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

Anerkannt durch/Recognized by
Zentralstelle der Länder für Gesundheitsschutz bei Arzneimitteln und Medizinprodukten
ZLG-AP-216.11.02

TEST REPORT

26/03/2019

Test report L19/0102aMV.2

Evaluation of the effectiveness of

Bacoban WB

Test virus: modified vaccinia virus Ankara (MVA)

Method: based on EN 14476:2013+A1:2015 (dirty conditions)

quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in human medicine

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

Norderoog 2, DE - 28259 Bremen
Tel.: +49 40-557631-0, Fax: +49 40-557631-11
info@brillhygiene.com, http://www.brillhygiene.com

1. Identification of test laboratory

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

2. Identification of sample

Manufacturer	ROPIMEX R. OPEL GmbH
Name of product	Bacoban WB
Confirmation no.	208396
Product diluent recommended by the manufacturer	-
Batch number	1902061
Application	surface disinfection
Production date	-
Expiry date	02/2021
Active compound (s) (100 g)	26 g benzalkonium chloride 2.5 g sodium pyrithione
Appearance, odour	clear, brownish liquid product specific
pH-values	undiluted: 5.72 (20 °C) 1.0 %: 6.25 (20 °C) 0.5 %: 6.57 (20 °C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	08/02/2019

3. Materials

3.1 Culture medium and reagents

- Eagle`s Minimum Essential Medium with Hank`s BSS (MEM, Biozym Scientific GmbH, catalogue no. 880144)
- fetal calf serum (Biochrom AG, article no. S 0115)
- 1.4 % formaldehyde solution (dilution of Roti®-Histofix 4 %, Carl Roth GmbH)
- Aqua bidest. (SG ultrapure water system, type Ultra Clear; serial no. 86996-1)
- PBS (Invitrogen, article no. 18912-014)

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- BSA (Sigma-Aldrich-Chemie GmbH, article no. CA-2153)
- sheep erythrocytes (Fiebig Nährstofftechnik).

3.2 Virus and cells

The modified vaccinia virus Ankara (MVA) originated from Dr. Manteufel, Institut für Tierhygiene und Öffentliches Veterinärwesen, DE - 04103 Leipzig. Before inactivation assays, virus had been passaged three times in *BHK 21-cells* (Baby Hamster Kidney).

BHK 21-cells (passage 108) originated from the Friedrich-Löffler-Institut, Bundesforschungsinstitut für Tiergesundheit (formerly Bundesforschungsanstalt für Viruskrankheiten der Tiere, isle of Riems).

The cells were inspected regularly for morphological alterations and for contamination by mycoplasmas. No morphological alterations of cells and no contamination by mycoplasmas could be detected.

3.3 Apparatus, glassware and small items of equipment

- CO_2 incubator, Nunc GmbH & Co. KG, model QWJ 350
- Agitator (Vortex Genie Mixer, type G 560E)
- pH measurement 315i (WTW, article no. 2A10-100)
- Centrifuge (Sigma-Aldrich-Chemie GmbH, type 113)
- Microscope (Olympus, type CK 30)
- Centrifuge 5804 R (Eppendorf AG)
- Water bath (JULABO, Julabo U 3)
- Adjustable and fixed-volume pipettes (Eppendorf AG)
- Polysterol 96-well microtitre plate (Nunc GmbH & Co. KG, Wiesbaden)
- Cell culture flask (Nunc GmbH & Co. KG, Wiesbaden)
- Sealed test tubes (Sarstedt AG & Co., Nümbrecht).

