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INSTITUT FÜR HYGIENE UND MIKROBIOLOGIE

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Anerkannt durch/Recognized by Zentralstelle der Länder für Gesundheitsschutz bei Arzneimitteln und Medizinprodukten
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Test report C15L0096aA

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Evaluation of the effectiveness of

Chemides Pulver

Test virus: adenovirus type 5

Method: EN 14476:2013/FprA1 2015

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, D - 28259 Bremen
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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	Chemides Pulver
Product diluent recommended by the manufacturer	-
Batch number	30180215
Application	disinfectant for instruments and surfaces
Production date	18.02.2015
Expiry date	2017/02
Active compound (s) (kg)	peracetic acid in reaction mixture
Appearance, odour	white powder product specific
pH-values (in WSH)	1.75 %: 8.21 (20 °C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	23.02.2015

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 erythrocytes (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 116) originated from Vircell, S.L., Spain, 18320 Santa Fe (now BIOTRIN International GmbH, D-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	1.75 %, 1.0 %, 0.5 %, 0,1 % and 0.01 % (demonstration of non-active range) solutions
Appearance of product dilutions	no precipitation
Contact times	5, 10 and 30 minutes
Interfering substance	3.0 g/l bovine serum albumin + 3.0 g/l erythrocytes (dirty conditions EN 14476:2013)
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 (1.75 % solution)	no flocculation, no precipitation
Virus strain	adenovirus type 5 strain adenoid 75 (ATCC VR-5)
Date of testing	23.02.2015 – 18.06.2015
End of testing	18.06.2015

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)

A working solution of the test product was prepared by dissolving 17.5 g of the powder (taking into account a factor of 1.25 due to addition of 1 part test virus and 1 part interfering substance to 8 parts of disinfectant) in pre-warmed WSH. Further dilutions (1.0 %, 0.5 %, 0.1 % and 0.01 %) were prepared out of this working solution 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 14476:2013, 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 as 1.75 %, 1.0 %, 0.5 %, 0.1 % and 0.01 % (demonstration of non-active range) solutions in WSH at 20 °C according to EN 14476:2013. 5, 10 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 after the longest exposure time (EN 5.5.7).

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 ≥ 4.88 ± 0.18).

b) The test product (1.75 % and 1.0 % solutions) showed cytotoxicity in the 1:10 dilutions 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 ≥ 4.13 ± 0.37 (between 3.0 – 5.0) after 30 min and ≥ 4.13 ± 0.37 (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: 7.63 ± 0.25 (PBS) versus 7.38 ± 0.49 (1:100 dilutions of disinfectant as 1.75 % solution) $log_{10}TCID_{50}$/ml.

e) The control of efficacy for suppression of disinfectant's activity (1.75 %) showed a decrease (< 0.5 log_{10}; EN 5.5.5.1) in virus titre (≤ 2.50 ± 0.00 versus 7.38 ± 0.25 $log_{10}TCID_{50}$/ml) due to the fact that even the 0.1 % solution showed a reduction of virus titre (RF ≥ 4.88 ± 0.18 after 30 minutes). In these experiments at the end of the defined exposure time the test mixture was immediately diluted and the dilutions transferred to the cell culture. Therefore, despite the insufficient control of efficacy for suppression the assay is valid.

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 14476:2013 is valid.

7. Results

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

The test product as 1.75 % solution was able to inactivate adenovirus type 5 after 5 minutes in this quantitative suspension test. The reduction factor was ≥ 4.88 ± 0.18 (Table 1).

The 1.0 % solution of the test product was also active within 5 minutes of exposure time (Table 2). The reduction factor was ≥ 4.88 ± 0.18.

In addition, even the 0.5 % solution of the test product was active within 5 minutes of exposure time (Table 3). The reduction factor was ≥ 4.88 ± 0.18. This corresponded to an inactivation of ≥ 99.99 %.

Tested as 0.1 % solution the test product was active within 30 minutes of exposure time (Table 4). The reduction factor was ≥ 4.88 ± 0.18.

Examined as 0.01 % solution the test product was not active within 30 minutes of exposure time (Table 5).

8. Conclusion

The instrument disinfectant Chemides Pulver tested as 0.5 % solution demonstrated effectiveness against adenovirus type 5 after an exposure time of 5 minutes under dirty conditions. The 0.1 % solution was active within 30 minutes.
Therefore, the instrument disinfectant Chemides Pulver can be declared as active against adenovirus type 5 as follows:

0.5 % 5 minutes

0.1 % 30 minutes

Bremen, 18.06.2015

- Dr. Jochen Steinmann -
Scientific Director

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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.

