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). As virus controls for the 97.0 % assays 0.1 parts by volume of test virus suspension were mixed with 0.2 parts interfering substance and 9.7 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 Determination of cytotoxicity

Determination of cytotoxicity was performed according to EN 5.5.4.1.

5.7 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. This mixture or PBS as control was 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.

5.8 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).

5.9 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 following 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 ≥ 6.00 ± 0.25).

b) The test product (80.0 % solution) showed no cytotoxicity in the 1:10 dilution thus allowing the detection of a 4 $\log_{10}$ reduction of virus titre.

c) The difference of the logarithmic titre of the virus control minus the logarithmic titre of the test virus in the reference inactivation test (see EN 5.7b) was ≥ 4.00 ± 0.25 (between 3.0 – 5.0) after 30 min and ≥ 4.00 ± 0.25 (between 3.5 – 5.5) after 60 min for adenovirus type 5.

d) The comparative titration on pre-treated (disinfectant) and non-pre-treated (PBS) *A549 cells* showed no significant difference (< 1 $\log_{10}$; EN 5.7) of virus titre: 7.75 ± 0.35 (PBS) versus 7.75 ± 0.33 (1:10 dilution of disinfectant as 80.0 % solution) $\log_{10}$ $TCID_{50}$/ml.

e) The control of efficacy for suppression of disinfectant's activity (80.0 % solution) showed no decrease (≤ 0.5 $\log_{10}$; EN 5.5.5.1) in virus titre (8.00 ± 0.38 versus 7.50 ± 0.35 $\log_{10}$ $TCID_{50}$/ml).

f) 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 adenovirus type 5 according to EN 14476 is valid.

7. Results

Results of examination are shown in tables 1 to 8. Tables 1 to 7 demonstrate the raw data, whereas table 8 (a+b) gives a summary of results.

The undiluted test product as 80.0 % solution was able to inactivate adenovirus type 5 after 30 seconds under clean conditions in this quantitative suspension test (tables 1 and 2). The reduction factors were 4.50 ± 0.58, and 4.75 ± 0.48 (mean value = 4.63 ± 0.38). This corresponded to an inactivation of ≥ 99.99 %.

Tested as 50.0 % solution, the test product was not able to inactivate adenovirus type 5 within 60 seconds under clean conditions in this quantitative suspension test (table 3).

Tested as 10.0 % solution, the test product was not active within 30 minutes of exposure time (table 4).

8. Conclusion

The surface disinfectant Bacticid AF tested undiluted demonstrated activity against adenovirus type 5 after an exposure time of 30 seconds under clean conditions.
Therefore, the surface disinfectant Bacticid AF can be declared as active against adenovirus type 5 as follows:

undiluted **30 seconds** **clean conditions**

Bremen, 06/02/2018

- Dr **ıann -**
He ınager

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

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.

11. Literature

1. EN 14476:2013+A1:2015: Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity of chemicals disinfectants and antiseptics in human medicine test - Test method and requirements (phase 2, step 1)

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

Appendix:

Legend to the Tables

Table 1: Raw data for Bacticid AF (80.0 %) tested against adenovirus type 5 (1st assay)

Table 2: Raw data for Bacticid AF (80.0 %) tested against adenovirus type 5 (2nd assay)

Table 3: Raw data for Bacticid AF (50.0 %) tested against adenovirus type 5

Table 4: Raw data for Bacticid AF (10.0 %) tested against adenovirus type 5

Table 5: Raw data for formaldehyde solution (0.7 %) tested against adenovirus type 5

Table 6: Raw data for control of efficacy for suppression of disinfectant's activity (80.0 %)

Table 7: Raw data (adenovirus type 5) for cell sensitivity (80.0 %)

Table 8 (a+b): Summary of results with Bacticid AF and adenovirus type 5

Legend to the Figures

Figure 1: Virus-inactivating properties of Bacticid AF (80.0 %)

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

Table 1: Raw data for Bacticid AF (80.0 %) tested against adenovirus type 5 at 20 °C (quantal test; 8 wells) (#5347) (1st assay)

Product	Concentration	Interfering substance	Contact time	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	80.0 %	clean conditions	15 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30 s	4333 3333	0000 0303	0300 0300	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			45 s	4203 3304	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			60 s	0000 0303	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
test product cytotoxicity	80.0 %	clean conditions	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	clean conditions	0 min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4033 3144	0100 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)

Table 2: Raw data for Bacticid AF (80.0 %) tested against adenovirus type 5 at 20 °C (quantal test; 8 wells) (#5369) (2nd assay)

Product	Concentration	Interfering substance	Contact time	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	80.0 %	clean conditions	15 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30 s	3333 3333	3000 3000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			45 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	80.0 %	clean conditions	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	clean conditions	0 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3433 3333	0000 1000	0000 3000	0000 0000
			60 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3331 3033	0000 0030	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)

Table 3: Raw data for Bacticid AF (50.0 %) tested against adenovirus type 5 at 20 °C (quantal test; 8 wells) (#5347)

Product	Concentration	Interfering substance	Contact time	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	50.0 %	clean conditions	15 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			20 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60 s	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3011 3233	0020 0000	n.d.	n.d.
test product cytotoxicity	50.0 %	clean conditions	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	clean conditions	0 min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4033 3144	0100 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)

Table 4: Raw data for Bacticid AF (10.0 %) tested against adenovirus type 5 at 20 °C (quantal test; 8 wells) (#5347)

Product	Concentration	Interfering substance	Contact time	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	10.0 %	clean conditions	15 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			20 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3033 3203	0000 0000	0000 0000	n.d.
test product cytotoxicity	10.0 %	clean conditions	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	clean conditions	0 min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4033 3144	0100 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)

Table 5: Raw data for formaldehyde solution (0.7 %) tested against adenovirus type 5 at 20 °C (quantal test; 8 wells) (#5369)