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4. Experimental conditions

Test temperature	20 °C ± 1.0 °C
Concentration of test product	1.0 %, 0.5 % and 0.1 % (demonstration of non-active range) solutions
Appearance of product dilutions	no precipitation
Contact times	5 and 30 minutes
Interfering substance	3.0 g/l bovine serum albumin + 3.0 ml/l erythrocytes (dirty conditions, EN 14476)
Procedure to stop action of disinfectant	immediate dilution
Diluent	water of standardised hardness (WSH)
Stability of product in the mix with virus and interfering substance (1.0 % solution)	no clouding, no precipitation
Virus strain	modified vaccinia virus Ankara (MVA) (ATCC VR-1508)
Date of testing	12/03/2019 – 26/03/2019
End of testing	26/03/2019

5. Methods

5.1 Preparation of test virus suspension

For preparation of test virus suspension, *BHK 21-cells* were cultivated with MEM and 10 % or 2 % fetal calf serum. *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.

5.2 Preparation of disinfectant (dilutions)

The test product was tested as 1.0 %, 0.75 % and 0.1 % (demonstrating of non-active range) solutions. Due to the addition of interfering substance and test virus suspension the solutions had to be prepared by the factor 1.25.

These solutions were prepared with WSH immediately before the inactivation tests.

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5.3 Infectivity assay

Infectivity was determined as endpoint titration according to EN 5.5 transferring 0.1 ml of each dilution into eight wells of a microtitre plate to 0.1 ml of freshly trypsinised *BHK 21-cells* (10-15 x 10^3 cells per well), beginning with the highest dilution. Microtitre plates were incubated at 37 °C in a 5 % CO_2-atmosphere. The cytopathic effect was read by using an inverted microscope after six days. Calculation of the infective dose $TCID_{50}$/ml was calculated with the method of Spearman (2) and Kärber (3) with the following formula:

$$- \log_{10}TCID_{50} = X_0 - 0.5 + \sum r/n$$

meaning

X_0 = $\log_{10}$ of the lowest dilution with 100 % positive reaction

r = number of pos. determinations of lowest dilution step with 100 % positive and all higher positive dilution steps

n = number of determinations for each dilution step.

5.4 Calculation and verification of virucidal activity

The virucidal activity of the test disinfectant was evaluated by calculating the decrease in titre in comparison with the control titration without disinfectant. The difference is given as reduction factor (RF).

According to the EN 14476, a disinfectant or a disinfectant solution at a particular concentration is having virus-inactivating efficacy if the titre is reduced at least by 4 $\log_{10}$ steps within the recommended exposure period. This corresponds to an inactivation of ≥ 99.99 %.

5.5 Inactivation assay (end point titration)

Determination of virucidal activity has been carried out according to EN 5.5. The test product was examined as 1.0 %, 0.5 % and 0.1 % (demonstration of non-active range) solutions in WSH at 20 °C based on EN 14476. 5 and 30 minutes were chosen as contact times.

Immediately at the end of a chosen contact time, activity of the disinfectant was stopped by dilution to 10^{-8}.

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Titrations of the virus control were performed at the beginning of the test and after the longest exposure time (EN 5.5.7). One part by volume of test virus suspension was mixed with one part interfering substance and eight parts by volume of WSH or Aqua bidest. (RTU products).

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

Inactivation tests were carried out in sealed test tubes in a water bath at 20 °C ± 1.0 °C. Aliquots were retained after appropriate exposure times and residual infectivity was determined.

5.6 Inactivation assay following the large volume plating method (LVP)

Following the large volume plating method (4) the inactivation assays were further diluted 1:5,000 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 (5, 6). This method is necessary for those products which demonstrate a great cytotoxicity.

12.5 µl of the inactivation assays were added to 62.5 ml medium (total dilution of 1:5,000) and then the total volume was distributed in 6 microtitre plates (54 µl / well, 1152 wells total (1.0 % solution) and 108 µl / well, 576 wells total (0.5 % solution, respectively)). After 6 days of inoculation cultures were observed for cytopathic effects.

The calculation of virus titre without residual virus followed the formula of Poisson:

$$c = \ln p / -V$$

c = number of virus particles

p = the probability to find no virus. The probability to find no virus should not greater than 5 % (p=0.05). By doing so, the number of virus particles can be calculated with a probability of 95 %.

