



26/02/2018

Test report L17/0629cA.1

Evaluation of the effectiveness of **Chemisept med**

Test virus: adenovirus type 5

Method: EN 14476:2013+A1:2015 (clean 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

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	Chemisept med
Confirmation no.	203850
Product diluent recommended by the manufacturer	-
Batch number	196101017
Application	hand disinfection
Production date	10/10/2017
Expiry date	10/10/2020
Active compound (s) (100 g)	72.5 g ethanol 7.5 g IPA
Appearance, odour	clear, colorless liquid product specific
pH-values	undiluted: 5.38 (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).

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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

- CO₂ 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	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 and 45 seconds and 30 minutes
Interfering substance	0.3 g/l bovine serum albumin (clean 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)	medium 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.

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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, 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 $10\text{-}15 \times 10^3$ cells per well. Microtitre plates were incubated at 37 °C in a 5 % CO₂-atmosphere. The cytopathic effect was read by using an inverted microscope after ten days. Calculation of the infective dose TCID₅₀/ml was calculated with the method of Spearman (2) and Kärber (3) with the following formula:

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

meaning

X_0 = log₁₀ 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 log₁₀ 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 and 45 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.

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Titration 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 $20\text{ °C} \pm 1.0\text{ °C}$. 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} .

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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 $\geq 4 \log_{10}$ reduction (maximal virus reduction $\geq 6.00 \pm 0.25$).
- b) The test product (80.0 % solution) showed no cytotoxicity in the 1:10 dilution thus allowing the detection of a \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 $\geq 4.00 \pm 0.25$ (between 3.0 – 5.0) after 30 min and $\geq 4.00 \pm 0.25$ (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 8.00 ± 0.38 (1:10 dilution of disinfectant as 80.0 % solution) \log_{10} TCID₅₀/ml.
- e) The control of efficacy for suppression of disinfectant's activity (80.0 % solution) showed no decrease ($\leq 0.5 \log_{10}$; EN 5.5.5.1) in virus titre (7.88 ± 0.45 versus $7.50 \pm 0.35 \log_{10}$ TCID₅₀/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.

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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 clean conditions in this quantitative suspension test (tables 1, 2, 3 and 4). The reduction factors were 3.63 ± 0.51 , $\geq 5.13 \pm 0.43$, $\geq 5.25 \pm 0.44$ and $\geq 5.00 \pm 0.35$ (mean value $\geq 4.75 \pm 0.22$). 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 30 seconds under clean 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 hand disinfectant Chemisept med tested undiluted demonstrated activity against adenovirus type 5 after an exposure time of 30 seconds under clean conditions.

Therefore, the hand disinfectant Chemisept med can be declared as active against adenovirus type 5 as follows:

undiluted 30 seconds clean conditions

Bremen, 26/02/2018



- 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 (BGBl. I, 1994, page 1703). Appendix revised at 14. 05. 1997 (BGBl. 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.

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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 Pharmac; 162, 1931, 480-487

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Appendix:

Legend to the Tables

Table 1:	Raw data for Chemisept med (80.0 %) tested against adenovirus type 5 (1 st assay)
Table 2:	Raw data for Chemisept med (80.0 %) tested against adenovirus type 5 (2 nd assay)
Table 3:	Raw data for Chemisept med (80.0 %) tested against adenovirus type 5 (3 rd assay)
Table 4:	Raw data for Chemisept med (80.0 %) tested against adenovirus type 5 (4 th assay)
Table 5:	Raw data for Chemisept med (50.0 %) tested against adenovirus type 5
Table 6:	Raw data for Chemisept med (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 Chemisept med and adenovirus type 5

Legend to the Figures

Figure 1:	Virus-inactivating properties of Chemisept med (80.0 %)
Figure 2:	Virus-inactivating properties of formaldehyde (0.7 %)

