DIPL.-BIOL. DR. RER. NAT. FLORIAN H. H. BRILL

C/O DR. BRILL + PARTNER GMBH
INSTITUT FÜR HYGIENE UND MIKROBIOLOGIE
STIEGSTÜCK 34, DE-22339 HAMBURG
TELEFON 0049-40/557631-0
TELEFAX 0049-40/557631-11
EMAIL INFO@BRILLHYGIENE.COM
INTERNET WWW.BRILLHYGIENE.COM

DR. F. H. H. BRILL · C/O DR. BRILL + PARTNER GMBH · STIEGSTÜCK 34 · DE-22339 HAMBURG

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

Hamburg, 27 January 2022

Expert opinion

Bactericidal Activity of **Bacoban WBUS** in the
quantitative suspension test according to DIN EN 1656:2019 (Phase 2, Step 1)

The disinfectant **Bacoban WBUS** was tested and evaluated according to DIN EN 1656:2019 "Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary area - Test method and requirements (phase 2, step 1)".

According to the test report no. L21/01041.5 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 1656:2019 (phase 2, step 1) with the following concentration-time relationship:

Bactericidal:	**clean conditions**	**0.5 %**	**30 minutes**

Dr. Florian H. H. Brill

Test report no L21/01041.5

Quantitative suspension test for the evaluation of bactericidal activity of **Bacoban WBUS** in the veterinary area (DIN EN 1656:2019; Phase 2, Step 1*)

In accordance with your order, we tested the preparation **Bacoban WBUS** for its activity in the quantitative suspension test according to DIN EN 1656:2019* under clean conditions.

1 General Information and Material

1.1 Client

Client:	ROPIMEX 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.-Ing. Dr. rer. nat. Andreas Kampe
Scientific assistant:	Dipl.-Biol. Henrik Gabriel
Laboratory technicians:	Elahe Saroukhani

1.3 Table of Contents

1.4 Identification of Sample

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

* Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Stiegstück 34, DE - 22339 Hamburg, Phone +49 40 557631-0, Telefax +49 40 557631-11, www.brillhygiene.com. No copying or transmission, in whole or in part, of this test report without the explicit prior written permission. The test results exclusively apply to the tested samples. Information on measurement uncertainty and Version history on request. © Dr. Brill + Partner GmbH 2022

DAkkS Deutsche Akkreditierungsstelle D-PL-13412-01-01

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):	6.2
pH value, 0.5 % (measured in WSH):	6.6
pH value, 0.1 % (measured in WSH):	7.0
Active agents (Manufacturer's data):	33.31 g benzalkoniumchloride

1.5 Test Conditions

Test period:	04/01/ - 07/01/2022	
Lab task no.:	L21/01041.5	
Product test concentrations:	0.1 + 0.5 + 1.0 %	
Exposure time:	30 minutes	
Test temperature:	10°C ± 1°C	
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:	*Staphylococcus aureus*	ATCC 6538
	Enterococcus hirae	ATCC 10541
	Proteus hauseri (vulgaris)	ATCC 13315
	Pseudomonas aeruginosa	ATCC 15442

* Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Stiegstück 34, DE - 22339 Hamburg, Phone +49 40 557631-0, Telefax +49 40 557631-11, www.brillhygiene.com. No copying or transmission, in whole or in part, of this test report without the explicit prior written permission. The test results exclusively apply to the tested samples. Information on measurement uncertainty and Version history on request. © Dr. Brill + Partner GmbH 2022

DAkkS Deutsche Akkreditierungsstelle D-PL-13412-01-01

2 Methods

The tests were carried out according to DIN EN 1656:2019 "Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary area - Test method and requirements (phase 2, step 1)".

3 Results

The test results based on DIN EN 1656:2019 are summarised in tables 1.

The test bacteria were sufficiently (RF >5) inactivated with the following concentration-time relationship:

Bactericidal:	clean conditions	0.5 %	30 minutes

Hamburg, 27/01/2022

i. V.

