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14.06.2010
Dr. St/BB

B. Braun Medical AG
Seesatz

CH-6204 Sempach

**Wirksamkeit von Helix ultra gegenüber dem Adenovirus Typ 5 im quantitativen
Suspensionsversuch nach EN 14476:2007-02 unter hoher Belastung**

GUTACHTEN

Diese Gutachten basiert auf dem Prüfbericht B10ML1055A vom 14.06.2010.

Das Instrumentendesinfektionsmittel Helix ultra der B. Braun Medical AG wurde gemäß Auftrag auf seine virusinaktivierenden Eigenschaften gegenüber dem Adenovirus Typ 5 nach der EN 14476:2007-02 untersucht.

In der EN 14476:2007-02 wird dann von einer Virus-Wirksamkeit eines Desinfektionsmittels ausgegangen, wenn nach einer bestimmten Einwirkzeit eine Reduktion des initialen Virustiters um $\geq 4 \log_{10}$ -Stufen (Inaktivierung $\geq 99,99 \%$) erfolgt ist.

Das Instrumentendesinfektionsmittel Helix ultra wurde als 1,0 %ige Lösung bei 20°C untersucht. Die Einwirkzeiten betrugen 5, 15 und 60 Minuten. Nach fünf Minuten war eine Reduktion des Virustiters um $\geq 4 \log_{10}$ -Stufen nachweisbar. Somit ergibt sich eine Adenovirus-Wirksamkeit unter hoher Belastung wie folgt:

1,0 % 5 Minuten



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Efficacy of Helix ultra against adenovirus type 5 in a quantitative suspension test at 20°C according to the EN 14476:2007-02 under dirty conditions

EXPERT OPINION


This expert opinion is based on the test report B10ML1055A dated 14.06.2010.

The virus-inactivating properties of the instrument disinfectant Helix ultra of B. Braun Medical AG against adenovirus type 5 were investigated by a quantitative suspension test according to the EN 14476:2007-02 under dirty conditions.

According to this suspension test, a disinfectant or a disinfectant solution at a particular concentration is considered as having virus-inactivating properties if within the recommended exposure period the titre is reduced by $\geq 4 \log_{10}$ (inactivation $\geq 99.99\%$).

Helix ultra was examined as 1.0 % solution at 20°C. The exposure times were 5, 15 and 60 minutes. After an exposure time of five minutes virus reduction exceeded 4 \log_{10} -steps. Therefore, a virucidal activity against adenovirus was measured under dirty conditions as follows:

1.0 % 5 min



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ZLG-P-429.08.10

14.06.2010

Test report B10ML1055A

Evaluation of the effectiveness of
Helix ultra

Test virus: adenovirus type 5

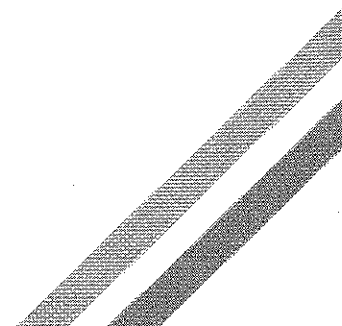
Method: EN 14476:2007-02 under dirty conditions

TEST REPORT

Sponsor:

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1. Introduction

The objective of this study was to evaluate the virus-inactivating properties of the instrument disinfectant Helix ultra against adenovirus type 5 using a quantitative suspension assay following EN 14476 (1) under dirty conditions.

2. Test laboratory

MikroLab GmbH, Norderoog 2, D-28259 Bremen

3. Identification of the sample

Manufacturer	B. Braun Medical AG
Name of product	Helix ultra
Batch number	1002BH0005
Application	instrument disinfection
Date of production	16.02.2010
Expiry date	02.2012
Active compound (s)	0.16 % peracetic acid (1.0 % solution)
Appearance and odour	white powder, neutral
pH-value (in WSH)	2.0 %: 7.72 (20°C) 1.0 %: 7.82 (20°C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	23.04.2010

4. Materials

4.1 Culture medium and reagents

- Eagle's Minimum Essential Medium with Earle's BSS (EMEM, Lonza Group Ltd., catalogue no. BE12-125F)
- Fetal calf serum (Biochrom AG, article no. S 0115)
- 1.4 % Formaldehyde solution (Chemisch-technologisches Laboratorium Dr. Melzer, D-28199 Bremen)
- Aqua bidest. (Fresenius Kabi Deutschland, article no. P2N 1636071)
- PBS (Invitrogen, article no. 18912-014)
- BSA (Sigma-Aldrich-Chemie GmbH, article no. CA-2153)



- sheep erythrocytes (Fiebig-Nährstofftechnik; article no. 31100100)

4.2 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* also originated from the Institute of Medical Virology, Hannover Medical School.