V = test volume (ml)

The titre to be used for calculating the reduction factor (RF) was finally calculated as followed: the determined number of virus particle is first converted with the aid of the dilution factor in the number of particle per ml. Subsequently, the numbers of particles per ml have to be converted in the tissue culture infectious dose per ml ($TCID_{50}$/ml) (1.0 $TCID_{50}$

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corresponds to 0.69 infectious virus particles). The common logarithm of this value results in the virus titre (log_{10} $TCID_{50}$/ml) used for calculating the reduction factor (RF).

In assays with residual virus, formula according to Taylor was used for calculating the virus titre:

$$c/ml = \frac{D}{Vw} \times \left(-\ln \frac{n - n_p}{n}\right)$$

c = number of virus particles

D = dilution

V_W = volume per well

n = number of inoculated wells

n_p = number of virus-positive wells

For calculating the reduction factor using the formula according to Taylor the number of virus particles is converted to the logarithmic titre ($log_{10}TCID_{50}$/ml) as described above.

5.7 Determination of cytotoxicity

Determination of cytotoxicity was performed according to EN 5.5.4.1.

5.8 Cell sensitivity to virus

For the control of cell sensitivity to virus two parts by volume of water were mixed with eight parts by volume of the lowest apparently non-cytotoxic dilution of the product. These mixtures or PBS as control were added to a volume of double concentrated cell suspension. After 1 h at 37 °C the cells were centrifuged and re-suspended in cell culture medium (EN 5.5.4.2b).

Finally, a comparative titration of the test virus suspension was performed on the pre-treated (disinfectant) and non-pre-treated (PBS) cells as described above.

5.9 Control of efficacy for suppression of disinfectant's activity

Furthermore, a control of efficiency for suppression of disinfectant's activity was included (EN 5.5.5).

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5.10 Reference virus inactivation test

As reference for test validation a 0.7 % formaldehyde solution according to EN 5.5.6 was included. 5, 15, 30 and 60 minutes were chosen as contact times. In addition, cytotoxicity of formaldehyde test solution was determined based on EN 5.5.6.2 with dilutions up to 10^{-5}.

6. Verification of the methodology

The following criteria as mentioned in EN 5.7 were fulfilled:

a) The titre of the test virus suspension allowed the determination of a ≥ 4 $\log_{10}$ reduction (maximal virus reduction $\geq 4.15 \pm 0.21$, LVP)

b) The test product (1.0 %) showed cytotoxicity in the 1:1,000 dilutions thus allowing the detection of a 4 $\log_{10}$ reduction of virus titre using the LVP method.

c) The comparative titration on pre-treated (disinfectant) and non-pre-treated (PBS) *BHK 21-cells* showed no significant difference (< 1 $\log_{10}$; EN 5.7) of virus titre: 6.63 ± 0.41 (PBS, LVP) versus 6.63 ± 0.25 (1:5,000 dilutions of disinfectant as 1.0 % solution, LVP) and 6.88 ± 0.37 (PBS, LVP) versus 7.00 ± 0.378 (1:5,000 dilutions of disinfectant as 0.5 % solution, LVP) $\log_{10}$ $TCID_{50}$/ml, respectively.

d) The control of efficacy for suppression of disinfectant's activity (1.0 %) showed a decrease of ≥ 2.13 ($\leq 4.50 \pm 0.00$ versus 6.63 ± 0.25 $\log_{10}$ $TCID_{50}$/ml) and failed the requirement of the EN (≤ 0.5 $\log_{10}$; EN 5.5.5.1). In these experiments at the end of the defined exposure time the test mixture was immediately diluted not 1:10 as described in the control of efficacy for suppression of disinfectant's activity but directly 1:5,000 (LVP) and the dilution transferred to the cell culture. For this reason this control is not relevant when using the LVP. Therefore, despite the insufficient control of efficacy for suppression of disinfectant's activity the assay is valid.

e) One concentration demonstrated a 4 $\log_{10}$ reduction and (at least) one concentration demonstrated a $\log_{10}$ reduction of less than 4.

