Contact time: **30 minutes** Test surface: stainless steel

Test and validation suspension (<i>N</i>)				Control concerning toxicity of neutraliser (<i>B</i>)				Control of neutralisation (<i>C</i>) At concentration: 1,0 %			
Dilution	V _{c1}	V _{c2}	Σ	Dilution	V _{c1}	V _{c2}	Σ	Dilution	V _{c1}	V _{c2}	Σ
1,00E-07	>330	>330	94	1,00E-04	78	78	179	1,00E-04	28	34	68
1,00E-08	46	48		1,00E-05	9	14		1,00E-05	3	3	
$\bar{x}_{xm} = 1,18E+08 =$			8,07	$\bar{x} = 8,14E+06 =$			6,91	$\bar{x} = 3,09E+06 =$			6,49
7,57 ≤ lg <i>N</i> ≤ 8,10 ?			Yes	x̄ of <i>B</i> ≥ 0,5 x x̄ von <i>N_w</i> ?			Yes	x̄ of <i>C</i> ≥ 0,5 x x̄ von <i>N_w</i> ?			Yes

Water control (<i>N_w</i>)	<i>N_w</i>	Microbial count				V _{c1}	V _{c2}	x̄	lg <i>N_w</i>	lg <i>N_w</i> ≥ lg 6,2 ?
	1,00E-04	26		31		26	31	2,85E+06	6,45	Yes
	1,00E-05	4		4		4	4			

Product-concentration [%]	Dilution	Microbial count				V _{c1}	V _{c2}	N _a	lg N _a = lg (x̄ oder x̄ _{wm})	lg <i>R</i> = (lg <i>N_w</i> = 6,45)
0,1	1,00E+00	22		28		22	28	2,50E+02	2,40	4,06
	1,00E-01	2		4		<14	<14			
	1,00E-02	0		0		<14	<14			
	N _{ts} =	0		N _{ts} < 1 ?	Yes	5 ≤ Quotient of x̄ _{wm} ≤ 15 ?		Na		
0,5	1,00E+00	0		0		<14	<14	<1,40E+02	< 2,15	≥ 4,31
	1,00E-01	0		0		<14	<14			
	1,00E-02	0		0		<14	<14			
	N _{ts} =	0		N _{ts} < 1 ?	Yes	5 ≤ Quotient of x̄ _{wm} ≤ 15 ?		Na		
1,0	1,00E+00	0		0		<14	<14	<1,40E+02	< 2,15	≥ 4,31
	1,00E-01	0		0		<14	<14			
	1,00E-02	0		0		<14	<14			
	N _{ts} =	0		N _{ts} < 1 ?	Yes	5 ≤ Quotient of x̄ _{wm} ≤ 15 ?		Na		

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Table 1.4: Validation, Controls and Evaluation

Product name: **Bacoban WBUS** Batch: 20210920_Bacoban
WBUS_imi
Test organism: *Pseudomonas aeruginosa* Temperature: 10°C ± 1°C
Organic load: clean conditions Neutraliser: TLSC-SDS
Contact time: **30 minutes** Test surface: stainless steel

Test and validation suspension (M)				Control concerning toxicity of neutraliser (B)				Control of neutralisation (C) At concentration: 1,0 %			
Dilution	V _{c1}	V _{c2}	Σ	Dilution	V _{c1}	V _{c2}	Σ	Dilution	V _{c1}	V _{c2}	Σ
1,00E-07	>330	>330	93	1,00E-04	>330	>330	66	1,00E-04	>330	>330	76
1,00E-08	42	51		1,00E-05	28	38		1,00E-05	36	40	
$\bar{x}_{xm} = 1,16E+08 = 8,07$				$\bar{x} = 3,30E+07 = 7,52$				$\bar{x} = 3,80E+07 = 7,58$			
$7,57 \leq \lg N \leq 8,10?$ Yes				\bar{x} of B $\geq 0,5 \times \bar{x}$ von N_W Yes				\bar{x} of C $\geq 0,5 \times \bar{x}$ von N_W Yes			
Water control (N_W)	N_W		Microbial count		V _{c1}	V _{c2}	\bar{x}	$\lg N_W$	$\lg N_W \geq \lg 6,2?$		
	1,00E-04	>330	>330	>330	>330	>330	4,15E+07	7,62	Yes		
	1,00E-05	38	45	38	45						
Product-concentration [%]	Dilution	Microbial count		V _{c1}	V _{c2}	N_a	$\lg N_a = \lg (\bar{x}$ oder $\bar{x}_{wm})$	$\lg R =$ ($\lg N_W = 7,62$)			
	0,1	1,00E+00	>330	>330	>330	>330	>3,30E+05	> 5,52	$\leq 2,10$		
		1,00E-01	>330	>330	>330	>330					
		1,00E-02	>330	>330	>330	>330					
	$N_{ts} =$	>330	$N_{ts} < 1?$	No	5 ≤ Quotient of $\bar{x}_{wm} \leq 15?$		Na				
0,5	1,00E+00	>330	>330	>330	>330	3,45E+04	4,54	3,08			
	1,00E-01	>330	>330	>330	>330						
	1,00E-02	34	35	34	35						
	$N_{ts} =$	>330	$N_{ts} < 1?$	No	5 ≤ Quotient of $\bar{x}_{wm} \leq 15?$		Na				
1,0	1,00E+00	10	12	<14	<14	<1,40E+02	< 2,15	$\geq 5,47$			
	1,00E-01	0	2	<14	<14						
	1,00E-02	0	0	<14	<14						
	$N_{ts} =$	0	$N_{ts} < 1?$	Yes	5 ≤ Quotient of $\bar{x}_{wm} \leq 15?$		Na				

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4 List of Abbreviations

cfu	=	colony forming units (viable microbial count)
N	=	test and validation suspension
N_w	=	\log_{10} cfu per test surface of water control
N_a	=	\log_{10} cfu per test surface of disinfection test
B	=	toxicity of neutraliser
C	=	neutralisation control
N_{ts}	=	remaining cfu on test surface
RF	=	germicidal activity ($\lg N_w - \lg N_a$)
Na	=	not applicable
n.t.	=	not tested
Σ	=	sum of V_c
V_c	=	viable microbial count per ml
\bar{x}	=	mean of V_c

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