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.

4.3 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)
- Water bath (JULABO, Julabo U 3)
- Adjustable volume automatic pipettes (Eppendorf AG)
- Polysterol 96-well microtiter plate (Nunc GmbH & Co. KG, Wiesbaden, Germany)
- Cell culture flask (Nunc GmbH & Co. KG, Wiesbaden, Germany)
- Sealed test tubes (Sarstedt AG & Co., Nümbrecht, Germany)



5. Experimental conditions

Test temperature(s)	20°C ± 1°C
Concentration(s) of test product	1.0 %, 0.5 % and 0.1 % (non-active range) solutions
Contact time(s)	5, 15 and 60 minutes
Interfering substance(s)	dirty conditions: 3.0 g/l BSA + 3.0 g/l erythrocytes
Diluent	water of standardised hardness
Procedure to stop action of product	immediate dilution
Test virus	adenovirus type 5 strain adenoid 75 (ATCC VR-5)
Period of analysis	23.04.2010 – 14.06.2010
End of testing	14.06.2010

6. Methods

6.1 Preparation of test virus suspension

For preparation of test virus suspension according to EN 6.3 *A549 cells* were cultivated in a 175 cm² flask with Eagle's Minimum Essential Medium with Earle's BSS and 10 % fetal calf serum (FCS). Adenovirus type 5 (stock virus suspension) was added to the monolayer for 1 h at 37°C with gentle shaking every 15 min. After cells showed a cytopathic effect, they were treated with ultrasound (HD 2200, Bandelin electronic GmbH & Co. KG, D-12207 Berlin) followed by a low speed centrifugation (10 min and 1000 x g) in order to sediment cell debris. After aliquotation, test virus suspension was stored at -80°C.

6.2 Disinfectant

The test product was solved and diluted before the inactivation experiments with water of standardised hardness to 1.0 %, 0.5 % and 0.1 % solutions immediately before the tests. These concentrations were multiplied by a factor of 1.25 due to the addition of test virus suspension and interfering substance.

6.3 Infectivity assay

Infectivity was determined as endpoint titration according to EN 6.5.1 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 10³ 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.

6.4 Inactivation assay

Investigations for determination of virucidal activity followed EN 6.6. The test product was examined as 1.0 %, 0.5 % and 0.1 % solutions at 20°C.

Contact times were 5, 15 and 60 minutes.

Due to a more convenient handling, the volumes in this assay were 0.1 ml test virus suspension, 0.1 ml interfering substance and 0.8 ml test product. Immediately at the end of a chosen contact time, activity of the disinfectant was stopped by dilution to 10⁻⁸.

Titration of the virus control was performed at contact times 0 min and 60 min (EN 6.6.8).

6.5 Determination of cytotoxicity

Determination of cytotoxicity was performed according to EN 6.6.4.1 with 200 µl hard water and 800 µl test product.

6.6 Cell sensitivity to virus

For the control of cell sensitivity to virus two parts by volume hard 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 resuspended in cell culture medium (EN 6.6.4.2b).

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

6.7 Control of efficacy for suppression of disinfectant activity

Furthermore, a control of efficiency for suppression of disinfectant activity was included (EN 6.6.6).



6.8 Reference virus inactivation test

As reference for test validation 0.7 % formaldehyde according to EN 6.6.7.1 was included. Contact times were 5, 15, 30 and 60 min. In addition, cytotoxicity of formaldehyde test solution was determined following EN 6.6.7.2 with dilutions up to 10^{-5} .

7. Verification of the methodology

The following criteria as mentioned in EN 8.3 were fulfilled:

- a) The titre of the test virus suspension allowed the determination of $\geq 4 \log_{10}$ reduction.
- b) The cytotoxicity of the test product was one \log_{10} step (both 1.0 % and 0.5 % solutions) and allowed the detection of a four \log_{10} reduction of virus titre.
- c) The comparative titration on pretreated (disinfectant) and non-pretreated (PBS) A549 cells showed an acceptable difference ($<1 \log_{10}$; EN 8.3) of virus titre: 7.50 (PBS) versus 7.75 (1:100 dilutions of disinfectant).
- d) The control of efficacy for suppression of disinfectant activity (1.0 %) showed a decrease in virus titre due to the fact that even the 0.1 % solution was active.