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	4444 4444	4444 4444	3333 3333	3000 0020	0000 0000	0000 0000	n.d.
			15	tttt tttt	tttt tttt	3313 3333	3000 0121	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			30	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			60	tttt tttt	tttt tttt	0000 0000	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	0000 0000	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	4444 4444	3333 2303	0000 0002	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)

Table 6: Raw data for control of efficacy for suppression of disinfectant's activity (80.0 %) (#5369)

Product	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
test product	clean conditions	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3333 3233	3030 3200	0000 0000	n.d.
corresponding virus control	clean conditions	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3331 3033	0000 0030	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)

Table 7: Raw data (adenovirus type 5) for cell sensitivity (80.0 %) (#5369)

Product	Dilution	Dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
PBS	-	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3333 3334	0300 0000	0003 0000	n.d.
test product	1:10	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4433 3344	0300 0200	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 8a: Summary of results with Bacticid AF and adenovirus type 5

Product*	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ...					> 4 log_{10} reduction after ...
				15 s	30 s	45 s	60 s	30 min	
test product (1)	80.0 %	clean conditions	1.50	n.d.	3.00±0.46	≤2.25±0.33	≤1.75±0.33	n.d.	30 s (RF = 4.50±0.58)
test product (2)	80.0 %	clean conditions	1.50	n.d.	2.75±0.33	n.d.	n.d.	n.d.	30 s (RF = 4.75±0.48)
test product (1)	50.0 %	clean conditions	1.50	n.d.	n.d.	n.d.	7.50±0.35	n.d.	> 60 s (RF = 0.00±0.50)
test product (1)	10.0 %	clean conditions	1.50	n.d.	n.d.	n.d.	n.d.	7.25±0.33	> 30 min (RF = 0.25±0.48)

*The number in brackets gives the number of the corresponding virus control, see Table 8b

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

Table 8b: Summary of results with Bacticid AF and adenovirus type 5

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	3.50	n.d.	6.75±0.33	5.00±0.38	≤ 3.50±0.00	≤ 3.50±0.00	30 (RF ≥ 4.00±0.25)
virus control	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	7.50±0.35	n.a.
virus control (1)	n.a.	clean conditions	n.a.	n.d.	n.d.	n.d.	n.d.	7.50±0.35	n.a.
virus control (2) (+ suppression)	n.a.	clean conditions	n.a.	7.75±0.35	n.d.	n.d.	n.d.	7.50±0.35	n.a.
suppression control	80.0 %	clean conditions	1.50	n.d.	n.d.	n.d.	8.00±0.38	n.d.	n.a.
sens.control PBS	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	7.75±0.35	n.a.
sens. control test product	80.0 % → 1:10	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	7.75±0.33	n.a.

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

Figure 1: Virus-inactivating properties of Bacticid AF (80.0 %)

