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17489 GREIFSWALD
DEUTSCHLAND - GERMANY

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Ropimex R. Opel GmbH

Bildstocker Straße 12 - 14

66538 Neunkirchen

Deutschland / Germany

CUSTOMER NUMBER	DATE
1155	May 08, 2023

REPORT 230790.VI

BACOBAN DL

YEASTICIDAL ACTIVITY AGAINST *CANDIDA AURIS*

EN 13624 (2021)

Purpose

The yeasticidal activity of the surface disinfectant **Bacoban DL** (Ropimex R. Opel GmbH, Neunkirchen, Germany) should be evaluated in accordance with the European Standard **EN 13624 (2021)** against the testorganism *C. auris*.

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Test description

Order number:	A23-0438
Manufacturer:	Ropimex R. Opel GmbH, Neunkirchen, Germany
Product:	**Bacoban DL**
Batch number:	2202037
Date of manufacture:	not provided
Best before:	02 / 2025
Sample number:	P 232071
Storage condition:	room temperature
Diluent:	water for injections
Date of order:	March 29, 2023
Date of delivery:	March 29, 2023
Test date:	April 13, 2023 – May 02, 2023
Basis:	**EN 13624 (2021: Version08/2022)**: Chemical disinfectants and antiseptics – quantitative suspension test for the evaluation of fungicidal or yeasticidal in the medical area – test method and requirements (phase 2, step 1)
Test organisms:	*Candida auris* DSM 21092
Test solutions:	80 %, 50 %, 25 %, 10 %
Active ingredients [1]:	0.26 g Benzalkoniumchloride CAS Nr. 68424-85-1 (Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC/BKC (C12-C16)) 0.025 g Natriumpyrithion CAS Nr. 3811-73-2 (Pyridine-2-thiol 1-oxide, sodium salt (Sodium pyrithione))
Odour:	product specific
Appearance of powder:	clear, colourless liquid
Appearance of dilutions:	clear, colourless liquids
pH – value (pH-meter):	100 %: 5.97 80 %: 5.94 50 %: 5.95 25 %: 6.02 10 %: 5.92 WFI: 8.17
Neutralizer:	4 % Tween 80 + 3 % Saponin + 0.4 % Lecithin + 0.25 % SDS (Neutralizer XXIV)
Contact times:	1 min
Interfering substance:	0.3 % albumin + 0.3 % sheep erythrocytes (dirty conditions)
Test temperature:	20 ± 1 °C
Incubation temperature:	30 ± 1 °C

Test Method

Testing is based on the European Standard **EN 13624 (2021)**. Validation and control procedures are therefore carried out in accordance with this standard, too.

For the test, to a sample of the product **Bacoban DL** (diluted with water for injections) is added to a suspension of test organisms in a solution of the interfering substance. The mixture is maintained at 20±1 °C for the required contact time. At the end of the contact time, an aliquot of 1 ml is taken; the microbicidal activity in this portion is immediately neutralized. Two 1 ml samples (per dilution step) of the resulting suspension are spread on at least 2 plates each. The number of surviving test organisms in the test mixture is calculated for each sample and the reduction is determined with respect to the corresponding test suspension N_0.

The experimental conditions (control A), the non-toxicity of the neutralizer (control B) and the dilution-neutralization method (control C) are validated in accordance with the EN 13624. The test is performed under dirty conditions (0.3 % albumin + 0.3 % sheep erythrocytes) using *C. auris* as test-organism. Results are presented in table 1 - 2.

Results and conclusion[2]

In accordance with the **EN 13624 (2021)**, the batch 2202037 of the test product **Bacoban DL**, when applied at the concentration / contact time - relation of at least **25 % / 1 min** at 20±1 °C under dirty conditions (0.3 % albumin + 0.3 % sheep erythrocytes), **possesses** yeasticidal efficacy ($\log_{10}$ RF $\geq$ 4) for the reference strain *C. auris* (Tab. 1 - 2).

