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

Bacoban DL

Evaluation of virucidal efficacy

Test report no.	L21/00889MV.2
Method	ISO 18184:2019-06
Endpoint	modified vaccinia virus, strain Ankara (MVA)
Client	ROPIMEX R. OPEL GmbH, Ms Jennifer Sahl, Bildstocker Straße 12, DE - 66538 Neunkirchen
Laboratory	Dr. Brill + Partner GmbH, Institute for Hygiene and Microbiology Norderoog 2, DE-28259 Bremen

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

DR. JOCHEN STEINMANN

C/O DR. BRILL + PARTNER GMBH
INSTITUT FÜR HYGIENE UND MIKROBIOLOGIE
NORDEROOG 2, DE 28259 BREMEN
TELEFON 0049-40/557631-0
TELEFAX 0049-40/557631-11
EMAIL INFO@BRILLHYGIENE.COM
INTERNET WWW.BRILLHYGIENE.COM

DR. J. STEINMANN · C/O DR. BRILL + PARTNER GMBH · NORDEROOG 2 · DE-28259 BREMEN

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

Bremen, 25 November 2021

Expert opinion

Activity of **Bacoban DL** against modified vaccinia virus, strain Ankara (MVA) in the quantitative carrier test according to ISO 18184:2019-06 without organic load.

This statement is based on the test report L21/00889MV.2 dated 25/11/2021.

The virus-inactivating properties of the test sample **Bacoban DL** of ROPIMEX R. OPEL GmbH against the modified vaccinia virus, strain Ankara (MVA) were investigated according to ISO 18184:2019.

According to the ISO 18184 standard the following assessment for the evaluation of an antiviral activity (Mv) is described:

Mv of ≥ 2.0 $\log_{10}$: a good antiviral effect of the treated textile product can be declared

Mv of ≥ 3.0 $\log_{10}$: an excellent antiviral effect can be declared.

The test sample **Bacoban DL** was examined at 25 °C ± 1 °C without organic load and achieved the following reduction in virus titre when comparing to an untreated control specimen:

Within 2 hours contact time the mean virus titre of MVA was reduced by

3.67 log steps (99.98%).

Dr. Jochen Steinmann

Table of contents

Test report no L21/00889MV.2

Quantitative carrier test for the evaluation of virucidal activity of **Bacoban DL** in the medical area (ISO 18184:2019-06)

1 General Information and Material

1.1 Client

Client ..... ROPIMEX R. OPEL GmbH, Ms Jennifer Sahl, Bildstocker Straße 12, DE - 66538 Neunkirchen

Date of order 01/10/2021

Confirmation no. 225367

1.2 Identification of Test Laboratory

Location ..... Dr. Brill + Partner GmbH, Institute for Hygiene and Microbiology, Norderoog 2, DE-28259 Hamburg, Germany

Study manager ..... Dr. Britta Becker

Scientific assistant ..... Dr. Dajana Paulmann

Laboratory technician(s) ..... Lena Schüler, Anja Wiegand-Zimmermann

1.3 Identification of Sample

Name of product ..... Bacoban DL

Internal product identifier 21/00952-001

Formulation code ..... not specified

Batch no. 2006051

Production date ..... not specified

Manufacturer ..... ROPIMEX R. OPEL GmbH, DE - 66538 Neunkirchen

Date of delivery 21/07/2021

Storage conditions ..... room temperature and darkness

Appearance of product ..... colorless liquid

Area of accreditation ..... DAkkS D-PL-13412-01-01

Coating material ..... Bacoban DL (Bacoban WB 1 %)

Active agents (Manufacturer's data) ..... 26 g benzalkonium chloride and 2.5 g sodium pyrithione

1.4 Identification of Reference product

Name of product	standard reference (as specified in ISO 105-F02)
Internal product identifier	L21/0649
Formulation code	Ökotex 100 class 1 certified fabric - No. CQ1049 / 1
Basis weight:	115 g/m²
Production date	not specified
Manufacturer	Perles & Co; https://www.perlesandco.de.com/Stoff-BIO-Stoffe-c-1635_5051.html
Date of delivery	10/05/2021
Storage conditions	room temperature and darkness
Appearance of product	light beige specimens made of 100% cotton