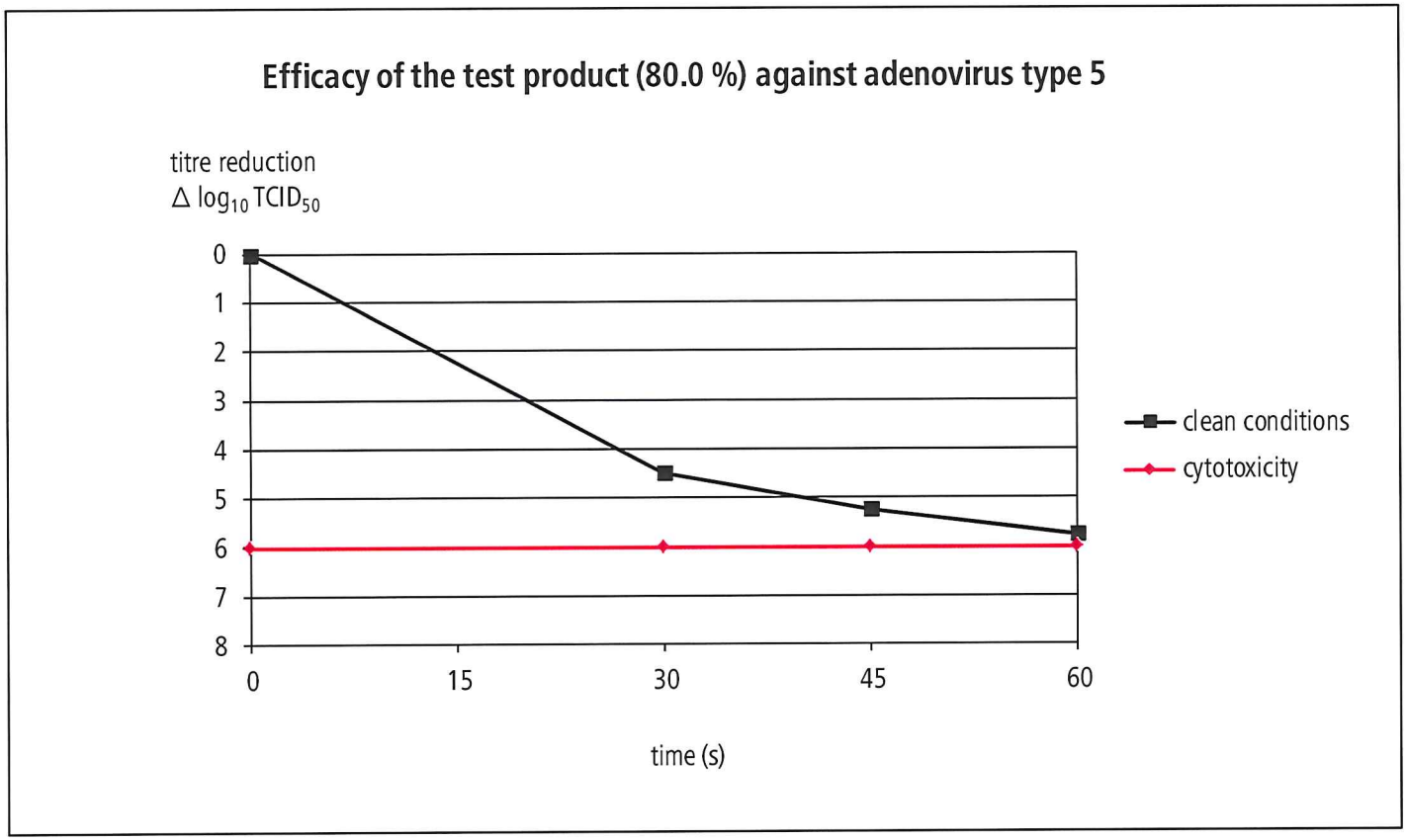
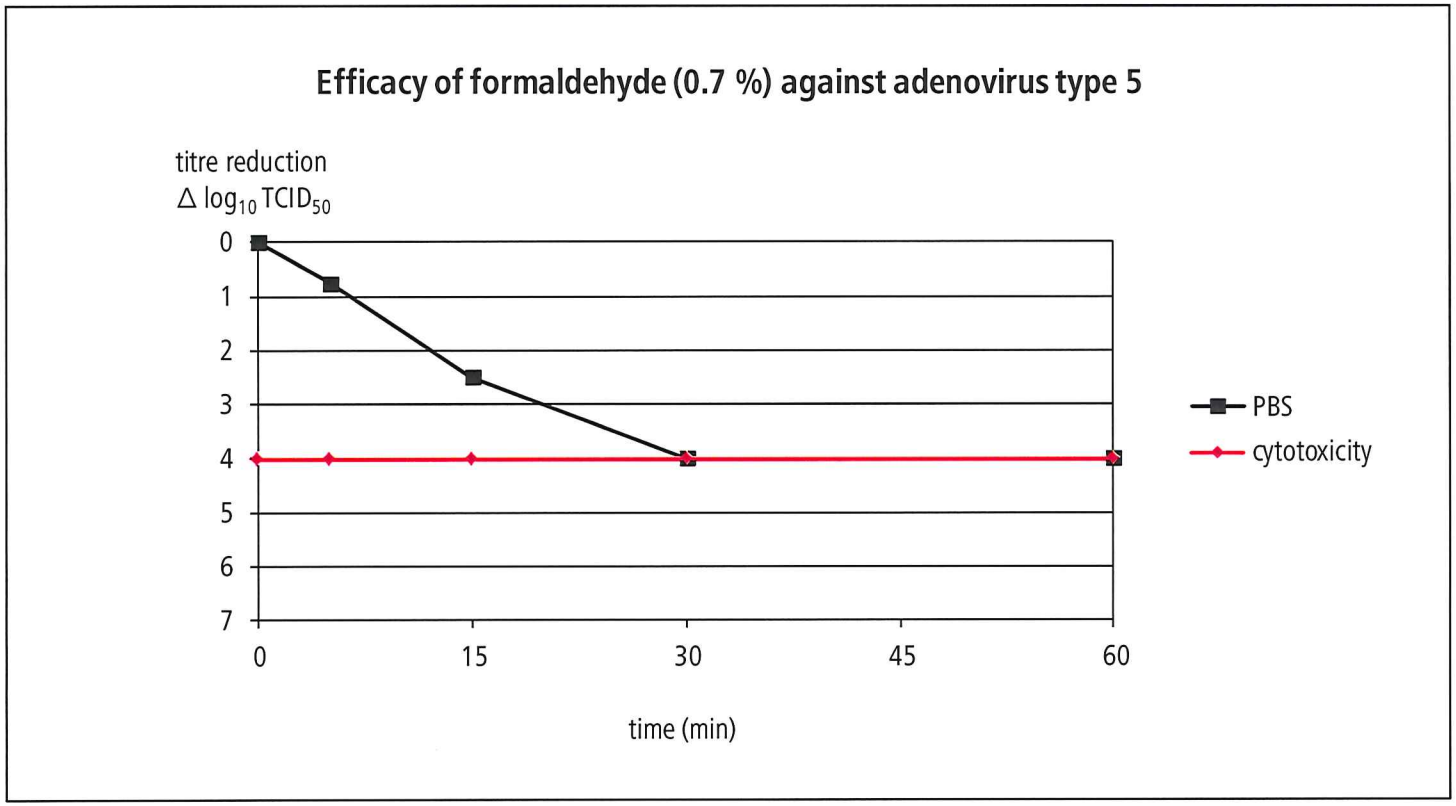


Figure 2: Virus-inactivating properties of formaldehyde (0.7 %)



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

ilac-MRA DAkkS Deutsche Akkreditierungsstelle D-PL-13412-01-01 D-PL-13412-01-02
Anerkannt durch/Recognized by Zentralstelle der Länder für Gesundheitsschutz bei Arzneimitteln und Medizinprodukten
ZLG-AP-216.11.02

26/02/2018

Test report L17/0629aA.2

Evaluation of the effectiveness of

Bacticid AF

Test virus: adenovirus type 5

Method: 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:
Chemi-Pharm AS
Pollu 132
EST – TALLINN 10917

TEST REPORT

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	Chemi-Pharm AS
Name of product	Bacticid AF
Confirmation no.	203850
Product diluent recommended by the manufacturer	-
Batch number	197101017
Application	surface disinfection
Production date	10/10/2017
Expiry date	10/10/2020
Active compound (s) (100 g)	57 g ethanol 6 g IPA
Appearance, odour	clear, colorless liquid product specific
pH-values	undiluted: 7.49 (20 °C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	13/10/2017

3. Materials

3.1 Culture medium and reagents

- Eagle`s Minimum Essential Medium with Earle`s BSS (EMEM, Biozym Scientific GmbH, catalogue no. 880121)
- 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)
- BSA (Sigma-Aldrich-Chemie GmbH, article no. CA-2153)

- sheep erythrocyte s (Fiebig Nährstofftechnik).

3.2 Virus and cells

The adenovirus type 5 strain adenoid 75 was obtained from PD Dr. A. Heim, Institute of Medical Virology, Hannover Medical School, Hannover, Germany. Before the inactivation assays, the virus had been passaged 3 times in *A549 cells* (human lung epithelial carcinoma cells).