Since all criteria according EN 5.7 were fulfilled, examination with MVA based on EN 14476 is valid.

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7. Results

Results of examination are shown in tables 1 to 10. Tables 1 to 8 demonstrate the raw data, whereas tables 9 (a+b) and 10 give a summary of results.

Since it was not possible to show a reduction in virus titre of 4 $\log_{10}$-steps testing the test product as 1.0 % and 0.5 % solutions using the endpoint dilution method due to cytotoxicity, this concentration was tested using the large volume plating method. The further dilution (non-active range) was examined using the end point dilution method.

The test product as 0.1 % solution was not active against MVA within 30 minutes in this quantitative suspension test using the end point dilution method (table 1).

In parallel to the end point dilution method the large volume plating method (LVP) was introduced testing the test product as 1.0 % and 0.5 % solutions with 5 minutes of exposure time. The mean virus titre in the twofold assays was $\log_{10}$ $TCID_{50}$/ml = 6.69 ± 0.21 (table 6).

The test product as 1.0 % was active after 5 minutes of exposure time. No residual virus was found in 1152 cell culture units at this time point (table 7). The result according to the formula of Poisson was ≤ 2.54 $\log_{10}$ $TCID_{50}$. The reduction factor was therefore ≥ 4.15 ± 0.21 (6.69 ± 0.21 $\log_{10}$ $TCID_{50}$ minus ≤ 2.54 $\log_{10}$). This corresponded to an inactivation of ≥ 99.99 %.

The test product as 0.5 % was not active within 5 minutes of exposure time (table 8). Since residual virus was found in 440 of 576 cell culture units at this time point, the result according to the formula of Taylor was 4.99 $\log_{10}$ $TCID_{50}$. The reduction factor was therefore 1.70 ± 0.21 (6.69 ± 0.21 $\log_{10}$ $TCID_{50}$ minus 4.99 $\log_{10}$).

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8. Conclusion

The surface disinfectant Bacoban WB tested as 1.0 % solution demonstrated effectiveness against MVA after an exposure time of 5 minutes under dirty conditions.

Therefore, the surface disinfectant Bacoban WB can be declared as active against MVA as follows:

1.0 % **5 minutes** **dirty conditions**

Bremen, 26/03/2019

- Dr. Britta Becker -
Head of Laboratory

- Dr. Dajana Paulmann -
Scientific Project Manager

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9. Quality control

The Quality Assurance of the results was maintained by performing the determination of the virus-inactivating properties of the disinfectant in accordance with Good Laboratory Practice regulations:

1) Chemicals Act of Germany, Appendix 1, dating of 01.08 1994 (BGBI. I, 1994, page 1703). Appendix revised at 14. 05. 1997 (BGBI. I, 1997, page 1060).
2) OECD Principles of Good Laboratory Practice (revised 1997); OECD Environmental Health and Safety Publications; Series on Principles of Good Laboratory Practice and Compliance Monitoring – Number 1. Environment Directorate, Organization for Economic Co-operation and Development, Paris 1998.

The plausibility of the results was additionally confirmed by controls incorporated in the inactivation assays.

10. Records to be maintained

All testing data, protocol, protocol modifications, the final report, and correspondence between Dr. Brill + Partner GmbH and the sponsor will be stored in the archives at Dr. Brill + Partner GmbH.

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The test results in this test report relate only to the items examined.