Dipl.-Ing. Dr. rer. nat. Andreas Kampe
Study Manager

Dipl.-Biol. Henrik Gabriel
Head of Laboratory

INSTITUTE FOR HYGIENE AND MICROBIOLOGY · DR. BRILL + DR. STEINMANN · CERTIFIED

* Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Stiegstück 34, DE - 22339 Hamburg, Phone +49 40 557631-0, Telefax +49 40 557631-11, www.brillhygiene.com. No copying or transmission, in whole or in part, of this test report without the explicit prior written permission. The test results exclusively apply to the tested samples. Information on measurement uncertainty and Version history on request. © Dr. Brill + Partner GmbH 2022

DAkkS Deutsche Akkreditierungsstelle D-PL-13412-01-01

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

Suspension for validation (N_{v0})				Control of test conditions (A)											
				5 minutes				30 minutes				n.t.			
Microbial count			$\bar{x}$	Microbial count			$\bar{x}$	Microbial count			$\bar{x}$	Microbial count			$\bar{x}$
V_{c1}	113		116,5	V_{c1}				V_{c1}	117		120,5	V_{c1}			
V_{c2}	120			V_{c2}				V_{c2}	124			V_{c2}			
$30 \leq \bar{x}$ of $N_{v0} \leq 160$			Yes	$\bar{x}$ of A(5') is $\geq 0,5 \times \bar{x}$ of N_{v0}?				$\bar{x}$ of A(30') is $\geq 0,5 \times \bar{x}$ of N_{v0}?			Yes	$\bar{x}$ of A(60') is $\geq 0,5 \times \bar{x}$ of N_{v0}?			
Control of neutraliser (B)				Validation of method (C) at highest product concentration: 1,0 %											
				5 minutes				30 minutes				n.t.			
Microbial count			$\bar{x}$	Microbial count			$\bar{x}$	Microbial count			$\bar{x}$	Microbial count			$\bar{x}$
V_{c1}	109		113	V_{c1}				V_{c1}	101		103	V_{c1}			
V_{c2}	117			V_{c2}				V_{c2}	105			V_{c2}			
$\bar{x}$ of B is $\geq 0,5 \times \bar{x}$ of N_{v0}?			Yes	$\bar{x}$ of C(5') is $\geq 0,5 \times \bar{x}$ of N_{v0}?				$\bar{x}$ of C(30') is $\geq 0,5 \times \bar{x}$ of N_{v0}?			Yes	$\bar{x}$ of C(60') is $\geq 0,5 \times \bar{x}$ of N_{v0}?			

Test suspension (N and N_0)	N	Microbial count of plates				V_{c1}	V_{c2}	$\bar{x}_{wm}$ / lg N	N_0=N/10; lg N_0	7,17 ≤ N0 ≤ 7,70 ?
	1,00E-06	>330		>330		>330	>330	4,40E+08	7,64	Yes
	1,00E-07	44		44		44	44	8,64		

Product concentration [%]	Exposure time [min]	Microbial count of plates				V_{c1}	V_{c2}	$N_a = \bar{x} \times 10$	lg N_a	lg R (lg N_0 =7,64)
0,10	30	0		0		<14	<14	<140	<2,15	≥ 5,49
0,50	30	0		0		<14	<14	<140	<2,15	≥ 5,49
1,00	30	0		0		<14	<14	<140	<2,15	≥ 5,49

* Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Stiegstück 34, DE - 22339 Hamburg, Phone +49 40 557631-0, Telefax +49 40 557631-11, www.brillhygiene.com. No copying or transmission, in whole or in part, of this test report without the explicit prior written permission. The test results exclusively apply to the tested samples. Information on measurement uncertainty and Version history on request. © Dr. Brill + Partner GmbH 2022

DAkkS Deutsche Akkreditierungsstelle D-PL-13412-01-01

Table 1.2: Validation, Controls and Evaluation

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

Suspension for validation (N_{v0})				Control of test conditions (A)											
				5 minutes				30 minutes				n.t.			
Microbial count			$\bar{x}$	Microbial count			$\bar{x}$	Microbial count			$\bar{x}$	Microbial count			$\bar{x}$
V_{c1}	84		86,5	V_{c1}				V_{c1}	80		81,5	V_{c1}			
V_{c2}	89			V_{c2}				V_{c2}	83			V_{c2}			
$30 \leq \bar{x}$ of $N_{v0} \leq 160$			Yes	$\bar{x}$ of A(5') is $\geq 0,5 \times \bar{x}$ of N_{v0}?				$\bar{x}$ of A(30') is $\geq 0,5 \times \bar{x}$ of N_{v0}?			Yes	$\bar{x}$ of A(60') is $\geq 0,5 \times \bar{x}$ of N_{v0}?			
Control of neutraliser (B)				Validation of method (C) at highest product concentration: 1,0 %											
				5 minutes				30 minutes				n.t.			
Microbial count			$\bar{x}$	Microbial count			$\bar{x}$	Microbial count			$\bar{x}$	Microbial count			$\bar{x}$
V_{c1}	79		88	V_{c1}				V_{c1}	77		79,5	V_{c1}			
V_{c2}	97			V_{c2}				V_{c2}	82			V_{c2}			
$\bar{x}$ of B is $\geq 0,5 \times \bar{x}$ of N_{v0}?			Yes	$\bar{x}$ of C(5') is $\geq 0,5 \times \bar{x}$ of N_{v0}?				$\bar{x}$ of C(30') is $\geq 0,5 \times \bar{x}$ of N_{v0}?			Yes	$\bar{x}$ of C(60') is $\geq 0,5 \times \bar{x}$ of N_{v0}?			