1.5 Experimental Conditions

Test period	02/11/2021 – 17/11/2021
Size of test samples	2.0 x 2.0 cm
Amount of test pieces per approach	0.4 g ± 0.05 g
Volume of virus inoculum	200 µl
Exposure time(s)	2 hours
Test temperature	25 °C ± 1 °C
Incubation temperature	36.0 °C – 37.1 °C
Organic load in the virus inoculum	without organic load
Procedure to stop action of disinfectant	immediate dilution in cell culture medium
Virus Strain	modified vaccinia virus, strain Ankara (MVA)[1]
Origin of Virus	Institut für Tierhygiene und Öffentliches Veterinärwesen, Leipzig, Germany
Passage of Virus	4
Cells name	BHK 21-cells (Baby Hamster Kidney)
Origin of Cells	Leibniz Institute, DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany

[1]Instead of the influenza A virus the modified vaccinia virus, strain Ankara (MVA) was selected as test virus because of in Europe, MVA represents the official model virus for all enveloped viruses, including influenza A virus (EN 14476:2013+A2:2019 (4)).

Passage of Cells 34

Cell culture medium ..... Eagle`s Minimum Essential Medium with Earle`s BSS (EMEM), Biozym Scientific GmbH, catalogue no. 880120

2 Methods

2.1 Preparation of test virus suspension

Cells were infected with a multiplicity of infection of 0.1. After cells showed a cytopathic effect, they were subjected to a freeze/thaw procedure followed by a low speed centrifugation in order to sediment cell debris. After aliquotation, test virus suspension was stored at – 80 °C.

2.2 Preparation of test pieces

The standard certified fabric were cut into pieces of 2.0 x 2.0 cm size with sterile scissors or a disinfected slitter. After that, pieces were coated (treated test specimens) or not coated (reference specimens) with **Bacoban DL** and stored at room temperature in the dark until use in the experiment.

For coating, cotton pieces (2.0 x 2.0 cm) were dipped into **Bacoban DL** for 30 seconds, dripped off and dried at room temperature for 5 hours in a steril perti dish. 10 days after the coating, 0.40 g ± 0.05 g of the treated and untreated test specimens were placed in a sterile petri dish (new ones for the treated specimens) and used in the experiment.

2.3 Preparation of virus inoculum

Test virus suspension was used without addition of an interfering substance solution.

2.4 Inactivation assay and controls

Tests were carried out at 25 °C ± 1 °C. For each test three pieces of 0.40 g ± 0.05 g per exposure time of the treated specimens and control specimens (non-treated) were prepared respectively.

Treated pieces, aseptically placed in a petri dish, were inoculated with 200 µl of the virus inoculum by spreading the virus in several small drops on the specimens. The petri dish was closed with the lid, edges were additionally sealed with parafilm and incubation took place at 25 °C ± 1 °C in an incubator. Immediately at the end of the exposure time (2 hours), specimens from a petri dish were transferred to a suitable reaction tube (eluate container) with 20 ml ice-cold cell culture medium and glass beads. After that, surface of petri dish was rinsed four times with cell culture medium out of the container, followed by addition of the medium back to the eluate container. Container was vortexed for 30 seconds to resuspend the virus. Directly after elution, series of ten-fold dilutions of the eluate in ice-cold maintenance medium were prepared and inoculated on cell culture.

The non-treated specimens (reference) were processed as described above. The test pieces were incubated for 2 hours (VC t2h), additional test pieces were eluated immediately after addition of the test virus suspension to the material (VC t0).

To determine the initial virus titre (VIC), 100 µl of the test virus suspension were added to 10 ml cell culture medium. Titrations of the VIC were performed before starting the test (t0) and after the longest exposure time (tx).

Determination of cytotoxicity was performed as described in ISO 18184 point 10.6.1. Test pieces (3 x 0.4 g for the treated and 3 x 0.4 g for the untreated specimen) were transferred to an eluate container, followed by addition of the elution medium, vortexing for 30 seconds and an endpoint titration as described above. For the large volume plating method (LVP; see 2.6) the CT-eluates were further diluted 1:1000 in cell culture medium, which is added to the cells. The cytotoxicity control is needed for definition of the lower detection limit.