Since all criteria according to EN 8.3 were fulfilled, examination with adenovirus type 5 according to EN 14476 was valid.

8. Results

Results of examinations are shown in tables 1 to 9. Tables 1 to 8 demonstrate the raw data, whereas table 9 gives a summary of results.

Helix ultra (1.0 %) was able to inactivate adenovirus type 5 after five minutes under dirty conditions in this quantitative suspension test. The reduction factors were measured: ≥ 5.00 and ≥ 5.13 . This corresponded to an inactivation of ≥ 99.999 %.

The 0.5 % solution was also active against adenovirus type 5 within five minutes incubation time (reduction factor: ≥ 5.13).

Even the 0.1 % solution was active against adenovirus type 5 after five minutes of exposure time (RF: ≥ 5.13).



9. Summary

In summary, a sufficient reduction of virus titre can be achieved by Helix ultra as 1.0 % solution after an exposure time of five minutes. Due to the lack of virological guidelines simulating practical conditions in Europe (phase 2, step 2 tests) the data of this quantitative suspension test lead to the recommendation to use the instrument disinfectant Helix ultra for inactivation of adenovirus type 5 as follows:

1.0 % dirty conditions 5 minutes

Bremen, 14.06.2010

Dr. Jochen Steinmann



10. 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.

11. Recorders to be maintained

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

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



Appendix

Table 1:	Raw data of Helix ultra (1.0 %) tested against adenovirus type 5 at 20°C (1 st assay) (dirty conditions)
Table 2:	Raw data of Helix ultra (1.0 %) tested against adenovirus type 5 at 20°C (2 nd assay) (dirty conditions)
Table 3:	Raw data of Helix ultra (1.0 %) tested against adenovirus type 5 at 20°C (3.0 % BSA)
Table 4:	Raw data of Helix ultra (0.5 %) tested against adenovirus type 5 at 20°C (dirty conditions)
Table 5:	Raw data of Helix ultra (0.1 %) tested against adenovirus type 5 at 20°C (dirty conditions)
Table 6:	Raw data of formaldehyde solution (0.7 %) tested against adenovirus type 5 at 20°C
Table 7:	Control of efficacy for suppression of disinfectant activity (1.0 %)
Table 8:	Raw data (adenovirus) for cell sensitivity to virus (1.0 %)
Table 9:	Results with Helix ultra and adenovirus type 5 (summary)



Table 1: Raw data of Helix ultra (1.0 %) tested against adenovirus type 5 (quantal test; 8 wells) at 20°C (2248) (1st assay)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log ₁₀)										
				1	2	3	4	5	6	7	8	9		
product	1.0%	3.0 g/l BSA + 3.0 g/l erythrocytes	5	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	
			15	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
product cytotoxicity	1.0%	3.0 g/l BSA + 3.0 g/l erythrocytes	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.	n.d.	
formaldehyde	0.7% (m/V)	PBS	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
			15	n.d.	n.d.	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.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
formaldehyde cytotoxicity	0.7% (m/V)	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
virus control	n.a.	3.0 g/l BSA + 3.0 g/l erythrocytes	0	n.d.	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	0223 3333	0003 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 2: Raw data of Helix ultra (1.0 %) tested against adenovirus type 5 (quantal test; 8 wells) at 20°C (2268) (2nd assay)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log ₁₀)									
				1	2	3	4	5	6	7	8	9	
product	1.0%	3.0 g/l BSA + 3.0 g/l erythrocytes	5	tttt	0000	0000	0000	0000	0000	0000	0000	0000	n.d.
			15	tttt	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.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product cytotoxicity	1.0%	3.0 g/l BSA + 3.0 g/l erythrocytes	n.a.	tttt	0000	0000	0000	0000	0000	n.d.	n.d.	n.d.	n.d.
			5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	n.d.	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.	n.d.
formaldehyde	0.7% (m/V)	PBS	60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			0	4444	4444	4444	4444	4444	2233	1000	0000	0000	0000
			60	4444	4444	4444	4444	4444	3323	0000	0300	0000	0000
formaldehyde cytotoxicity	0.7% (m/V)	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			0	4444	4444	4444	4444	4444	4444	4444	4444	4444	4444
			60	4444	4444	4444	4444	4444	3333	3000	0000	0000	0000
			n.a.	4444	4444	4444	4444	4444	3313	0000	0000	0000	0000