Test suspension (N and N_0)	N	Microbial count of plates				V_{c1}	V_{c2}	$\bar{x}_{wm}$ / lg N	N_0=N/10; lg N_0	7,17 ≤ N0 ≤ 7,70 ?
	1,00E-06	>330		>330		>330	>330	3,30E+08	7,52	Yes
	1,00E-07	29		37		29	37	8,52		

Product concentration [%]	Exposure time [min]	Microbial count of plates				V_{c1}	V_{c2}	$N_a = \bar{x} \times 10$	lg N_a	lg R (lg N_0 =7,52)
0,10	30	0		0		<14	<14	<140	<2,15	≥ 5,37
0,50	30	0		0		<14	<14	<140	<2,15	≥ 5,37
1,00	30	0		0		<14	<14	<140	<2,15	≥ 5,37

* Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Stiegstück 34, DE - 22339 Hamburg, Phone +49 40 557631-0, Telefax +49 40 557631-11, www.brillhygiene.com. No copying or transmission, in whole or in part, of this test report without the explicit prior written permission. The test results exclusively apply to the tested samples. Information on measurement uncertainty and Version history on request. © Dr. Brill + Partner GmbH 2022

DAkkS Deutsche Akkreditierungsstelle D-PL-13412-01-01

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

Suspension for validation (N_{v0})				Control of test conditions (**A**)											
				5 minutes				30 minutes				n.t.			
Microbial count			$\bar{x}$	Microbial count			$\bar{x}$	Microbial count			$\bar{x}$	Microbial count			$\bar{x}$
V_{c1}	103		110	V_{c1}				V_{c1}	84		92	V_{c1}			
V_{c2}	117			V_{c2}				V_{c2}	100			V_{c2}			
30 ≤ $\bar{x}$ of N_{v0} ≤ 160			**Yes**	$\bar{x}$ of A(5') is ≥ 0,5 x $\bar{x}$ of N_{v0}?				$\bar{x}$ of A(30') is ≥ 0,5 x $\bar{x}$ of N_{v0}?			**Yes**	$\bar{x}$ of A(60') is ≥ 0,5 x $\bar{x}$ of N_{v0}?			
Control of neutraliser (**B**)				Validation of method (C) at highest product concentration: 1,0 %											
				5 minutes				30 minutes				n.t.			
Microbial count			$\bar{x}$	Microbial count			$\bar{x}$	Microbial count			$\bar{x}$	Microbial count			$\bar{x}$
V_{c1}	95		95,5	V_{c1}				V_{c1}	89		93	V_{c1}			
V_{c2}	96			V_{c2}				V_{c2}	97			V_{c2}			
$\bar{x}$ of B is ≥ 0,5 x $\bar{x}$ of N_{v0}?			**Yes**	$\bar{x}$ of C(5') is ≥ 0,5 x $\bar{x}$ of N_{v0}?				$\bar{x}$ of C(30') is ≥ 0,5 x $\bar{x}$ of N_{v0}?			**Yes**	$\bar{x}$ of C(60') is ≥ 0,5 x $\bar{x}$ of N_{v0}?			

Test suspension (N and N_0)	N	Microbial count of plates				V_{c1}	V_{c2}	$\bar{x}_{wm}$ / lg N	N_0=N/10; lg N_0	7,17 ≤ N0 ≤ 7,70 ?
	1,00E-06	>330		>330		>330	>330	4,70E+08	7,67	**Yes**
	1,00E-07	46		48		46	48	8,67		

Product concentration [%]	Exposure time [min]	Microbial count of plates				V_{c1}	V_{c2}	$N_a = \bar{x}$ x 10	lg N_a	lg R (lg N_0 =7,67)
0,10	30	0		0		<14	<14	<140	<2,15	≥ 5,52
0,50	30	0		0		<14	<14	<140	<2,15	≥ 5,52
1,00	30	0		0		<14	<14	<140	<2,15	≥ 5,52

* Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Stiegstück 34, DE - 22339 Hamburg, Phone +49 40 557631-0, Telefax +49 40 557631-11, www.brillhygiene.com. No copying or transmission, in whole or in part, of this test report without the explicit prior written permission. The test results exclusively apply to the tested samples. Information on measurement uncertainty and Version history on request. © Dr. Brill + Partner GmbH 2022

DAkkS Deutsche Akkreditierungsstelle D-PL-13412-01-01

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

Suspension for validation (N_{v0})				Control of test conditions (**A**)											
				5 minutes				30 minutes				n.t.			
Microbial count			$\bar{x}$	Microbial count			$\bar{x}$	Microbial count			$\bar{x}$	Microbial count			$\bar{x}$
V_{c1}	85		85	V_{c1}				V_{c1}	83		87,5	V_{c1}			
V_{c2}	85			V_{c2}				V_{c2}	92			V_{c2}			
$30 \leq \bar{x}$ of $N_{v0} \leq 160$			**Yes**	$\bar{x}$ of A(5') is $\geq 0{,}5 \times \bar{x}$ of N_{v0}?				$\bar{x}$ of A(30') is $\geq 0{,}5 \times \bar{x}$ of N_{v0}?			**Yes**	$\bar{x}$ of A(60') is $\geq 0{,}5 \times \bar{x}$ of N_{v0}?			
Control of neutraliser (**B**)				Validation of method (C) at highest product concentration: 1,0 %											
				5 minutes				30 minutes				n.t.			
Microbial count			$\bar{x}$	Microbial count			$\bar{x}$	Microbial count			$\bar{x}$	Microbial count			$\bar{x}$
V_{c1}	84		89,5	V_{c1}				V_{c1}	92		92,5	V_{c1}			
V_{c2}	95			V_{c2}				V_{c2}	93			V_{c2}			
$\bar{x}$ of B is $\geq 0{,}5 \times \bar{x}$ of N_{v0}?			**Yes**	$\bar{x}$ of C(5') is $\geq 0{,}5 \times \bar{x}$ of N_{v0}?				$\bar{x}$ of C(30') is $\geq 0{,}5 \times \bar{x}$ of N_{v0}?			**Yes**	$\bar{x}$ of C(60') is $\geq 0{,}5 \times \bar{x}$ of N_{v0}?			

Test suspension (N and N_0)	N	Microbial count of plates				V_{c1}	V_{c2}	$\bar{x}_{wm}$ / lg N	N_0=N/10; lg N_0	7,17 ≤ N0 ≤ 7,70 ?
	1,00E-06	>330		>330		>330	>330	4,60E+08	7,66	**Yes**
	1,00E-07	43		49		43	49	8,66		

Product concentration [%]	Exposure time [min]	Microbial count of plates				V_{c1}	V_{c2}	$N_a = \bar{x} \times 10$	lg N_a	lg R (lg N_0 =7,66)
0,10	30	>330		>330		>330	>330	>3300	>3,52	≤ 4,14
0,50	30	20		21		20	21	205	2,31	5,35
1,00	30	0		0		<14	<14	<140	<2,15	≥ 5,51

* Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Stiegstück 34, DE - 22339 Hamburg, Phone +49 40 557631-0, Telefax +49 40 557631-11, www.brillhygiene.com. No copying or transmission, in whole or in part, of this test report without the explicit prior written permission. The test results exclusively apply to the tested samples. Information on measurement uncertainty and Version history on request. © Dr. Brill + Partner GmbH 2022

DAkkS Deutsche Akkreditierungsstelle D-PL-13412-01-01

4 List of Abbreviations

A	=	control of test conditions
B	=	control of neutraliser
C	=	validation of method at highest product concentration
N	=	test suspension
N_{V0}	=	suspension for validation
n.t.	=	not tested
N_0	=	microbial count of test suspension N / 10 (microbial count at time index 0)
R	=	germ reduction in $\log_{10}$-steps
V_C	=	viable microbial count per ml
$\bar{x}$	=	weighted mean of N

* Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Stiegstück 34, DE - 22339 Hamburg, Phone +49 40 557631-0, Telefax +49 40 557631-11, www.brillhygiene.com. No copying or transmission, in whole or in part, of this test report without the explicit prior written permission. The test results exclusively apply to the tested samples. Information on measurement uncertainty and Version history on request. © Dr. Brill + Partner GmbH 2022

DAkkS Deutsche Akkreditierungsstelle D-PL-13412-01-01