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

Hamburg, 15 June 2022

Expert opinion

Activity of **Bacoban DL impregnated cotton lobs** in the test for absorbent surfaces according to DIN EN ISO 20743:2021.

Bacoban DL impregnated cotton lobs were tested and evaluated according to DIN EN ISO 20743:2021-10 "Determination of antibacterial activity of textile products" (German version EN ISO 20743:2021). For evaluation the absorption method (Chapter 8.1; an evaluation method in which the test bacterial suspension is inoculated directly onto specimens) was selected. *Staphylococcus aureus* and *Klebsiella pneumoniae* were used as test bacteria.

Cubic shaped samples with an edge length of 20 mm weighing 0.04 g were prepared and dipped for 30 seconds in 100 ml of **Bacoban DL**. After a short drain period to remove excess fluid, the soaked lobs were incubated for 10 days at room temperature in the dark. Weight changes were documented for all steps. The tests were carried out in triplicates. Reduction values were calculated against untreated control sample **Testgewebe 19101**.

According to the test report no. L21/00863.2 dated 15/06/2022 of Dr. Brill + Partner GmbH **Bacoban DL impregnated cotton lobs** complies with the requirements of DIN EN ISO 20743:2021 (appendix F) for strong antibacterial properties (antimicrobial activity A ≥ 3 log steps) with an exposure time of 24 hours at 20°C ± 1°C test temperature.

Dr. Florian H. H. Brill

Test report no L21/00863.2

Determination of antibacterial activity of textile products (DIN EN ISO 20743:2021)*

In accordance with your order, we tested **Bacoban DL impregnated cotton lobs** for their activity in the test for absorbent surfaces according to DIN EN ISO 20743:2021.

1 General Information and Material

1.1 Client

Client:	ROPIMEX R. OPEL GmbH, Bildstocker Straße 12, DE-66538 Neunkirchen
Date of order:	25/01/2022, 04/04/2022
Confirmation no.:	226764, 227794

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. Henrik Gabriel
Scientific assistant:	Dipl.-Ing. Dr. rer. nat. Andreas Kampe
Laboratory technicians:	Carmela Jänicke

1.3 Table of Contents

1.4 Identification of Sample Series

Name of series:	**Bacoban DL impregnated cotton lobs**
Dates of delivery:	21/07/2021
Storage conditions:	room temperature and darkness

Product type: Surface disinfectant

1.5 Identification of samples:

Designation of sample	Batch No.	Internal No.	Active Agents
Bacoban DL	2006051	21/00957	No data
Testgewebe 19101 according to ISO 105 F02	10101.091-242	21/00933	Untreated
Testgewebe 19101 soaked with Bacoban DL	22112021	21/00933	Treated

1.6 Test Conditions

Test period:	14/03/ - 28/03/2022, 23/05/ - 07/06/2022	
Lab task no.:	L21/00863.2+.3	
Exposure time:	24 hours	
Test temperature:	20°C ± 1°C	
Incubation temperature:	36°C ± 1°C	
Neutralizer:	SCDLP-broth	
Test organisms:	*Staphylococcus aureus*	ATCC 6538
	Klebsiella pneumoniae	ATCC 4352

2 Methods

The tests were carried out according to DIN EN ISO 20743:2021-10 "Determination of antibacterial activity of textile products" (German version EN ISO 20743:2021). For evaluation the absorption method (Chapter 8.1; an evaluation method in which the test bacterial suspension is inoculated directly onto specimens) was selected.

Cubic shaped samples with an edge length of 20 mm weighing 0.04 g were prepared and dipped for 30 seconds in 100 ml of **Bacoban DL**. After a short drain period to remove excess fluid, the soaked lobs were incubated for 10 days at room temperature in the dark. Weight changes were documented for all steps. The tests were carried out in triplicates.

Reduction values were calculated against untreated control sample **Testgewebe 19101**.

3 Results

3.1 Determination of cotton lobs weight at different test times test period 1 (*Staphylococcus. aureus*)

Test conditions		1	2	3	4	5	6	7	8	9	10	Mean	Std. Dev.
Dry lobs	-	0,044	0,043	0,040	0,043	0,041	0,042	0,041	0,042	0,043	0,042	0,042 g	0,001 g
Soaked lobs	0 h	0,129	0,141	0,143	0,122	0,119	0,128	0,140	0,144	0,125	0,124	0,131 g	0,009 g
	24 h	0,045	0,043	0,043	0,043	0,041	0,043	0,042	0,042	0,044	0,043	0,043 g	0,001 g

The following weight differences were determined:

1. Weight gain of dry cotton lobs directly after soaking: 0,089 g
2. Weight loss of freshly soaked cotton lobs 24 h after soaking: 0,088 g
3. Weight change between dry lobs and 24 h: 0,001 g