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Table 1: Raw data for Chemisept med (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.	n.d.	n.d.	
			20 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
			30 s	4444	4333	0003	0000	0000	0000	0000	0000	0000	0000	0000	0000
				4444	4444	0340	0000	0000	0000	0000	0000	0000	0000	0000	0000
test product cytotoxicity	80.0 %	clean conditions	60 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
			n.a.	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.	n.d.	n.d.	
			60 min	4444	4444	4444	4444	4444	4444	4033	0100	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 Chemisept med (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.	n.d.	n.d.		
			30 s	3333	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	
			45 s	3043	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.
			60 s	3000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.
test product cytotoxicity	80.0 %	clean conditions	n.a.	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.		
virus control	n.a.	clean conditions	0 min	4444	4444	4444	4444	4444	4444	4444	4444	4444	4444	4444	0000	
			60 min	4444	4444	4444	4444	4444	4444	4444	4444	4444	4444	4444	4444	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 Chemisept med (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 %	clean conditions	15 s	n.d.	n.d.	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.	n.d.	n.d.	n.d.	
			30 s	4343 3444	0000 0003	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.
test product cytotoxicity	80.0 %	clean conditions	60 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
			n.a.	0000 0000	0000 0000	0000 0000	0000 0000	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.	n.d.	n.d.	n.d.	n.d.
			60 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3343 3444	2000 2300	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 4: Raw data for Chemisept med (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 %	clean conditions	15 s	n.d.	n.d.	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.	n.d.	n.d.	
			30 s	4334	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000
				4433	0003	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000
60 s	n.d.	n.d.	n.d.	n.d.	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	0000	
			0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	
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.	n.d.	n.d.	
			60 min	4444	4444	4444	4444	4444	4444	4444	4444	4444	4444	4444	

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 Chemisept med (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.	n.d.	n.d.
			20 s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30 s	4444 4444	4444 4444	4444 4444	4444 4444	4343 3344	3330 3323	0000 0000	n.d.	n.d.	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.	n.d.	
			0 min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	clean conditions	60 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4033 3144	0100 0000	0000 0000	0000 0000	

n.a. = not applicable
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 Chemisept med (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.	n.d.	n.d.
			20 s	n.d.	n.d.	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.	n.d.	n.d.
test product cytotoxicity	10.0 %	clean conditions	30 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3032 0434	2002 0100	0000 0000	n.d. n.d.	
			n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	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.	n.d.	n.d.
			60 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4033 3144	0100 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)





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	4444	4444	3333	3000	0000	0000	0000	n.d.	
				tttt	tttt	4444	4444	3333	0020	0000	0000	0000	0000	
			15	tttt	tttt	3313	3000	0000	0000	0000	0000	0000	0000	n.d.
				tttt	tttt	3333	0121	0000	0000	0000	0000	0000	0000	0000
formaldehyde cytotoxicity	0.7 % (m/V)	PBS	30	tttt	tttt	0000	0000	0000	0000	0000	0000	0000	n.d.	
				tttt	tttt	0000	0000	0000	0000	0000	0000	0000	0000	
			60	tttt	tttt	0000	0000	0000	0000	0000	0000	0000	0000	n.d.
				tttt	tttt	0000	0000	0000	0000	0000	0000	0000	0000	0000
virus control	n.a.	PBS	n.a.	tttt	tttt	0000	0000	0000	n.d.	n.d.	n.d.	n.d.		
				tttt	tttt	0000	0000	0000	n.d.	n.d.	n.d.	n.d.		
virus control	n.a.	PBS	60	4444	4444	4444	4444	4444	3333	0000	0000	0000		
				4444	4444	4444	4444	4444	2303	0002	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	clean conditions	4444	4444	4444	4444	4444	3333	1220	0000	n.d.
		4444	4444	4444	4444	4444	3310	0004	0000	
corresponding virus control	clean conditions	4444	4444	4444	4444	4444	3331	0000	0000	0000
		4444	4444	4444	4444	4444	3033	0030	0000	0000

n.a. = not applicable

0 = no virus present; t = cytotoxic

1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

n.d. = not done



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	4444 4444	0300 0000	0003 0000	n.d.
test product	1:10	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4443 3334	3300 0024	0000 0000	n.d.

n.a. = not applicable

0 = no virus present; t = cytotoxic

n.d. = not done
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)



Table 10a: Summary of results with Chemisept med and adenovirus type 5

Product*	Concentration	Interfering substance	Level of cytotoxicity	log ₁₀ TCID ₅₀ /ml after ...					> 4 log ₁₀ reduction after ...
				15 s	20 s	30 s	45 s	30 min	
test product (1)	80.0 %	clean conditions	1.50	n.d.	n.d.	3.88±0.37	n.d.	n.d.	> 30 s (RF = 3.63±0.51)
test product (2)	80.0 %	clean conditions	1.50	n.d.	n.d.	≤2.38±0.25	≤1.75±0.33	n.d.	30 s (RF ≥ 5.13±0.43)
test product (3)	80.0 %	clean conditions	1.50	n.d.	n.d.	≤2.63±0.25	n.d.	n.d.	30 s (RF ≥ 5.25±0.44)
test product (4)	80.0 %	clean conditions	1.50	n.d.	n.d.	≤2.63±0.25	n.d.	n.d.	30 s (RF = 5.00±0.35)
test product (1)	50.0 %	clean conditions	1.50	n.d.	n.d.	7.38±0.25	n.d.	n.d.	> 30 s (RF = 0.13±0.43)
test product (1)	10.0 %	clean conditions	1.50	n.d.	n.d.	n.d.	n.d.	7.63±0.49	> 30 min (RF = 0.00±0.61)