11. Literature

1. EN 14476:2013/FprA1 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 Chemides Pulver (1.75 %) tested against adenovirus type 5
Table 2:	Raw data for Chemides Pulver (1.0 %) tested against adenovirus type 5
Table 3:	Raw data for Chemides Pulver (0.5 %) tested against adenovirus type 5
Table 4:	Raw data for Chemides Pulver (0.1 %) tested against adenovirus type 5
Table 5:	Raw data for Chemides Pulver (0.01 %) tested against adenovirus type 5
Table 6:	Raw data for formaldehyde solution (0.7 %) tested against adenovirus type 5
Table 7:	Raw data for control of efficacy for suppression of disinfectant activity (1.75 %)
Table 8:	Raw data (adenovirus type 5) for cell sensitivity (1.75 %)
Table 9 (a+b):	Summary of results with Chemides Pulver and adenovirus type 5

Legend to the Figures

Figure 1:	Virus-inactivating properties of Chemides Pulver (0.5 %)
Figure 2:	Virus-inactivating properties of formaldehyde (0.7 %)

Table 1: Raw data for Chemides Pulver (1.75 %) tested against adenovirus type 5 at 20 °C (quantal test; 8 wells) (3938)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	1.75%	dirty conditions	5	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			10	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	1.75%	dirty conditions	n.a.	tttt tttt	0000 0000	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	4444 4444	4440 4444	4300 0304	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4440 4444	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 Chemides Pulver (1.0 %) tested against adenovirus type 5 at 20 °C (quantal test; 8 wells) (3938)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	1.0%	dirty conditions	5	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			10	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	1.0%	dirty conditions	n.a.	tttt tttt	0000 0000	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	4444 4444	4440 4444	4300 0304	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4440 4444	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 3: Raw data for Chemides Pulver (0.5 %) tested against adenovirus type 5 at 20 °C (quantal test; 8 wells) (3938)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	0.5%	dirty conditions	5	n.d.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	0.5%	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	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4440 4444	4300 0304	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4440 4444	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 Chemides Pulver (0.1 %) tested against adenovirus type 5 at 20 °C (quantal test; 8 wells) (3938)

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	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	n.d.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	0.1%	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	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4440 4444	4300 0304	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4440 4444	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 5: Raw data for Chemides Pulver (0.01 %) tested against adenovirus type 5 at 20 °C (quantal test; 8 wells) (3938)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	0.01%	dirty conditions	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4422 4044	3000 0000	0400 0000	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	0.01%	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	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4440 4444	4300 0304	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4440 4444	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 formaldehyde solution (0.7 %) tested against adenovirus type 5 at 20 °C (quantal test; 8 wells) (3938)

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	0420 3333	0300 0000	0000 0000	0000 0000	n.d.
			15	tttt tttt	tttt tttt	3333 3424	0000 0302	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	4403 4440	0400 0040	0004 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 control of efficacy for suppression of disinfectant's activity (1.75 %) (3938)

Product	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
test product	PBS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product	clean conditions	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product	dirty conditions	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	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 8: Raw data (adenovirus type 5) for cell sensitivity (1.75 %) (3938)

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	4423 3444	0000 0002	0000 0000	n.d.
test product	1:10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product	1:100	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4440 0404	0400 4000	0000 0000	n.d.
test product	1:1,000	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	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 9a: Summary of results with Chemides Pulver 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
				5	10	15	30	60	
test product	1.75%	dirty conditions	2.50	≤2.50±0.00	≤2.50±0.00	n.d.	n.d.	n.d.	5 (RF ≥ 4.88±0.18)
test product	1.0%	dirty conditions	2.50	≤2.50±0.00	≤2.50±0.00	n.d.	n.d.	n.d.	5 (RF ≥ 4.88±0.18)
test product	0.5%	dirty conditions	1.50	≤2.50±0.00	n.d.	n.d.	n.d.	n.d.	5 (RF ≥ 4.88±0.18)
test product	0.1%	dirty conditions	1.50	n.d.	n.d.	n.d.	≤2.50±0.00	n.d.	30 (RF ≥ 4.88±0.18)
test product	0.01%	dirty conditions	1.50	n.d.	n.d.	n.d.	7.63±0.43	n.d.	> 30

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

Table 9b: Summary of results with Chemides Pulver 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.	6.38±0.41	4.75±0.33	≤3.50±0.00	≤3.50±0.00	30 (RF ≥4.13±0.37)
virus contr.	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	7.63±0.43	n.a.
virus contr. 1	n.a.	dirty conditions	n.a.	7.88±0.45	n.d.	n.d.	n.d.	7.38±0.25	n.a.
suppression control	1.75%	dirty conditions	2.50	n.d.	n.d.	n.d.	≤2.50±0.00	n.d.	n.a.
sens.control PBS	n.a.	dirty conditions	n.a.	n.d.	n.d.	n.d.	n.d.	7.63±0.25	n.a.
sens. control test product	1.75% → 1:100	dirty conditions	n.a.	n.d.	n.d.	n.d.	n.d.	7.38±0.49	n.a.