3.2 Determination of cotton lobs weight at different test times test period 2 (*Klebsiella pneumoniae*)

Test conditions		1	2	3	4	5	6	7	8	9	10	Mean	Std. Dev.
Dry lobs	-	0,045	0,044	0,045	0,045	0,045	0,046	0,046	0,046	0,044	0,046	0.045	0,001
Soaked lobs	0 h	0,151	0,149	0,136	0,136	0,127	0,115	0,114	0,130	0,122	0,125	0,131	0,013
	24 h	0,051	0,059	0,055	0,060	0,049	0,051	0,051	0,047	0,049	0,045	0,052	0,005

The following weight differences were determined:

4. Weight gain of dry cotton lobs directly after soaking: 0,086 g
5. Weight loss of freshly soaked cotton lobs 24 h after soaking: 0,079 g
6. Weight change between dry lobs and 24 h: 0,007 g

3.3 Determination of antibacterial activity of textile products

The following criteria must have been met for valid testing:

1. Initial cell count was between 1.0E+05 and 3.0E+05 CFU/ml.
2. The logarithmic value of the number of viable bacteria recovered immediately after inoculation and after 24 h from the untreated test specimens shall satisfy the following requirement: Lmax – Lmin < 1.0
3. The difference between log of arithmetic mean of colony count after 24 h and immediately after inoculation shall be equal to 1.0 or greater.

The test results based on DIN EN ISO 20743:2021 are shown in tables and summarised below (for abbreviations please see last page, results with failed validation tests are marked red).

Material	Test organism	Contact Time				Reduction		
		C_0	C_t	T_0	T_t	F	G	A
		0 h	24 h	0 h	24 h	C_t-C_0/T_0	T_t - T_0	
		[log CFU / test piece]				log	log	log
Testgewebe 19101 soaked with Bacoban DL	*S. aureus*	6.20	8.29	2.60	2.60	2.08	0.00	**5.68**
	K. pneumoniae	6.17	8.66	3.71	2.60	2.49	-1.10	**6.06**

According to appendix F of DIN EN ISO 20743:2021 an antimicrobial activity A ≥ 3 log steps indicates strong antibacterial properties of the test pieces.

Hamburg, 15/06/2022

Dipl.-Biol. Henrik Gabriel
Study Manager

Dipl.-Biol. Dr. rer. nat. Jan-Hendrik Klock
Deputy Head of Laboratory

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Table 1: Validation, Controls and Evaluation

Product name: **Bacoban DL** Batch: 2006051
Test organism: *Staphylococcus aureus* Lab task no.: L21/00863.1
Test product: **Testgewebe 19101 soaked with Bacoban DL** Untreated control: **Testgewebe 19101**

Inital cell count and test inoculum	Dilution	Microbial count				$\bar{x}$	$\bar{x}$ ICC / TI	lg ICC / TI	5.00 > ICC < 5,48
	1,00E-03	>330		>330		>330	2,90E+05	5,46	**Yes**
	1,00E-04	22		36		29	1,45E+04	4,16	

Test	Dilution	Cell Count		$\bar{x}$	C_0	Cell Count		$\bar{x}$	C_0	Cell Count		$\bar{x}$	C_0
1	1,00E+00	>330	>330	>330	1,80E+06	>330	>330	>330	1,69E+06	>330	>330	>330	1,27E+06
2	1,00E-01	>330	>330	>330		>330	>330	>330		>330	>330	>330	
3	1,00E-02	28	42	35		27	52	40		36	31	34	
	1,00E-03	6	5	6		5	4	5		2	4	3	

Test	Dilution	Cell Count		$\bar{x}$	C_t	Cell Count		$\bar{x}$	C_t	Count U_0		$\bar{x}$	C_t
1	1,00E-02	>330	>330	>330	9,88E+07	>330	>330	>330	2,91E+08	>330	>330	>330	1,89E+08
2	1,00E-03	248	270	259		>330	>330	>330		>330	>330	>330	
3	1,00E-04	21	26	24		58	63	61		56	33	45	
	1,00E-05	3	6	5		6	11	9		4	6	5	

Test	Dilution	Cell Count		$\bar{x}$	T_0	Cell Count		$\bar{x}$	T_0	Count T_0		$\bar{x}$	T_0
1	1,00E+00	0	0	0	≤4,00E+02	0	0	0	≤4,00E+02	0	0	0	≤4,00E+02
2	1,00E-01	0	0	0		0	0	0		0	0	0	
3	1,00E-02	0	0	0		0	0	0		0	0	0	
	1,00E-03	0	0	0		0	0	0		0	0	0	

Test	Dilution	Cell Count		$\bar{x}$	T_t	Cell Count		$\bar{x}$	T_t	Count T_t		$\bar{x}$	T_t
1	1,00E+00	0	0	0	≤4,00E+02	0	0	0	≤4,00E+02	0	0	0	≤4,00E+02
2	1,00E-01	0	0	0		0	0	0		0	0	0	
3	1,00E-02	0	0	0		0	0	0		0	0	0	
	1,00E-03	0	0	0		0	0	0		0	0	0	

Mean C_0	log mean C_0	L_{max}-L_{min} < 1,0 ?
1,59E+06	6,20	0,15 **Yes**

Mean C_t	log mean C_t	L_{max}-L_{min} < 1,0 ?	F=logC_t - logC_0	F ≥ 1,0 ?
1,93E+08	8,29	0,47 **Yes**	2,08	**Yes**