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

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



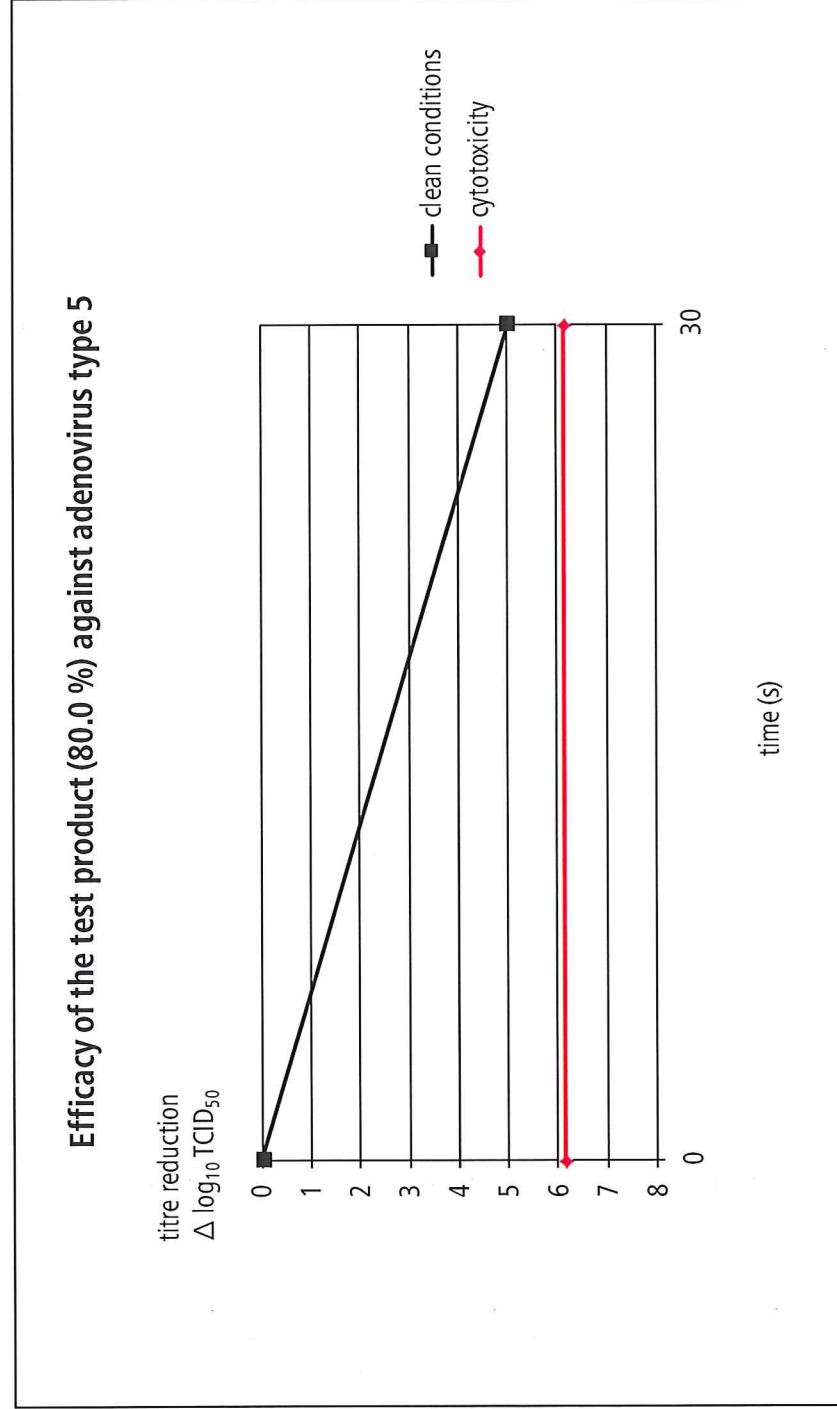
Table 10b: Summary of results with Chemisept med and adenovirus type 5

Product	Con- centration	Interfering substance	Level of cytotoxicity	log ₁₀ TCID ₅₀ /ml aftermin					> 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.
virus control (3)	n.a.	clean conditions	n.a.	n.d.	n.d.	n.d.	n.d.	7.88±0.37	n.a.
virus control (4)	n.a.	clean conditions	n.a.	n.d.	n.d.	n.d.	n.d.	7.63±0.25	n.a.
suppression control	80.0 %	clean conditions	1.50	n.d.	n.d.	n.d.	7.88±0.45	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.	8.00±0.38	n.a.