11. Literature

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5. Bekanntmachung über die Zulassung von Arzneimitteln, Anforderungen an Validierungsstudien zum Nachweis der Virussicherheit von Arzneimitteln aus menschlichem Blut oder Plasma vom 20. Dezember 1993/21. Januar 1994. Bundesanzeiger Nr. 84: 4740-4744 bzw. CPMP/BWP/268/95: Note for Guidance on virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses. http://www.ema.europa.eu

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Appendix:

Legend to the Tables

Table 1:	Raw data for Bacoban WB (0.1 %) tested against MVA
Table 2:	Raw data for formaldehyde solution (0.7 %) tested against MVA
Table 3:	Raw data for control of efficacy for suppression of disinfectant's activity (1.0 %)
Table 4:	Raw data (MVA) for cell sensitivity (1.0 %) (LVP)
Table 5:	Raw data (MVA) for cell sensitivity (0.5 %) (LVP)
Table 6:	Determination of virus titre (LVP)
Table 7:	Inactivation of MVA by Bacoban WB (1.0 %) (5 minutes) (LVP)
Table 8:	Inactivation of MVA by Bacoban WB (0.5 %) (5 minutes) (LVP)
Table 9 (a+b):	Summary of results (end point dilution method) with Bacoban WB and MVA
Table 10:	Summary of results (LVP) with Bacoban WB and MVA

Legend to the Figures

Figure 1:	Virus-inactivating properties of Bacoban WB (1.0 %) (LVP)
Figure 2:	Virus-inactivating properties of formaldehyde (0.7 %)

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Table 1: Raw data for Bacoban WB (0.1 %) tested against MVA at 20 °C (quantal test; 8 wells) (#5963)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	0.1 %	dirty conditions	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			5	tttt tttt	tttt tttt	4444 4444	4444 4444	3303 3333	0203 4000	0000 0000	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	tttt tttt	tttt tttt	4444 4444	4444 4444	3423 2020	0000 0000	0000 0000	n.d.	n.d.
test product cytotoxicity	0.1 %	dirty conditions	n.a.	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	3433 3233	0000 0000	0000 0000	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	3333 3332	0020 0000	0000 0000	0000 0000	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

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Table 2: Raw data for formaldehyde solution (0.7 %) tested against MVA at 20 °C (quantal test; 8 wells) (#5963)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
formaldehyde	0.7 % (m/V)	PBS	5	tttt tttt	tttt tttt	tttt tttt	0010 1000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			15	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			30	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			60	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
formaldehyde cytotoxicity	0.7 % (m/V)	PBS	n.a.	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	PBS	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	3333 3233	1000 2020	0000 0000	0000 0000	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

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Table 3: Raw data for control of efficacy for suppression of disinfectant's activity (1.0 %) (#5963)

Product	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
test product	dirty conditions	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
corresponding virus control	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	3333 3332	0020 0000	0000 0000	0000 0000	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

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Table 4: Raw data (MVA) for cell sensitivity (1.0 % solution) (#5963) (LVP)

Product	Dilution	Dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
PBS	-	4444 4444	4444 4444	4444 4444	4444 4444	3034 3443	3000 2000	0000 0000	0000 0000	n.d.
test product	1:5,000	4444 4444	4444 4444	4444 4444	4444 4444	3333 4444	0000 2000	0000 0000	0000 0000	n.d.

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 5: Raw data (MVA) for cell sensitivity (0.5 % solution) (#5963) (LVP)

Product	Dilution	Dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
PBS	-	4444 4444	4444 4444	4444 4444	4444 4444	3343 2332	0302 3000	0000 0000	0000 0000	n.d.
test product	1:5,000	4444 4444	4444 4444	4444 4444	4444 4444	4334 4443	4030 2400	0000 0000	0000 0000	n.d.