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 3: Raw data of Helix ultra (1.0 %) tested against adenovirus type 5 (quantal test; 8 wells) at 20°C (2268)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log ₁₀)							
				1	2	3	4	5	6	7	8
product	1.0%	3.0% BSA	5	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d. 0000
			15	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d. 0000
			30	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.
product cytotoxicity	1.0%	3.0% BSA	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.
			5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	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.
formaldehyde	0.7% (m/V)	PBS	60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3334 3334	1000 2033	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	2232 1332	0000 0000	0000 0000
formaldehyde cytotoxicity	0.7% (m/V)	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	3.0% BSA	n.a.	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 4: Raw data of Helix ultra (0.5 %) tested against adenovirus type 5 (quantal test; 8 wells) at 20°C (2268)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log ₁₀)								
				1	2	3	4	5	6	7	8	9
product	0.5%	3.0 g/l BSA + 3.0 g/l erythrocytes	5	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d. n.d.
			15	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	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.
product cytotoxicity	0.5%	3.0 g/l BSA + 3.0 g/l erythrocytes	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
formaldehyde	0.7% (m/V)	PBS	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	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.
formaldehyde cytotoxicity	0.7% (m/V)	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	3.0 g/l BSA + 3.0 g/l erythrocytes	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	2233 3323	1000 0000	0000 0300	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4443 3444	3333 3313	3000 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 of Helix ultra (0.1 %) tested against adenovirus type 5 (quantal test; 8 wells) at 20°C (2268)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log ₁₀)								
				1	2	3	4	5	6	7	8	9
product	0.1%	3.0 g/l BSA + 3.0 g/l erythrocytes	5	n.d.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			15	n.d.	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.	
			60	n.d.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	
product cytotoxicity	0.1%	3.0 g/l BSA + 3.0 g/l erythrocytes	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	
formaldehyde	0.7% (m/V)	PBS	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	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.
formaldehyde cytotoxicity	0.7% (m/V)	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
virus control	n.a.	3.0 g/l BSA + 3.0 g/l erythrocytes	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	2233 3323	1000 0000	0000 0300	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4443 3444	3333 3313	3000 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 of formaldehyde solution (0.7 %) tested against adenovirus type 5 (quantal test; 8 wells) (2253)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log ₁₀)									
				1	2	3	4	5	6	7	8	9	
product	n.a.	n.a.	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	n.d.	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.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product cytotoxicity	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
formaldehyde	0.7% (m/V)	PBS	5	tttt	tttt	4444	4444	2322	0002	0000	0000	0000	n.d.
			15	tttt	tttt	3333	3000	0000	0000	0000	0000	0000	n.d.
			30	tttt	tttt	0000	0000	0000	0000	0000	0000	0000	n.d.
			60	tttt	tttt	0000	0000	0000	0000	0000	0000	0000	n.d.
formaldehyde cytotoxicity	0.7% (m/V)	PBS	n.a.	tttt	tttt	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	3223	0022	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: Control of efficacy for suppression of disinfectant activity (1.0 %) (2268)

Product	Interfering substance	Dilutions (log ₁₀)								
		1	2	3	4	5	6	7	8	9
product	PBS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product	0.3 g/l BSA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product	3.0 g/l BSA + 3.0 g/l erythrocytes	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 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 8: Raw data (adenovirus) for cell sensitivity to virus (1.0 %) (2268)

Product	Interfering substance	Dilution	Dilutions (log ₁₀)								
			1	2	3	4	5	6	7	8	9
PBS	PBS	without	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PBS	0.3 g/l BSA	without	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PBS	3.0 g/l BSA + 3.0 g/l erythrocytes	without	4444 4444	4444 4444	4444 4444	4444 4444	4334 3334	0332 2324	0010 0000	0000 0000	0000 0000
test product	PBS	1:100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		1:1,000	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product	0.3 g/l BSA	1:100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		1:1,000	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product	3.0 g/l BSA + 3.0 g/l erythrocytes	1:100	4444 4444	4444 4444	4444 4444	4444 4444	4344 4444	2223 2331	0002 0000	0000 0300	0000 0000
		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)