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



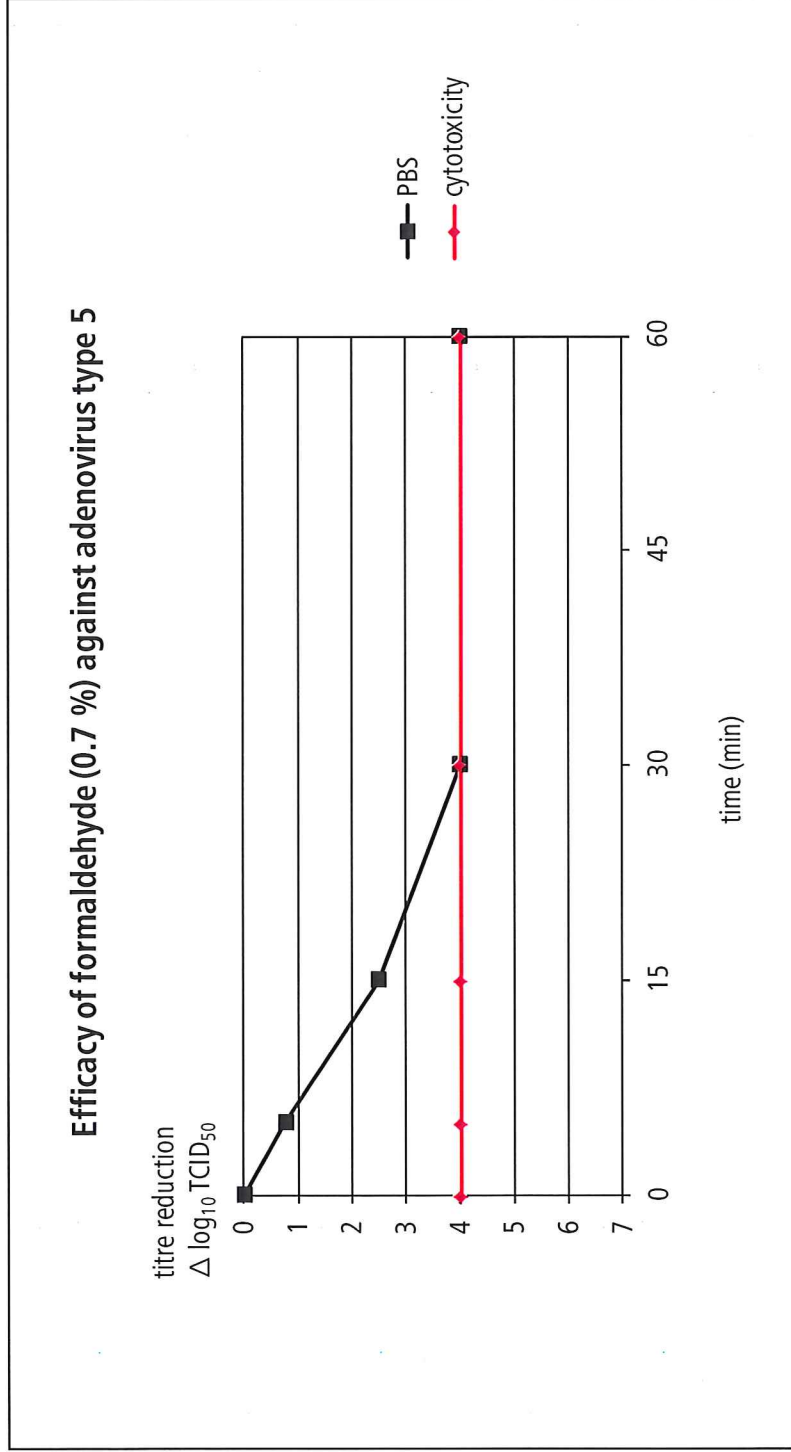
Figure 1: Virus-inactivating properties of Chemisept med (80.0 %)



* Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Nordroog 2, DE – 28259 Bremen, Germany, Telephone +49. 40. 557631-0, Telefax +49. 40. 557631-11, www.brillhygiene.com. 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 on request. © Dr. Brill + Partner GmbH 2018



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



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