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

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Table 6: Determination of virus titre (LVP) at 20 °C (#5963)

Virus titration	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
1st control	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	3333 3332	0020 0000	0000 0000	0000 0000	0000 0000
2nd control	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	1322 4334	2000 3000	0000 0000	0000 0000	0000 0000

n.a. = not applicable t = cytotoxic 0 = no virus detectable
n.d. = not done 1 to 4 = virus detectable (degree of CPE in 8 wells of a microtitre plate)

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Table 7: Inactivation of MVA by Bacoban WB (1.0 %) at 20 °C (5 minutes) (LVP, 1:5,000) (#5963)

Interfering substance	Row	1	2	3	4	5	6	7	8	9	10	11	12
dirty conditions	plate 1/12	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 2/12	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 3/12	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 4/12	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 5/12	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 6/12	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 7/12	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 8/12	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 9/12	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 10/12	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 11/12	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 12/12	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000

t = cytotoxic

0 = no virus detectable
1 to 4 = virus detectable (degree of CPE in 8 wells of a microtitre plate)

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Table 8: Inactivation of MVA by Bacoban WB (0.5 %) at 20 °C (5 minutes) (LVP, 1:5,000) (#5963)

Interfering substance	Row	1	2	3	4	5	6	7	8	9	10	11	12
dirty conditions	plate 1/6	4444 0440	4444 4044	0404 0444	0044 4400	4440 4444	4444 0440	4444 4444	0004 0404	4444 0044	4004 4440	0404 4400	0404 0444
	plate 2/6	0440 4444	0444 4440	4004 4400	4444 4444	4444 0440	4400 4444	4444 0404	0404 4444	4044 4044	4404 4444	4440 4004	0404 4444
	plate 3/6	4444 4440	4040 4044	4444 4444	4444 4444	4404 4444	4404 4404	4440 4444	4004 4444	4444 4404	4440 4444	4444 4444	0444 4440
	plate 4/6	4444 4040	0404 4404	4044 4044	4444 0444	0404 4404	4404 0444	4044 4044	4444 4444	4404 4444	0444 4040	4044 4444	4004 4440
	plate 5/6	4444 4400	4044 4444	4444 4444	0044 0404	4444 0444	4444 0444	4440 4444	0444 4400	4444 4444	4044 4444	4444 4444	4404 4044
	plate 6/6	4404 4440	4444 4044	0444 0444	0440 4000	4044 4044	4444 4044	0440 4040	4044 4444	4040 4440	0444 4040	4404 4044	4004 4404

t = cytotoxic

0 = no virus detectable
1 to 4 = virus detectable (degree of CPE in 8 wells of a microtitre plate)

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Table 9a: Summary of results (end point dilution method) with Bacoban WB and MVA

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after …min
				1	5	15	30	60	
test product	0.1 %	dirty conditions	3.50	n.d.	6.75±0.44	n.d.	6.25±0.33	n.d.	> 30 (RF = 0.38±0.41)

n.a. = not applicable n.d. = not done

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Table 9b: Summary of results (end point dilution method) with Bacoban WB and MVA

Product	Con-centration	Interfering substance	Level of cytotoxicity	$\log_{10}$ TCID$_{50}$/ml after ....min					> 4 $\log_{10}$ reduction after … min
				0	5	15	30	60	
formaldehyde	0.7 % (w/v)	PBS	4.50	n.d.	≤ 4.75±0.33	≤ 4.50±0.00	≤ 4.50±0.00	≤ 4.50±0.00	≥ 15 (RF ≥ 2.38±0.26)
virus control	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	6.88±0.37	n.a.
virus control (+ suppression)	n.a.	dirty conditions	n.a.	6.50±0.00	n.d.	n.d.	n.d.	6.63±0.25	n.a.
suppression control	1.0 %	dirty conditions	4.50	n.d.	n.d.	n.d.	≤ 4.50±0.00	n.d.	n.a.

n.a. = not applicable n.d. = not done sens. = sensitivity

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Table 10: Summary of results (LVP, 1:5,000) with Bacoban WB and MVA