Results are considered validated in accordance with the requirements of the **EN 13624 (2021).**

Greifswald, May 08, 2023

AKKREDITIERTES PRÜFLABOR HYGIENE NORD GMBH W.-RATHENAU-STR. 49A 17489 GREIFSWALD NACH DIN EN ISO/IEC 17025 D-PL-18411-01

Dr. rer med. (Dipl. Biol.) T. Koburger-Janssen
- General Manager -

Prof. Dr. med. A. Kramer
- MD for Hygiene and Environmental Medicine -

Table 1: Results of the quantitative suspension test according to EN 13624 (2021)

Date:	April 17, 2023	**Order number:**	A23-0438
Product:	**Bacoban DL**	**Sample number:**	P 232071
Test organism:	*C. auris*	**Batch number:**	2202037
Interfering substance:	0.3 % albumin + 0.3 % sheep erythrocytes		
Incubation temperature:	30 ± 1 °C	**Neutralizer:**	**XXIV**
Test suspension (N_0):	$5.10*10^6$ cfu/ml (6.71 log) *	**Incubation time:**	48 h – 72 h
Validation Suspension (N_V):	$1.40*10^3$ cfu/ml (3.15 log)	**Test temperature:**	20 ± 1 °C
Validation Suspension (N_{VB}):	$1.52*10^5$ cfu/ml (5.18 log)		

* slightly exceeding limits ; but Ok with regard to efficacy being achieved

contact time: 1 min									
concentration	**dilution**	**cfu / plate 1**	**cfu / plate 2**	**cfu / plate 3**	**cfu / plate 4**	**V_{c1}**	**V_{c2}**	**$\log_{10}$ Na**	**$\log_{10}$ R**
80 %	1 ml (10^0)	0	0	0	0	< 14	< 14	< 2.15	**> 4.56**
	1 ml (10^{-1})	0	0	0	0	< 14	< 14		
50 %	1 ml (10^0)	0	0	0	0	< 14	< 14	< 2.15	**> 4.56**
	1 ml (10^{-1})	0	0	0	0	< 14	< 14		
25 %	1 ml (10^0)	0	0	0	0	< 14	< 14	< 2.15	**> 4.56**
	1 ml (10^{-1})	0	0	0	0	< 14	< 14		
10 %	1 ml (10^0)	11	11	10	12	22	22	2.34	**4.37**
	1 ml (10^{-1})	4	1	2	1	< 14	< 14		

Controls and validation:

Validation - Suspension (N_{VO})					Experimental condition control (A)					Neutralizer control (B)					Method validation (C) Product concentration: 80 %				
	cfu /plate 1 & 2		V_c	$\bar{x}$		cfu /plate 1 & 2		V_c	$\bar{x}$		cfu /plate 1 & 2		V_c	$\bar{x}$		cfu /plate 1 & 2		V_c	$\bar{x}$
V_{c1}	74	65	139	**140**	V_{c1}	84	64	148	**150**	V_{c1}	77	62	139	**143.5**	V_{c1}	0	0	0	**0**
V_{c2}	69	72	141		V_{c2}	75	77	152		V_{c2}	73	75	148		V_{c2}	0	0	0	
$30 \leq \bar{x}$ of $N_{VO} \leq 160$?					$\bar{x}$ of A is ≥ 0.5 $\bar{x}$ of N_{VO}?					$\bar{x}$ of B is ≥ 0.0005 $\bar{x}$ of N_{VB}?					$\bar{x}$ of C is ≥ 0.5 $\bar{x}$ of N_{VO}?				
X yes			no		X yes			no		X yes			no		yes			X no **	

** results below limits; suggesting insufficient neutralization; test was repeat once again using membrane filtration for neutralization - see table 2

Method validation (C) Product concentration: 50 %				
	cfu /plate 1 & 2		V_c	$\bar{x}$
V_{c1}	0	0	0	**0**
V_{c2}	0	0	0	
$\bar{x}$ of C is ≥ 0.5 $\bar{x}$ of N_{VO}?				
yes			X no **	

Table 2: Results of the quantitative suspension test according to EN 13624 (2021)

Date:	May 02, 2023	**Order number:**	A23-0438
Product:	**Bacoban DL**	**Sample number:**	P 232071
Test organism:	*C. auris*	**Batch number:**	2202037
Interfering substance:	0.3 % albumin + 0.3 % sheep erythrocytes		
Incubation temperature:	30 ± 1 °C	**Neutralizer:**	**membrane filtration**
Test suspension (N_0):	$5.75*10^6$ cfu/ml (6.76 log) *	**Incubation time:**	48 h – 72 h
Validation Suspension (N_V):	$1.52*10^3$ cfu/ml (3.18 log)	**Test temperature:**	20 ± 1 °C