The *A549 cells* (passage 111) originated from Vircell, S.L., Spain, 18320 Santa Fe (now BIOTRIN International GmbH, DE - 69126 Heidelberg).

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).

4. Experimental conditions

Test temperature	20 °C ± 1.0 °C
Concentration of test product	undiluted (80.0 %) and as 50.0 % and 10.0 % (demonstration of non-active range) solutions
Appearance of product dilutions	no precipitation
Contact times	30, 45 and 60 seconds and 30 minutes
Interfering substance	3.0 g/l bovine serum albumin + 3.0 g/l eryhtrocytes (dirty conditions, EN 14476)
Procedure to stop action of disinfectant	immediate dilution
Diluent	water
Stability of product in the mix with virus and interfering substance (80.0 % solution)	no clouding, no precipitation
Virus strain	adenovirus type 5 strain adenoid 75 (ATCC VR-5)
Date of testing	19/12/2017 – 26/02/2018
End of testing	26/02/2018

5. Methods

5.1 Preparation of test virus suspension

For preparation of test virus suspension according to EN 5.4.1 *A549 cells* were infected with a multiplicity of infection of 0.1 at 37 °C. After cells showed a cytopathic effect, they were subjected to a threefold freeze/thaw procedure followed by a low speed centrifugation in order to sediment cell debris. After aliquotation of the supernatant, test virus suspension was stored at -80 °C.

5.2 Preparation of disinfectant (dilutions)

The test product was tested undiluted. Due to the addition of interfering substance and test virus suspension an 80.0 % solution resulted.

Furthermore, the product was evaluated as 50.0 % and 10.0 % solutions (demonstrating of non-active range). These solutions were prepared with water immediately before the inactivation tests.

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, beginning with the highest dilution. This was followed by the addition of 0.1 ml of freshly trypsinized *A549 cells*. This cell suspension was adjusted to reach 10-15 x 10^3 cells per well. Microtitre plates were incubated at 37 °C in a 5 % CO_2-atmosphere. The cytopathic effect was read by using an inverted microscope after ten 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 four $\log_{10}$ steps within the recommended exposure period. This corresponds to an inactivation of ≥ 99.99 %.

5.5 Inactivation assay

Determination of virucidal activity has been carried out in accordance to EN 5.5. The test product was examined undiluted (80.0 %) and as 50.0 % and 10.0 % (demonstration of non-active range) solutions in water at 20 °C according to EN 14476. 30, 45 and 60 seconds 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}.

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 Determination of cytotoxicity

Determination of cytotoxicity was performed according to EN 5.5.4.1.

5.7 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. This mixture or PBS as control was 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.

5.8 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).

5.9 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 following 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 ≥ 6.50 ± 0.27).

b) The test product (80.0 % solution) showed no cytotoxicity in the 1:10 dilution thus allowing the detection of a 4 $\log_{10}$ reduction of virus titre.

c) The difference of the logarithmic titre of the virus control minus the logarithmic titre of the test virus in the reference inactivation test (see EN 5.7b) was ≥ 4.00 ± 0.25 (between 3.0 – 5.0) after 30 min and ≥ 4.00 ± 0.25 (between 3.5 – 5.5) after 60 min for adenovirus type 5.

d) The comparative titration on pre-treated (disinfectant) and non-pre-treated (PBS) *A549 cells* showed no significant difference (< 1 $\log_{10}$; EN 5.7) of virus titre: 7.50 ± 0.00 (PBS) versus 7.38 ± 0.41 (1:10 dilution of disinfectant as 80.0 % solution) $\log_{10}$ $TCID_{50}$/ml.

e) The control of efficacy for suppression of disinfectant's activity (80.0 % solution) showed no decrease (≤ 0.5 $\log_{10}$; EN 5.5.5.1) in virus titre (7.50 ± 0.00 versus 7.63 ± 0.25 $\log_{10}$ $TCID_{50}$/ml).

f) 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 adenovirus type 5 according to EN 14476 is valid.

7. Results

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

The undiluted test product as 80.0 % solution was able to inactivate adenovirus type 5 after 30 seconds under dirty conditions in this quantitative suspension test (tables 1, 2, 3 and 4). The reduction factors were 3.00 ± 0.00, 4.75 ± 0.48, ≥ 5.38 ± 0.61 and ≥ 5.50 ± 0.52 (mean value ≥ 4.66 ± 0.23). This corresponded to an inactivation of ≥ 99.99 %.

Tested as 50.0 % solution, the test product was not able to inactivate adenovirus type 5 within 60 seconds under dirty conditions in this quantitative suspension test (table 5).

Tested as 10.0 % solution, the test product was not active within 30 minutes of exposure time (table 6).

8. Conclusion

The surface disinfectant Bacticid AF tested undiluted demonstrated activity against adenovirus type 5 after an exposure time of 30 seconds under dirty conditions.

Therefore, the surface disinfectant Bacticid AF can be declared as active against adenovirus type 5 as follows:

undiluted 30 seconds dirty conditions

Bremen, 26/02/2018

- Dr
He

-
er

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

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.

11. Literature

1. EN 14476:2013+A1:2015: Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity of chemicals disinfectants and antiseptics in human medicine test - Test method and requirements (phase 2, step 1)

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

Appendix:

Legend to the Tables

Table 1: Raw data for Bacticid AF (80.0 %) tested against adenovirus type 5 (1st assay)

Table 2: Raw data for Bacticid AF (80.0 %) tested against adenovirus type 5 (2nd assay)

Table 3: Raw data for Bacticid AF (80.0 %) tested against adenovirus type 5 (3rd assay)

Table 4: Raw data for Bacticid AF (80.0 %) tested against adenovirus type 5 (4th assay)

Table 5: Raw data for Bacticid AF (50.0 %) tested against adenovirus type 5

Table 6: Raw data for Bacticid AF (10.0 %) tested against adenovirus type 5

Table 7: Raw data for formaldehyde solution (0.7 %) tested against adenovirus type 5