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

Figure 1: Virus-inactivating properties of Chemides Pulver (0.5 %)

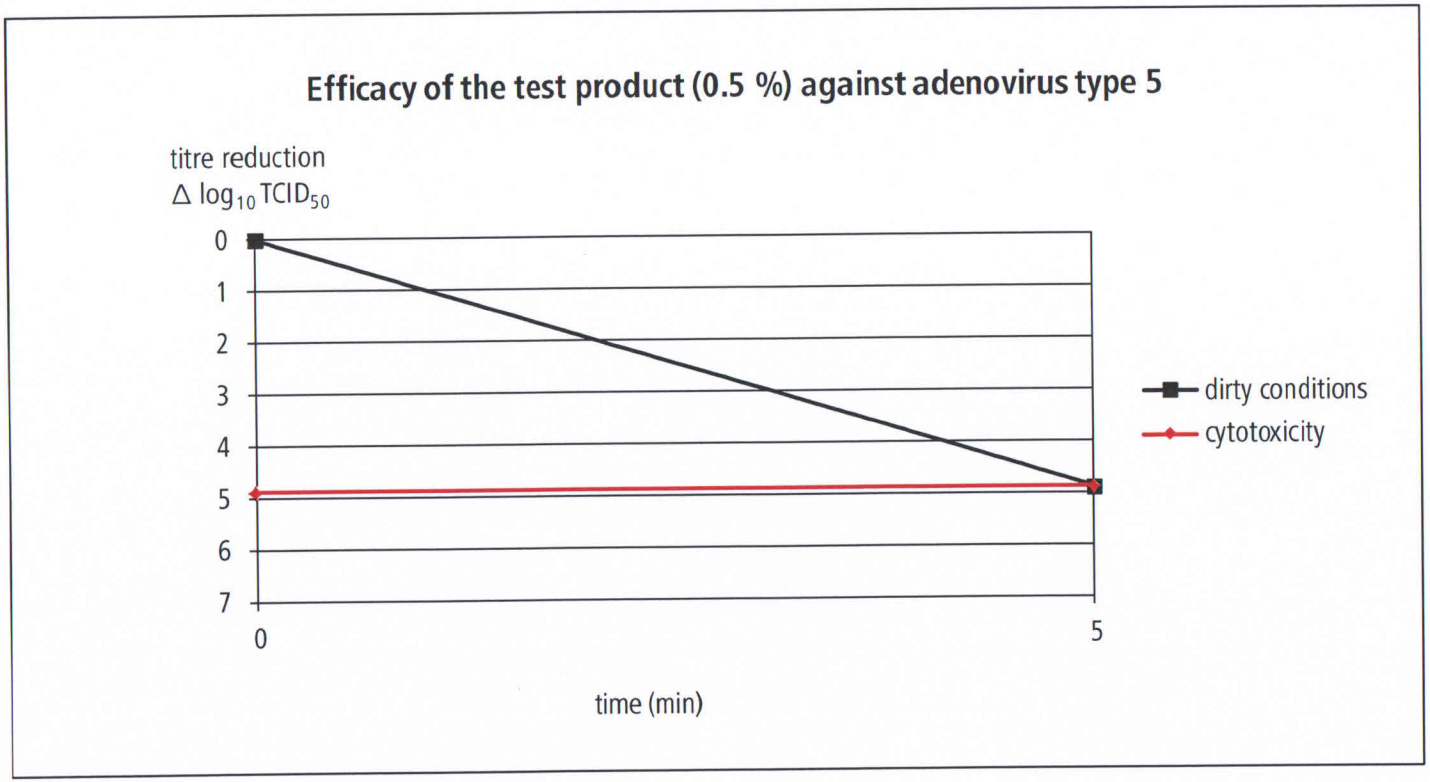

Figure 2: Virus-inactivating properties of formaldehyde (0.7 %)