In addition, two controls for verification of cell sensitivity and the inactivation of viral activity were included. One control for determination of infectivity by end point titration titration (see 2.5) as described in ISO 18184 point 10.6.2 and one control for verifying results evaluated with the large volume plating method (LVP; see 2.6).
For the endpoint titration 10 ml of the undiluted eluate from the cytotoxicity control (3 x for the treated specimen and 3 x for the untreated specimen) (see above) were mixed with 100 µl pre-diluted test virus suspension (1:100 dilution to reach about 4-6 x 10^4 $TCID_{50}$/ml) and incubated for 30 minutes at 25 °C ± 1 °C. For LVP, 100 µl of the pre-diluted test virus suspension were added to 10 ml of the 1:1000 dilution and incubated for 30 minutes at 25 °C ± 1 °C. Afterwards, approaches were diluted and the infectivity was determined. The results of the treated and untreated specimens are compared and the difference should be ≤ 0.5 log_{10}.

Furthermore, a cell control (only addition of medium) was incorporated.

2.5 Determination of infectivity by end point titration

Infectivity was determined as endpoint titration transferring 0.1 ml of each dilution into eight wells of a microtitre plate containing 0.1 ml of cell suspension. Microtitre plates were incubated at 37 °C in a 5 % CO_2-atmosphere. The cytopathic effect was read by using an inverted microscope. The infective dose $TCID_{50}$/ml was calculated with the method of Spearman (2) and Kärber (3).

2.6 Determination of infectivity following the large volume plating method (LVP)

Following the large volume plating method (EN 14476, section 5.5.4.3) the inactivation assays (eluates) were further diluted 1:1000 in cell culture medium. The total volume was added (without any further dilution) to the permissive cells. By introducing such a huge dilution, it is possible to eliminate cytotoxicity of the test product in order to demonstrate a 4 log_{10} reduction of virus titre. Calculation of virus titre follows formula of Taylor or Poisson (EN 14476, section B.3). This method is necessary for those products which demonstrate a great cytotoxicity.

30 µl of the inactivation assay were added to 30 ml medium and then the total volume was distributed in 3 microtitre plates (104 µl / well, 288 wells total). After 5 days of inoculation cultures were observed for cytopathic effects.

2.7 Calculation and verification of virucidal activity

The antiviral activity of the treated **Bacoban DL** was calculated by Formula 8, ISO 18184, 14.3.2:

$$Mv = lg\ (Vb/Vc) = lg(Vb) - lg(Vc)$$

Mv is the antiviral activity value

lg(Vb) is the common logarithm average of 3 infectivity titre value after 2 hours contacting with the control specimen

lg(Vc) is the common logarithm average of 3 infectivity titre value after 2 hours contacting with the antiviral fabric specimen

According to the ISO 18184 standard the following assessment for the evaluation of an antiviral activity is described:

Mv of ≥ 2.0 log_{10}: a good antiviral effect of the treated textile product can be declared

Mv of ≥ 3.0 log_{10}: an excellent antiviral effect can be declared

This corresponds to an inactivation of ≥ 99 % (Mv of ≥ 2.0 log_{10}) or 99.9 % (Mv of ≥ 3.0 log_{10}).

3 Results

3.1 Verification of results and pass criteria

The following criteria according to ISO 18184, 14.3.1 were fulfilled/not fullfilled:

a) Titre of the virus stock solution was > 10^7 log_{10} $TCID_{50}$/ml (ISO 18184, 14.3.1 a)

b) The difference of the average virus titres of the control of efficiency for suppression of disinfectant's activity with the untreated and treated specimens for test results using the endpoint titration was not ≤ 0.5 after 30 minutes (ISO 18184, 10.6.2.6 and 14.3.1 b) and after a shortened contact time of 5 minutes (see table 2). So, results evaluated with the enpoint titration are not valid.