Table 8: Raw data for control of efficacy for suppression of disinfectant's activity (80.0 %)

Table 9: Raw data (adenovirus type 5) for cell sensitivity (80.0 %)

Table 10 (a+b): Summary of results with Bacticid AF and adenovirus type 5

Legend to the Figures

Figure 1: Virus-inactivating properties of Bacticid AF (80.0 %)

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

Table 1: Raw data for Bacticid AF (80.0 %) tested against adenovirus type 5 at 20 °C (quantal test; 8 wells) (#5347) (1st assay)

Product	Concentration	Interfering substance	Contact time	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	80.0 %	dirty conditions	15 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30 s	4444 4444	4444 4444	3341 3214	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			45 s	3234 4444	0003 0030	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			60 s	3333 0110	0000 0300	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
test product cytotoxicity	80.0 %	dirty conditions	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0 min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3433 3423	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)

Table 2: Raw data for Bacticid AF (80.0 %) tested against adenovirus type 5 at 20 °C (quantal test; 8 wells) (#5369) (2nd assay)

Product	Concentration	Interfering substance	Contact time	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	80.0 %	dirty conditions	15 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30 s	3333 3333	3000 3000	3000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			45 s	0103 3003	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			60 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	80.0 %	dirty conditions	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3333 3043	0000 0300	0000 0000	0000 0000
			60 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3332 3334	0003 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)

Table 3: Raw data for Bacticid AF (80.0 %) tested against adenovirus type 5 at 20 °C (quantal test; 8 wells) (#5407) (3rd assay)

Product	Concentration	Interfering substance	Contact time	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	80.0 %	dirty conditions	15 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30 s	0200 2200	0000 3300	0030 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			45 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	80.0 %	dirty conditions	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0 min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3434 3333	0000 3000	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)

Table 4: Raw data for Bacticid AF (80.0 %) tested against adenovirus type 5 at 20 °C (quantal test; 8 wells) (#5407) (4th assay)

Product	Concentration	Interfering substance	Contact time	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	80.0 %	dirty conditions	15 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30 s	3203 3233	0000 3000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			45 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	80.0 %	dirty conditions	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0 min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4333 4334	2043 0002	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)

Table 5: Raw data for Bacticid AF (50.0 %) tested against adenovirus type 5 at 20 °C (quantal test; 8 wells) (#5347)

Product	Concentration	Interfering substance	Contact time	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	50.0 %	dirty conditions	15 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			20 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60 s	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4434 3443	3200 0004	n.d.	n.d.
test product cytotoxicity	50.0 %	dirty conditions	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0 min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3433 3423	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)

Table 6: Raw data for Bacticid AF (10.0 %) tested against adenovirus type 5 at 20 °C (quantal test; 8 wells) (#5347)

Product	Concentration	Interfering substance	Contact time	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	10.0 %	dirty conditions	15 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			20 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3040 2430	3200 0000	0000 0000	n.d.
test product cytotoxicity	10.0 %	dirty conditions	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0 min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3433 3423	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)

Table 7: Raw data for formaldehyde solution (0.7 %) tested against adenovirus type 5 at 20 °C (quantal test; 8 wells) (#5369)

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	4444 4444	4444 4444	3333 3333	3000 0020	0000 0000	0000 0000	n.d.
			15	tttt tttt	tttt tttt	3313 3333	3000 0121	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			30	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			60	tttt tttt	tttt tttt	0000 0000	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	0000 0000	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	4444 4444	3333 2303	0000 0002	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)

Table 8: Raw data for control of efficacy for suppression of disinfectant's activity (80.0 %) (#5369)

Product	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
test product	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3444 3233	0000 0000	0000 0000	n.d.
corresponding virus control	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3332 3334	0003 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)

Table 9: Raw data (adenovirus type 5) for cell sensitivity (80.0 %) (#5369)

Product	Dilution	Dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
PBS	-	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3333 3313	0000 0000	0000 0000	n.d.
test product	1:10	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3333 3300	0000 0002	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 10a: Summary of results with Bacticid AF and adenovirus type 5

Product*	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ...					> 4 log_{10} reduction after ...
				15 s	30 s	45 s	60 s	30 min	
test product (1)	80.0 %	dirty conditions	1.50	n.d.	4.50±0.00	2.75±0.33	≤2.38±0.41	n.d.	45 s (RF = 4.75±0.33)
test product (2)	80.0 %	dirty conditions	1.50	n.d.	2.88±0.41	≤2.00±0.33	n.d.	n.d.	30 s (RF = 4.75±0.48)
test product (3)	80.0 %	dirty conditions	1.50	n.d.	≤2.25±0.55	n.d.	n.d.	n.d.	30 s (RF ≥ 5.38±0.61)
test product (4)	80.0 %	dirty conditions	1.50	n.d.	≤2.50±0.35	n.d.	n.d.	n.d.	30 s (RF ≥ 5.50±0.52)
test product (1)	50.0 %	dirty conditions	1.50	n.d.	n.d.	n.d.	7.88±0.37	n.d.	> 60 s (RF = 0.00±0.37)
test product (1)	10.0 %	dirty conditions	1.50	n.d.	n.d.	n.d.	n.d.	7.38±0.49	> 30 min (RF = 0.13±0.49)

*The number in brackets gives the number of the corresponding virus control, see Table 10b

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

Table 10b: Summary of results with Bacticid AF and adenovirus type 5

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	3.50	n.d.	6.75±0.33	5.00±0.38	≤ 3.50±0.00	≤ 3.50±0.00	30 (RF ≥ 4.00±0.25)
virus control	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	7.50±0.35	n.a.
virus control (1)	n.a.	dirty conditions	n.a.	n.d.	n.d.	n.d.	n.d.	7.50±0.00	n.a.
virus control (2) (+ suppression)	n.a.	dirty conditions	n.a.	7.50±0.35	n.d.	n.d.	n.d.	7.63±0.25	n.a.
virus control (3)	n.a.	dirty conditions	n.a.	n.d.	n.d.	n.d.	n.d.	7.63±0.25	n.a.
virus control (4)	n.a.	dirty conditions	n.a.	n.d.	n.d.	n.d.	n.d.	8.00±0.38	n.a.
suppression control	80.0 %	dirty conditions	1.50	n.d.	n.d.	n.d.	7.50±0.00	n.d.	n.a.
sens.control PBS	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	7.50±0.00	n.a.
sens. control test product	80.0 % → 1:10	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	7.38±0.41	n.a.