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after …min
				5	10	15	30	60	
test product	1.0 %	dirty conditions	n.a.	≤ 2.54	n.d.	n.d.	n.d.	n.d.	5 (RF ≥ 4.15±0.21)
test product	1.0 %	dirty conditions	n.a.	4.99	n.d.	n.d.	n.d.	n.d.	> 5 (RF ≥ 1.70±0.21)
virus control	n.a.	dirty conditions	n.a.	n.d.	n.d.	n.d.	n.d.	6.63±0.25 6.75±0.33 (Ø6.69±0.21)	n.a.
sens. PBS	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	6.63±0.41	n.a.
sens. product	1.0 % → 1:5,000	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	6.63±0.25	n.a.
sens. PBS	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	6.88±0.37	n.a.
sens. product	0.5% → 1:5,000	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	7.00±0.38	n.a.

n.a. = not applicable n.d. = not done sens. = sensitivity n.c. = not calculable

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Figure 1: Virus-inactivating properties of Bacoban WB (1.0 %) (LVP)

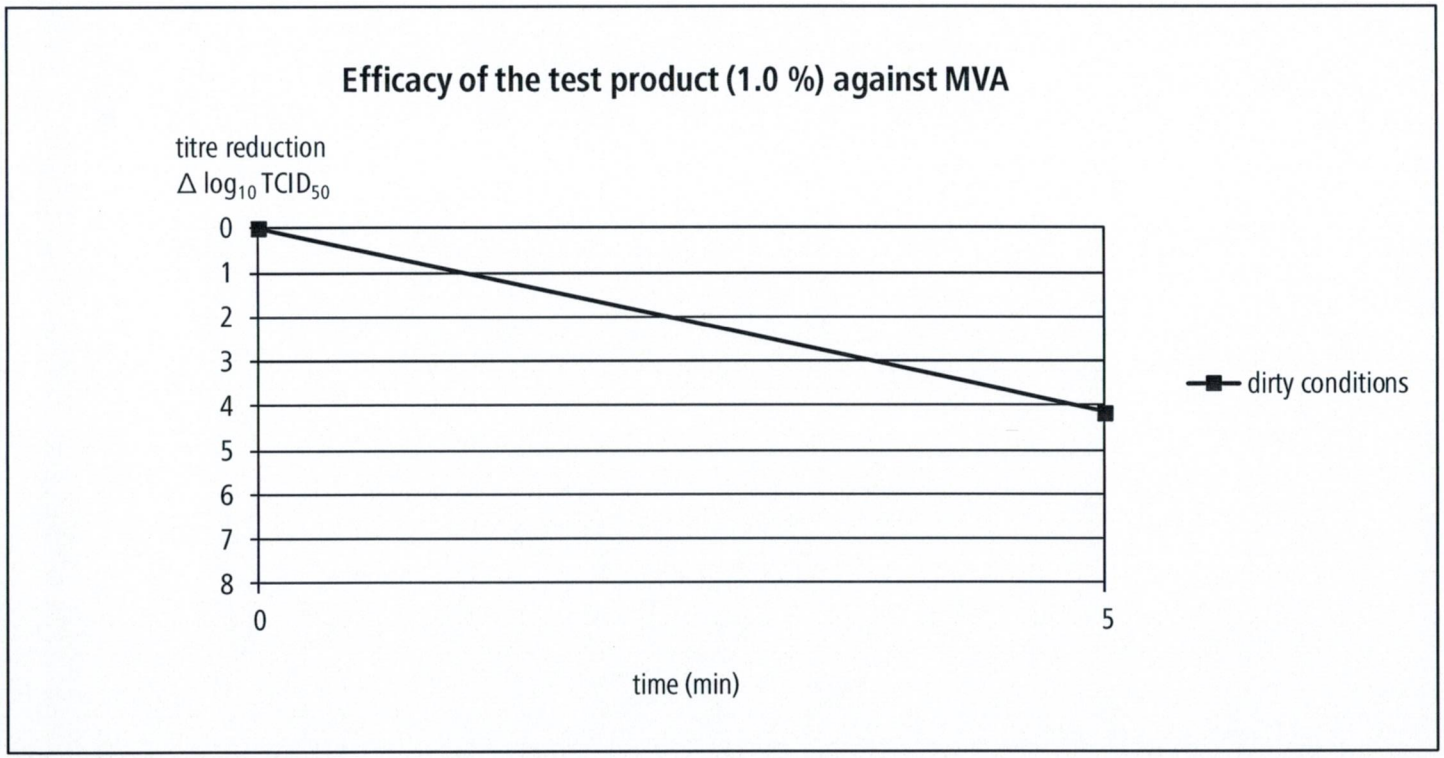

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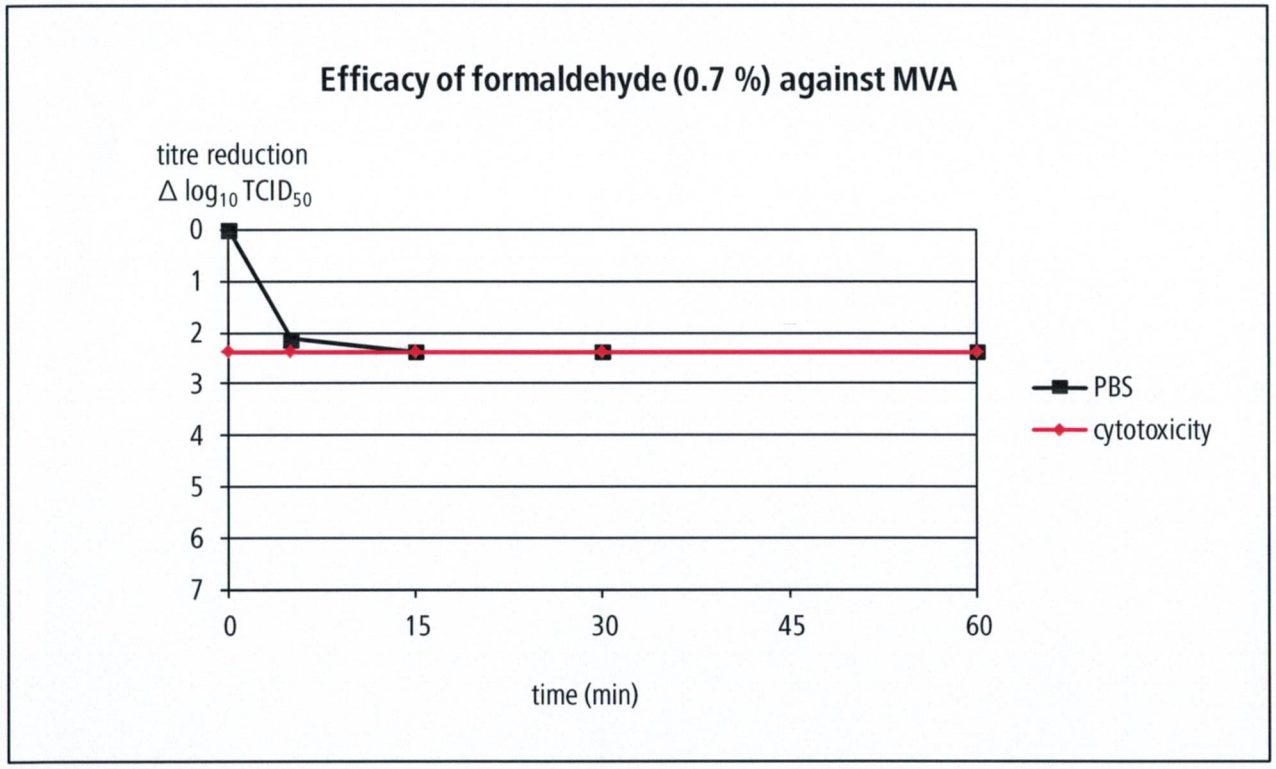
Figure 2: Virus-inactivating properties of formaldehyde (0.7 %)



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