* slightly exceeding limits ; but Ok with regard to efficacy being achieved

contact time: 1 min

concentration	dilution	cfu / plate 1	cfu / plate 2	V_{c1}	V_{c2}	$\log_{10}$ Nd	$\log_{10}$ R
80 %	1 ml (10^0)	0	0	< 14	< 14	< 1.15	**> 5.61**
	1 ml (10^{-1})	0	0	< 14	< 14		
	1 ml (10^{-2})	0	0	< 14	< 14		
50 %	1 ml (10^0)	0	0	< 14	< 14	< 1.15	**> 5.61**
	1 ml (10^{-1})	0	0	< 14	< 14		
	1 ml (10^{-2})	0	0	< 14	< 14		
25 %	1 ml (10^0)	7	6	< 14	< 14	< 1.15	**> 5.61**
	1 ml (10^{-1})	1	0	< 14	< 14		
	1 ml (10^{-2})	0	0	< 14	< 14		
10 %	1 ml (10^0)	> 165	> 165	> 165	> 165		
	1 ml (10^{-1})	> 165	> 165	> 165	> 165		
	1 ml (10^{-2})	> 165	> 165	> 165	> 165	> 4.22	**< 2.54**
water-control 0 %	1 ml (10^{-3})	> 165	> 165	> 165	> 165		
	1 ml (10^{-4})	> 165	> 165	> 165	> 165		
	1 ml (10^{-5})	58	51	58	51	6.74	

Table 2, continued: Results of the quantitative suspension test according to EN 13624 (2021)

Date:	May 02, 2023	**Order number:**	A23-0438
Product:	**Bacoban DL**	**Sample number:**	P 232071
Test organism:	*C. auris*	**Batch number:**	2202037
Interfering substance:	0.3 % albumin + 0.3 % sheep erythrocytes		
Incubation temperature:	30 ± 1 °C	**Neutralizer:**	**membrane filtration**
Test suspension (N_0):	$5.75*10^6$ cfu/ml (6.76 log) *	**Incubation time:**	48 h – 72 h
Validation Suspension (N_V):	$1.52*10^3$ cfu/ml (3.18 log)	**Test temperature:**	20 ± 1 °C

* slightly exceeding limits ; but Ok with regard to efficacy being achieved

Controls and validation:

Validation - Suspension (N_{VO})		
	cfu / plate	$\bar{x}$
V_{c1}	> 165	**152**
V_{c2}	139	
$30 \leq \bar{x}$ of $N_{VO} \leq 160$?		
[X] yes	[ ] no	

Experimental condition control (A)		
	cfu / plate	$\bar{x}$
V_{c1}	> 165	**149.5**
V_{c2}	134	
$\bar{x}$ of C is $\geq 0.05 * \bar{x}$ of N_V?		
[X] yes	[ ] no	

Neutralizer control (B)		
	cfu / plate	$\bar{x}$
V_{c1}	159	**149.5**
V_{c2}	140	
$\bar{x}$ of C is $\geq 0.05 * \bar{x}$ of N_V?		
[X] yes	[ ] no	

Method validation (C); Product concentration: 80 %		
	cfu / plate	$\bar{x}$
V_{c1}	127	**115.5**
V_{c2}	104	
$\bar{x}$ of C is $\geq 0.05 * \bar{x}$ of N_V?		
[X] yes	[ ] No	

Method validation (C); Product concentration: 50 %		
	cfu / plate	$\bar{x}$
V_{c1}	120	**114**
V_{c2}	108	
$\bar{x}$ of C is $\geq 0.05 * \bar{x}$ of N_V?		
[X] yes	[ ] No	

Method validation (C); Product concentration: 25 %		
	cfu / plate	$\bar{x}$
V_{c1}	145	**146.5**
V_{c2}	148	
$\bar{x}$ of C is $\geq 0.05 * \bar{x}$ of N_V?		
[X] yes	[ ] no	

Method validation (C); Product concentration: 10 %		
	cfu / plate	$\bar{x}$
V_{c1}	153	**155.5**
V_{c2}	158	
$\bar{x}$ of C is $\geq 0.05 * \bar{x}$ of N_V?		
[X] yes	[ ] no	

Legend:

[1]	=	as provided by the sponsor / manufacturer (unless stated otherwise)
[2]	=	According to EN 17025, § 7.8.2.1 l, we are required to state that the results presented in this report relate to the item(s) tested only. That is quite obvious in the first place, anyway. And it is also ridiculous, of course, with regard to these tests and reports typically being used for a product's generalized efficacy evaluation and market authorization. Which, as such, is then fully acceptable by all other relevant authorizing and responsible parties (other than EN 17025), too. Which therefore is why this disclaimer is only to be found at the very back end of this report.
$\bar{x}$	=	average value
RF	=	reduction factor
> 330	=	not countable
> 660	=	not countable
n.a.	=	not applicable
n.d.	=	not determined
n.p.	=	not provided
WFI	=	water for injections
WSH	=	water of standardized hardness