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

Hamburg, 16 November 2021

Expert opinion

Yeasticidal Activity of **Bacoban WBUS** in the
quantitative surface test according to DIN EN 16438:2014 (Phase 2, Step 2)

The disinfectant **Bacoban WBUS** was tested and evaluated according to DIN EN 16438:2014 „ Chemical disinfectants and antiseptics – Quantitative surface test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in the veterinary area on non-porous surfaces without mechanical action – Test method and requirements (phase 2, step 2)".

According to the test report no. L21/01041.3 dated 16/11/2021 of Dr. Brill + Partner GmbH the preparation showed yeasticidal activity under clean conditions at a test temperature of 10°C ± 1°C.

Bacoban WBUS complies with the requirements of DIN EN 16438:2014 (phase 2, step 2) with the following concentration-time relationship:

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

Dr. Florian H. H. Brill

Test report no L21/01041.3

Quantitative surface test for the evaluation of fungicidal or yeasticidal activity of **Bacoban WBUS** in veterinary area according to DIN EN 16438:2014 (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 16438:2014 * under clean conditions.

1 General Information and Material

1.1 Client

Client:	ROPIMEX R. OPEL GmbH, Frau Jennifer Sahl, Bildstocker Straße 12, DE - 66538 Neunkirchen, Germany
Date of order:	24/09/2021
Confirmation no.:	225183

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. Dr. rer. nat. Jan-Hendrik Klock
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

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):	6.4
pH value, 0.5 % (measured in WSH):	6.7
pH value, 0.1 % (measured in WSH):	7.0
Active agents (Manufacturer's data):	33.31 g benzalkoniumchloride

1.5 Test Conditions

Test period:	27/10/ - 01/11/2021
Lab task no.:	L21/01041.3
Product test concentrations:	0.1 + 0.5 + 1.0 %
Exposure time:	30 minutes
Germ carrier:	stainless steel disc
Test temperature:	10°C ± 1°C
Incubation temperature:	30°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 histidine, 2.5 g/L SDS (TLSH-SDS)
Test organisms:	*Candida albicans* ATCC 10231

2 Methods

The tests were carried out according to DIN EN 16438:2014 " Chemical disinfectants and antiseptics – Quantitative surface test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in the veterinary area on non-porous surfaces without mechanical action – Test method and requirements (phase 2, step 2); German version EN 16438:2014".

3 Results

The test results based on DIN EN 16438:2014 are summarised in table 1.

The test yeast *C. albicans* was sufficiently (RF >3) inactivated with the following concentration-time relationship:

Yeasticidal:	clean conditions	0.5 %	30 minutes

Hamburg, 16/11/2021

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 · HAMBURG

Table 1: Validation, Controls and Evaluation (DIN EN 16438:2014*)

Product name:	**Bacoban WBUS**	Batch:	20210920_Bacoban WBUS_imi
Test organism:	*Candida albicans*	Temperature:	10°C ± 1°C
Organic load:	clean conditions	Neutraliser:	TLSH-SDS
Contact time:	**30 minutes**	Germ carrier:	stainless steel disc

Test and validation suspension (N)			**Toxicity control (B)**			**Validation neutralisation medium / Neutralisation control (C)** **Product**		**1,00 %**
	V_{c1}	V_{c2}		V_{c1}	V_{c2}		V_{c1}	V_{c2}
1,00E-06	>330	>330	1,00E-03	>330	>330	1,00E-03	>330	>330
1,00E-07	27	28	1,00E-04	35	42	1,00E-04	40	45
$\bar{x}$ =	6,88E+06 =	6,84 lg	$\bar{x}$ =	3,85E+06 =	6,59 lg	$\bar{x}$ =	4,25E+06 =	6,63 lg
6,57 ≤ lg *N* ≤ 7,1 ?		**Yes**	B > 0,5 x N_W ?		**Yes**	C > 0,5 x N_W ?		**Yes**

Water control

Water control (N_W):	N_W	V_{c1}	V_{c2}	$\bar{x}$ = 4,00E+06
	1,00E-03	>330	>330	lgN_W = **6,60**
	1,00E-04	39	41	$lgN_W \geq 5,27$ log ? **Yes**

Test

Concentration of product test solution [%]	Dilution step	V_{c1}	V_{c2}	**N_a** ($\bar{x}$ v $\bar{x}$wm) cfu/ml	**lg N_a** lg ($\bar{x}$ *v* $\bar{x}$*wm*)	**R** (lgN_W =6,60) lgN_W- lgN_a	Exposure time (min)
0,10	1,00E+00	>330	>330	> 3,30E+05	> 5,52	≤ 1,08	30
	1,00E-01	>330	>330				
	1,00E-02	>330	>330				
0,50	1,00E+00	6	10	< 1,40E+02	< 2,15	≥ 4,45	30
	1,00E-01	0	0				
	1,00E-02	0	0				
1,00	1,00E+00	0	0	< 1,40E+02	< 2,15	≥ 4,45	30
	1,00E-01	0	0				
	1,00E-02	0	0				

4 List of Abbreviations

$\bar{x}$	=	mean of a and a' and x and x'
B	=	control of toxicity of neutraliser
C	=	neutralisation control
cfu	=	colony forming units (viable microbial count)
N	=	test suspension
n.t.	=	not tested
N_a	=	$\log_{10}$ cfu per test surface of disinfection test
N_W	=	$\log_{10}$ cfu per test surface of water control
R	=	germicidal activity ($N_W - N_a$)
Vc	=	viable microbial count per ml of the test suspension