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

Figure 1: Virus-inactivating properties of Bacticid AF (80.0 %)

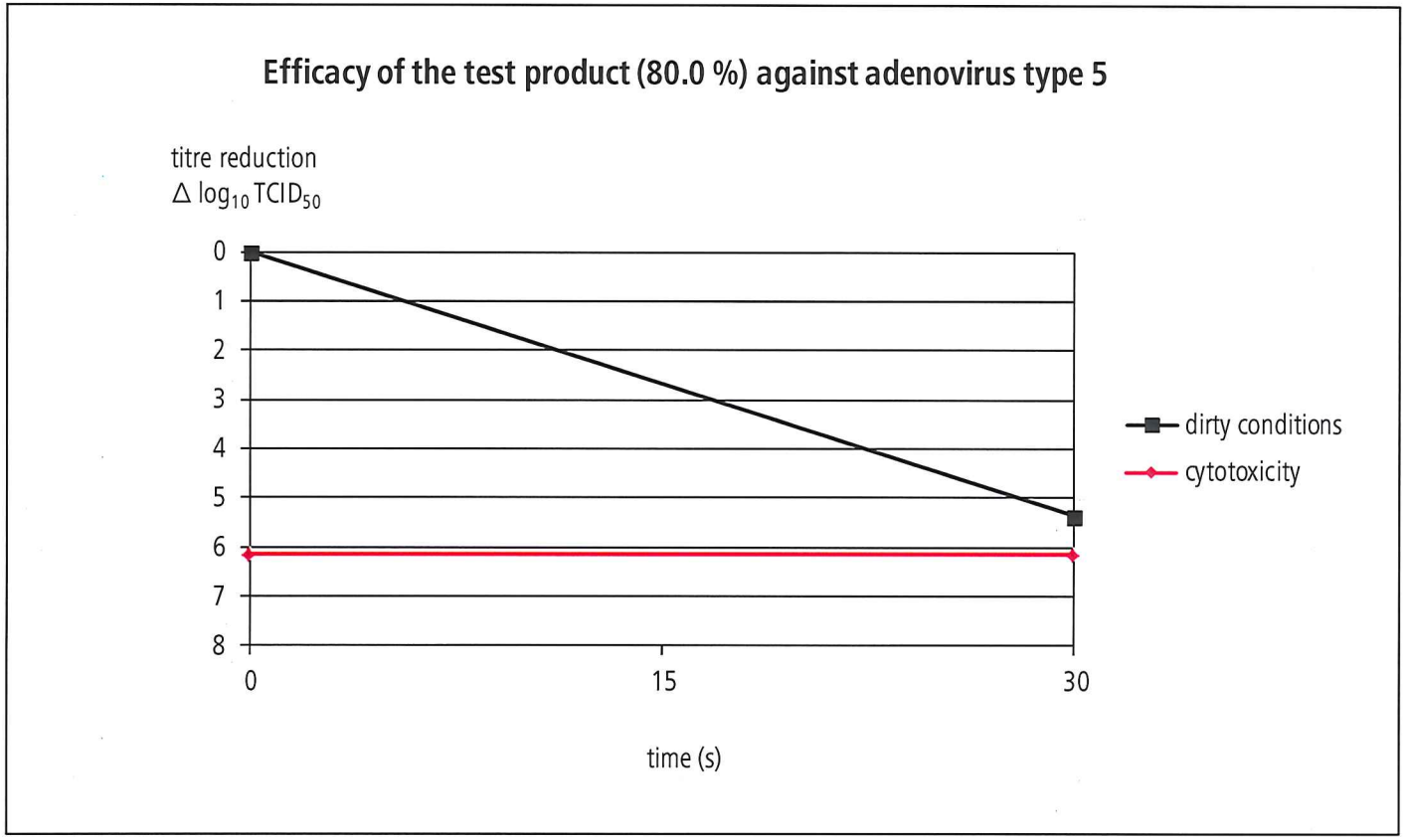
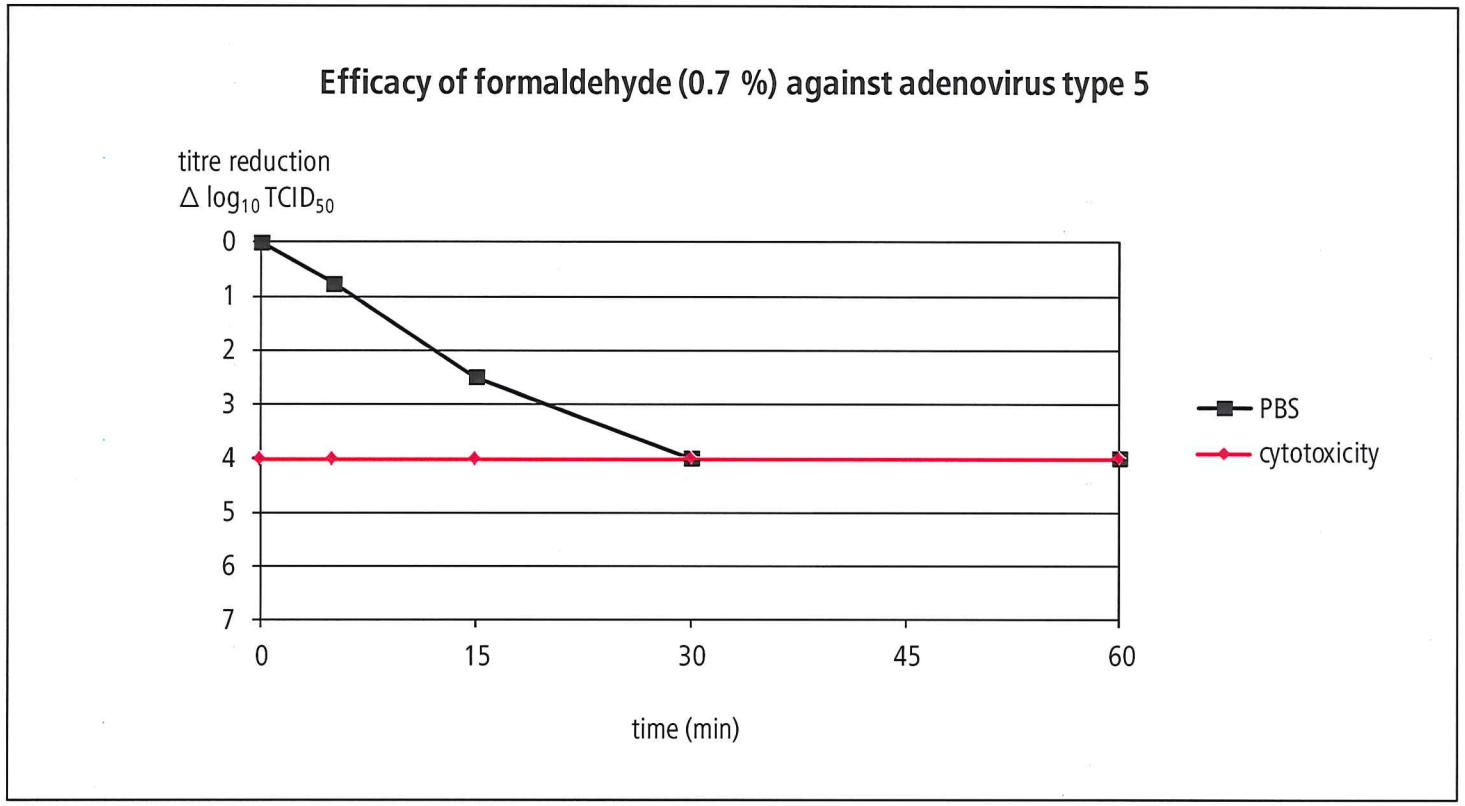