Mean T_0	log mean T_0	L_{max}-L_{min} < 2,0 ?
4,00E+02	2,60	0,00 **Yes**

Mean T_t	log mean T_t	L_{max}-L_{min} < 2,0 ?	G=logT_t - logT_0	R = (Ct-T0) - (Tt-T0) = F - G
4,00E+02	2,60	0,00 **Yes**	0,00	5,68

Table 2: Validation, Controls and Evaluation

Product name: **Bacoban DL** Batch: 2006051
Test organism: *Klebsiella pneumoniae* Lab task no.: L21/00863.3
Test product: **Testgewebe 19101 soaked with Bacoban DL** Untreated control: **Testgewebe 19101**

Inital cell count and test inoculum	Dilution	Microbial count				$\bar{x}$	$\bar{x}$ ICC / TI	lg ICC / TI	5.00 > ICC < 5,48
	1,00E-03	>330		>330		>330	2,85E+05	5,45	**Yes**
	1,00E-04	33		24		29	1,43E+04	4,15	

Test	Dilution	Cell Count		$\bar{x}$	C_0	Cell Count		$\bar{x}$	C_0	Cell Count		$\bar{x}$	C_0
1	1,00E-01	284	230	257	1,38E+06	304	334	319	1,43E+06	>330	>330	>330	1,62E+06
2	1,00E-02	43	44	44		29	50	40		35	47	41	
3	1,00E-03	2	5	4		2	3	3		3	5	4	
	1,00E-04	0	0	0		0	0	0		0	0	0	

Test	Dilution	Cell Count		$\bar{x}$	C_t	Cell Count		$\bar{x}$	C_t	Count U_0		$\bar{x}$	C_t
1	1,00E-02	>330	>330	>330	6,10E+08	>330	>330	>330	3,16E+08	>330	>330	>330	4,45E+08
2	1,00E-03	>330	>330	>330		>330	>330	>330		>330	>330	>330	
3	1,00E-04	128	112	120		94	22	58		76	79	78	
	1,00E-05	23	14	19		9	11	10		19	10	15	

Test	Dilution	Cell Count		$\bar{x}$	T_0	Cell Count		$\bar{x}$	T_0	Count T_0		$\bar{x}$	T_0
1	1,00E-01	41	42	42	9,15E+03	18	22	20	4,50E+03	4	8	6	1,60E+03
2	1,00E-02	4	6	5		2	3	3		1	1	1	
3	1,00E-03	0	0	0		0	0	0		0	0	0	
	1,00E-04	0	0	0		0	0	0		0	0	0	

Test	Dilution	Cell Count		$\bar{x}$	T_t	Cell Count		$\bar{x}$	T_t	Count T_t		$\bar{x}$	T_t
1	1,00E+00	0	0	0	≤4,00E+02	0	0	0	≤4,00E+02	0	0	0	≤4,00E+02
2	1,00E-01	0	0	0		0	0	0		0	0	0	
3	1,00E-02	0	0	0		0	0	0		0	0	0	
	1,00E-03	0	0	0		0	0	0		0	0	0	

Mean C_0	log mean C_0	L_{max}-L_{min} < 1,0 ?
1,48E+06	6,17	0,07 **Yes**

Mean C_t	log mean C_t	L_{max}-L_{min} < 1,0 ?	$F=\log C_t - \log C_0$	F ≥ 1,0 ?
4,57E+08	8,66	0,29 **Yes**	2,49	**Yes**

Mean T_0	log mean T_0	L_{max}-L_{min} < 2,0 ?
5,08E+03	3,71	0,76 **Yes**

Mean T_t	log mean T_t	L_{max}-L_{min} < 2,0 ?	$G=\log T_t - \log T_0$	R = (Ct-T0) - (Tt-T0) = F - G
4,00E+02	2,60	0,00 **Yes**	-1,10	6,06

4 List of Abbreviations

A_t	=	Average of the common logarithm of the number of viable bacteria, in CFU/test piece, recovered from the treated test specimens after 24 h
C_0	=	Average of the common logarithm of the number of viable bacteria, in CFU/ test piece, recovered from the untreated test specimens immediately after inoculation
C_t	=	Average of the common logarithm of the number of viable bacteria, in CFU/ test piece, recovered from the untreated test specimens after 24 h
CFU	=	Colony forming unit
ICC	=	Initial cell count
F	=	is the increase value of the untreated control
L_{max}	=	is the common logarithm (i.e. base 10 logarithm) of the maximum number of viable bacteria found on a specimen
L_{mean}	=	is the common logarithm of the mean number of viable bacteria found on the specimens
L_{min}	=	is the common logarithm of the minimum number of viable bacteria found on a specimen
log	=	logarithm
R	=	Antimicrobial activity
T_0	=	Average of the common logarithm of the number of viable bacteria, in CFU/ test piece, recovered from the treated test specimens immediately after inoculation
T_t	=	Average of the common logarithm of the number of viable bacteria, in CFU/ test piece, recovered from the treated test specimens after 24 h