In contrast the difference of the virus titres for the after-effect control using the LVP-method was ≤ 0.5 after 30 minutes (ISO 18184, 10.6.2.6 and 14.3.1 b), so that the requirement of the ISO 18184 is met (see point 8 and table 4). To exclude an virus-eliminating efficacy during the elution process (rinsing and mixing) an additional after-effect-control was performed with an incubation time of 1.5 minutes. Since the difference of the average virus titres of the control of efficiency for suppression of disinfectant's activity with the untreated and treated specimens was ≤ 0.5 log_{10} (see table 2), results with the LVP-method are valid.

c) The difference of the logarithmic virus titre recovered immediately after inoculation of the untreated test specimens and after selected contact time was ≤ 1.0 after 2 h and/or ≤ 2.0 after 24 h (ISO 18184, 14.3.1 c) (see table 3).

$M = lg\ (Va/Vb) = lg(Va) - lg(Vb)$ is the reduction value

lg(Va) is the common logarithm average of 3 infectivity titre value immediate after inoculation of the control specimen

lg(Vb) is the common logarithm average of 3 infectivity titre value after 2 hours contacting with the control specimen

Since all criteria (for LVP) were fulfilled, examination with MVA according to ISO 18184 was valid.

3.2 Results of efficacy testing with the LVP-method

The untreated standard reference (reference) and treated specimens with **Bacoban DL** were examined for 2 hours at 25 °C ± 1 °C.

The mean virus titre on the non-treated specimens was 5.83 log_{10} $TCID_{50}$/ml after 2 hours. The mean virus titre on the treated specimen with **Bacoban DL** was 2.16 log_{10} $TCID_{50}$/ml after 2 hours of incubation (see table 1). Accordingly, the antiviral activity value Mv was 3.67 (5.83 minus 2.16) after 2 hours.

3.3 Conclusion

The treated specimen with **Bacoban DL** provided by ROPIMEX R. OPEL GmbH reached a RF of 3.67 log steps and was able to demonstrate a sufficient reduction of MVA after an exposure time of 2 hours. That means, by the performance standard according to the ISO 18184 the **Bacoban DL** has an excellent antiviral efficacy.

4 Signature page

Bremen, 25/11/2021

Dr. Britta Becker
Director of Laboratory - Virology

Dr. Dajana Paulmann
Deputy Head of Laboratory - Virology

Table 1: Summary of results with the treated specimens

Product name:	**Bacoban DL**	Batch:	2006051
Test organism:	modified vaccinia virus, strain Ankara (MVA)	Temperature:	25 °C ± 1 °C
Organic load:	without organic load	Test Run:	#7679
No. of assay:	1st assay		

	contact time	log_{10} CD_{50}/ml	log_{10} $TCID_{50}$/ml before		log_{10} $TCID_{50}$/ml after incubation, approach						
					1	2	3	4	MV	2xSD	RF
VIC	n.a.	n.a.	6.38	6.00	n.a.	n.a.	n.a.	n.a.	6.19	0.53	n.a.
VC t0 (untreated material)	0 hours	0.50	n.a.	n.a.	6.25	6.25	5.88	n.d.	6.13	0.43	0.06
VC t2.0h (untreated material)	2 hours	0.50	n.a.	n.a.	6.38	5.75	5.38	n.d.	5.83	1.01	0.35
cotton specimens coated with Bacoban DL* -end point dilution-	2 hours	3.50	n.a.	n.a.	≤ 3.50	≤ 3.50	≤ 3.50	n.d.	≤ 3.50	0.00	≥ 2.33
cotton specimens coated with Bacoban DL* -LVP 1:1000-	2 hours	n.a.	n.a.	n.a.	2.16	2.16	2.16	n.d.	2.16	0.00	3.67

VIC = virus input control
VC = virus control
CD = cytotoxic dose
TCID = tissue culture infectious dose
RF = reduction factor
MV = mean value
n.a. = not applicable
n.d. = not done
SD = standard deviation

* cotton specimens (standard reference as specified in ISO 105-F02) were dipped into Bacoban DL (Bacoban WB 1%) for 30 seconds and dried. The test was started 10 days after the specimens were coated.