Figure 2: Virus-inactivating properties of formaldehyde (0.7 %)



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

Dr. Brill + Partner GmbH, Norderoog 2, DE - 28259 Bremen

Tel: +49 (40) 557631-0
Fax: +49 (40) 557631-11
E-mail: jochen.steinmann@brillhygiene.com
http://www.brillhygiene.com

Chemi-Pharm AS
Pollu 132
EST – TALLINN 10917

Bremen, 07/02/2018

Summary: Virus-inactivating properties of Bacticid AF of Chemi-Pharm AS according to EN 14476:2013+A1:2015/prA2:2016 under clean conditions

This summary is based on the following test reports of Dr. Brill + Partner GmbH for the surface disinfectant Bacticid AF produced by Chemi-Pharm AS:

adenovirus type 5 test report L17/0629aA.1 dating 06/02/2018

murine norovirus (MNV) test report L17/0629aM.1 dating 07/02/2018

The following concentration and exposure time are necessary for the inactivation of these test viruses:

undiluted 30 seconds

in order to achieve a 4 $\log_{10}$ reduction (inactivation ≥ 99.99 %) under clean conditions in a quantitative suspension test according to EN 14476:2013+A1:2015/prA2:2016.

After evaluation with adenovirus type 5 and MNV the surface disinfectant Bacticid AF can be declared as having **"limited spectrum virucidal activity"** according to EN 14476:2013+A1:2015 /prA2:2016.

The declaration **"limited spectrum virucidal activity"** covers the specified test organisms and all enveloped humanpathogenic viruses like HBV, HCV, HIV and Ebola virus.

Dr. Jo

From Annex A in EN 14476
Examples of viruses which may contaminate human medical instruments, hands, surfaces (*Enveloped viruses are in bold*)

NOTE This list is not exhaustive.

Blood

Enterovirus
Filoviridae
Flavivirus
Herpesviridae
Hepatitis A Virus (HAV)
Hepatitis B virus (HBV)
Hepatitis C virus (HCV)
Hepatitis Delta virus (HDV)
Human Immunodeficiency Virus (HIV)
Human T Cell Leukemia Virus (HTLV)
Parvovirus B 19

Respiratory tract

Adenovirus (Mast-)
Coronavirus
Enterovirus
Herpesviridae
Influenza Virus
Paramyxoviridae
Rhinovirus
Rubella Virus

Neural tissue, ear & nose, eye

Adenovirus (Mast-)
Enterovirus
Herpesviridae
Measles Virus
Human Immunodeficiency Virus (HIV)
Polyomavirus
Rabies Virus
Rubella Virus

Gastro-intestinal

Adenovirus (Mast-)
Caliciviridae
Coronavirus
Astrovirus
Enterovirus
Hepatitis A Virus (HAV)
Hepatitis E Virus (HEV)
Rotavirus

Skin, breast and/or milk

Enterovirus
Herpesviridae
Human Immunodeficiency Virus (HIV)
Human T Cell Leukemia Virus (HTLV)
Papillomavirus
Poxviridae

Spleen and lymph nodes (see also „Blood")

Human T Cell Leukemia Virus (HTLV)
Human Immunodeficiency Virus (HIV)

Dental procedure

Adenovirus (Mast-)
Enterovirus
Herpesviridae
Hepatitis B virus (HBV)
Hepatitis C Virus (HCV)
Hepatitis Delta Virus (HDV)
Human Immunodeficiency Virus (HIV)

Urogenital tract

Hepatitis B Virus (HBV)
Herpesviridae
Human Immunodeficiency Virus (HIV)
Human T Cell Leukemia Virus (HTLV)
Papillomavirus
Polyomavirus

Reference:
Van Regenmortel MHV et al., Eds.: Virus Taxonomy, Classification and Nomenclature of Viruses, seventh report of the international committee on taxonomy of viruses.
Academic Press, San Diego, 2000

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

Dr. Brill + Partner GmbH, Norderoog 2, DE - 28259 Bremen

Tel: +49 (40) 557631-0
Fax: +49 (40) 557631-11
E-mail: jochen.steinmann@brillhygiene.com
http://www.brillhygiene.com

Chemi-Pharm AS
Pollu 132
EST – TALLINN 10917

Bremen, 07/02/2018

Summary: Virus-inactivating properties of Bacticid AF of Chemi-Pharm AS according to EN 14476:2013+A1:2015/prA2:2016 under clean conditions

This summary is based on the following test reports of Dr. Brill + Partner GmbH for the surface disinfectant Bacticid AF produced by Chemi-Pharm AS:

adenovirus type 5 test report L17/0629aA.1 dating 06/02/2018

murine norovirus (MNV) test report L17/0629aM.1 dating 07/02/2018

The following concentration and exposure time are necessary for the inactivation of these test viruses:

undiluted 30 seconds

in order to achieve a 4 $\log_{10}$ reduction (inactivation ≥ 99.99 %) under clean conditions in a quantitative suspension test according to EN 14476:2013+A1:2015/prA2:2016.

After evaluation with adenovirus type 5 and MNV the surface disinfectant Bacticid AF can be declared as having **"limited spectrum virucidal activity"** according to EN 14476:2013+A1:2015 /prA2:2016.

The declaration **"limited spectrum virucidal activity"** covers the specified test organisms and all enveloped humanpathogenic viruses like HBV, HCV, HIV and Ebola virus.

Dr.

From Annex A in EN 14476
Examples of viruses which may contaminate human medical instruments, hands, surfaces (*Enveloped viruses are in bold*)

NOTE This list is not exhaustive.

Blood

Enterovirus
Filoviridae
Flavivirus
Herpesviridae
Hepatitis A Virus (HAV)
Hepatitis B virus (HBV)
Hepatitis C virus (HCV)
Hepatitis Delta virus (HDV)
Human Immunodeficiency Virus (HIV)
Human T Cell Leukemia Virus (HTLV)
Parvovirus B 19

Respiratory tract

Adenovirus (Mast-)
Coronavirus
Enterovirus
Herpesviridae
Influenza Virus
Paramyxoviridae
Rhinovirus
Rubella Virus

Neural tissue, ear & nose, eye

Adenovirus (Mast-)
Enterovirus
Herpesviridae
Measles Virus
Human Immunodeficiency Virus (HIV)
Polyomavirus
Rabies Virus
Rubella Virus

Gastro-intestinal

Adenovirus (Mast-)
Caliciviridae
Coronavirus
Astrovirus
Enterovirus
Hepatitis A Virus (HAV)
Hepatitis E Virus (HEV)
Rotavirus

Skin, breast and/or milk

Enterovirus
Herpesviridae
Human Immunodeficiency Virus (HIV)
Human T Cell Leukemia Virus (HTLV)
Papillomavirus
Poxviridae

Spleen and lymph nodes (see also „Blood")

Human T Cell Leukemia Virus (HTLV)
Human Immunodeficiency Virus (HIV)

Dental procedure

Adenovirus (Mast-)
Enterovirus
Herpesviridae
Hepatitis B virus (HBV)
Hepatitis C Virus (HCV)
Hepatitis Delta Virus (HDV)
Human Immunodeficiency Virus (HIV)

Urogenital tract

Hepatitis B Virus (HBV)
Herpesviridae
Human Immunodeficiency Virus (HIV)
Human T Cell Leukemia Virus (HTLV)
Papillomavirus
Polyomavirus

Reference:
Van Regenmortel MHV et al., Eds.: Virus Taxonomy, Classification and Nomenclature of Viruses, seventh report of the international committee on taxonomy of viruses.
Academic Press, San Diego, 2000

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

ilac-MRA DAkkS Deutsche Akkreditierungsstelle D-PL-13412-01-01 D-PL-13412-01-02
Anerkannt durch/Recognized by Zentralstelle der Länder für Gesundheitsschutz bei Arzneimitteln und Medizinprodukten www.zlg.de
ZLG-AP-216.11.02

07/02/2018

Test report L17/0629aM.2

Evaluation of the effectiveness of

Bacticid AF

Test virus: murine norovirus (as surrogate of human norovirus)

Method: 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:
Chemi-Pharm AS
Pollu 132
EST – TALLINN 10917

TEST REPORT

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	Chemi-Pharm AS
Name of product	Bacticid AF
Confirmation no.	203850
Product diluent recommended by the manufacturer	-
Batch number	197101017
Application	surface disinfection
Production date	10/10/2017
Expiry date	10/10/2010
Active compound (s) (100 g)	57 g ethanol 6 g IPA
Appearance, odour	clear, colorless liquid product specific
pH-values	undiluted: 7.49 (20 °C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	13/10/2017

3. Materials

3.1 Culture medium and reagents

- Dulbecco´s Modified Eagle´s Medium (DMEM, Biozym Scientific GmbH, catalogue no. 880006)
- Fetal calf serum (Thermo Fisher, article no. CH30160.02)
- 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)
- BSA (Sigma-Aldrich-Chemie GmbH, article no. CA-2153)

- sheep erythrocyte s (Fiebig Nährstofftechnik).

3.2 Virus and cells

Murine norovirus (MNV) was obtained from PD. Dr. E. Schreier, Head of FG15 Molecular Epidemiology of Viral Pathogens at the Robert Koch-Institute (RKI) in Berlin. Prior to inactivation, MNV was passaged three times in *RAW 264.7 cells* (a macrophage-like, Abelson leukemia virus transformed cell line derived from BALB/c mice, ATCC TIB-71). *RAW 264.7 cells* were cultured with Dulbecco's Modified Eagle's Medium with 4.5 g/l glucose and fetal calf serum with low endotoxin. Furthermore, cells (passage 25) 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).