Table 2: Data for verification of cell sensitivity and inactivation of viral activity

Product name:	**Bacoban DL**	Batch:	2006051
Test organism:	modified vaccinia virus, strain Ankara (MVA)	Temperature:	25 °C ± 1 °C
Organic load:	without organic load	Test Run:	#7679
No. of assay:	1st assay		

	contact time	log_{10} CD_{50}/ml	log_{10} $TCID_{50}$/ml before		log_{10} $TCID_{50}$/ml after incubation, approach						
					1	2	3	4	MV	2xSD	Δ RF
RA medium control	30 min	n.d.	n.a.	n.a.	4.75	5.00	5.25	n.d.	5.00	0.50	n.a.
RA untreated specimen	30 min	n.d.	n.a.	n.a.	5.00	5.13	5.13	n.d.	5.08	0.14	-0.08
RA treated specimen	30 min	n.d.	n.a.	n.a.	4.13	4.00	≤ 3.25	n.d.	≤ 3.79	0.95	≥ 1.21
RA treated specimen	5.0 min	n.d.	n.a.	n.a.	4.50	4.50	4.50	n.d.	4.50	0.00	0.50
RA treated specimen	1.5 min	n.d.	n.a.	n.a.	5.00	5.13	4.50	n.d.	4.88	0.66	0.13
RA treated specimen - LVP	30 min	n.d.	n.a.	n.a.	5.13	5.13	5.13	n.d.	5.13	0.00	-0.13

RA = residual viral activity
RF = reduction factor
SD = standard deviation
MV = mean value
n.a. = not applicable
n.d. = not done
Δ = Difference compared to medium control

Test validity: Δ RA treated vs RA untreated specimen = ≤ 0.5 log_{10} (LVP-method)		
RA t30' untreated specimen	5.08	
RA t30' treated specimen	5.13	
Δ =	-0.04	OK

Test validity: Δ RA treated vs RA untreated specimen = ≤ 0.5 log_{10}		
RA t30' untreated specimen	5.08	
RA t5' treated specimen	4.50	
Δ =	0.58	not OK

Test validity: Δ RA treated vs RA untreated specimen = ≤ 0.5 log_{10} (endpoint titration)		
RA t30' untreated specimen	5.08	
RA t30' treated specimen	3.79	
Δ =	1.29	not OK

Test validity: Δ RA treated vs RA untreated specimen = ≤ 0.5 log_{10}		
RA t30' untreated specimen	5.08	
RA t1.5' treated specimen	4.88	
Δ =	0.21	OK

RA = residual viral activity

Table 3: Results of test validity by comparing the control virus titres (VC t0 vs VC tx)

Product name:	**Bacoban DL**	Batch:	2006051
Test organism:	modified vaccinia virus, strain Ankara (MVA)	Temperature:	25 °C ± 1 °C
Organic load:	without organic load	Test Run:	#7679
No. of assay:	1st assay		

Test validity: Δ VCt0 vs VCtx ≤ 1.0 log_{10} after 2 h and/or ≤ 2.0 log_{10} after 24 h		
VC t0:	6.13	
VC t2h	5.83	
Δ =	0.29	OK

VC = virus control

5 Appendix

5.1 Version history

Previous versions are replaced by most recent version.

Version	Date	Reason of change and changelog	Author
01	25/11/2021	-	BB

5.2 Accreditation and certificates

ISO 18184:2019-06 is included in our accriditation according to DIN EN ISO/IEC 17025.

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

5.3 Terms of use

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.

5.4 Literature

1 ISO 18184:2019-06 "International Standard ISO 18184. Textiles - Determination of antiviral activity of textile products. ISO 18184:2019; Second edition"
2 Spearman, C.: The method of "right or wrong cases" (constant stimuli) without Gauss's formulae. Brit J Psychol; 2 1908, 227-242
3 Kärber, G.: Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Arch Exp Path Pharmak; 162, 1931, 480-487
4 DIN EN 14476:2019-10 "Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical area – Test method and requirements (Phase 2/Step 1); German version EN 14476:2013+A2:2019"

5.5 List of Abbreviations

DIN	=	Norm by German institute for normation (German: Deutsches Institut für Normung)
EN	=	European norm (issued by European Committee for Standardization – CEN)
IEC	=	International Electrotechnical Commission
ISO	=	International Organization for Standardization