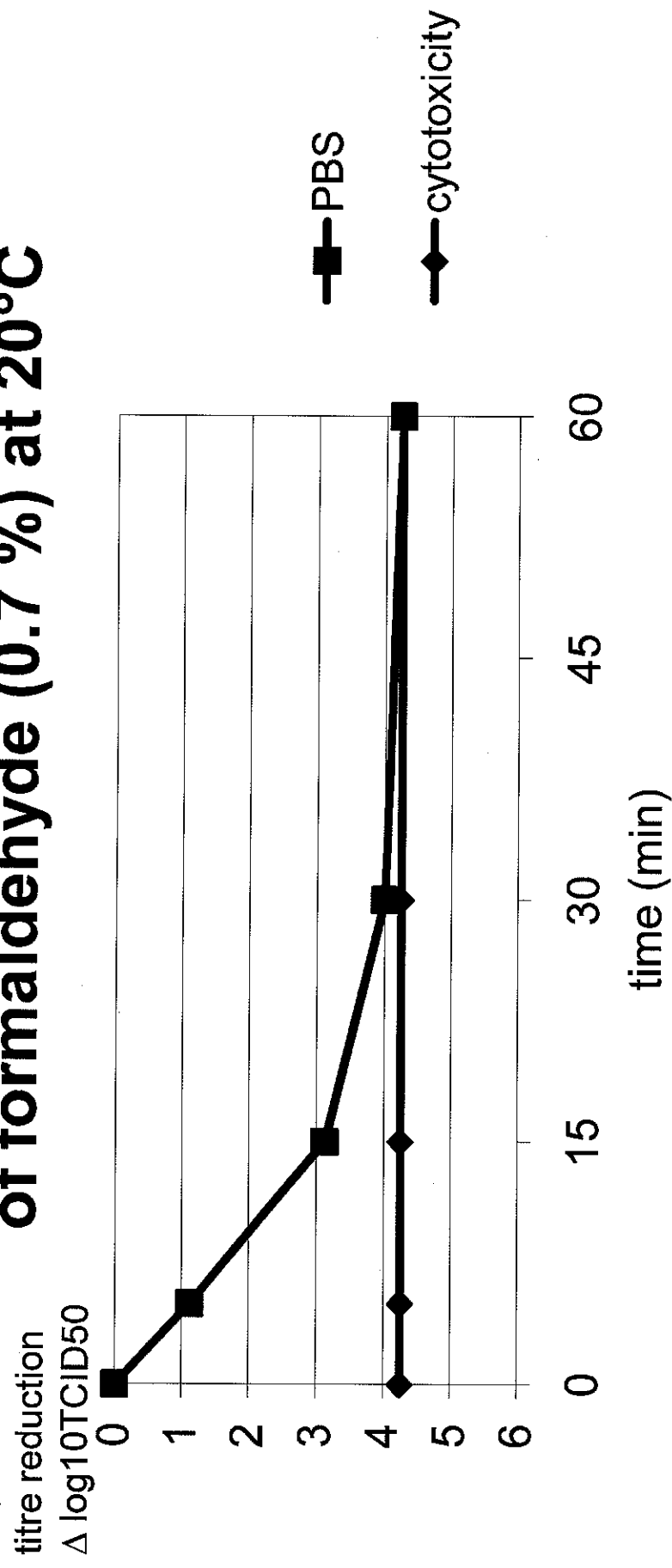
Table 9: Results with Helix ultra and adenovirus type 5 (summary)

Product	Con- centration	Interfering substance	Level of cytoto- xicity	log ₁₀ TCID ₅₀ /ml aftermin							≥ 4 log ₁₀ reduction after ... min	
				0	0.5	1.0	2.0	5.0	15.0	30.0		60.0
product	1.0%	3.0 g/l BSA + 3.0 g/l erythrocytes	2.50	n.d.	n.d.	n.d.	n.d.	≤2.50	≤2.50	n.d.	≤2.50	5.0
product	1.0%	3.0 g/l BSA + 3.0 g/l erythrocytes	2.50	n.d.	n.d.	n.d.	n.d.	≤2.50	≤2.50	n.d.	n.d.	5.0
product	1.0%	3.0% BSA	2.50	n.d.	n.d.	n.d.	n.d.	≤2.50	≤2.50	n.d.	n.d.	5.0
product	0.5%	3.0 g/l BSA + 3.0 g/l erythrocytes	2.50	n.d.	n.d.	n.d.	n.d.	≤2.50	≤2.50	n.d.	n.d.	5.0
product	0.1%	3.0 g/l BSA + 3.0 g/l erythrocytes	≤1.50	n.d.	n.d.	n.d.	n.d.	≤2.50	≤2.50	n.d.	≤2.50	5.0
form- aldehyde	0.7% (m/V)	PBS	3.50	n.d.	n.d.	n.d.	n.d.	6.63	4.63	≤3.75	≤3.50	30
virus control	n.a.	3.0 g/l BSA + 3.0 g/l erythrocytes	n.a.	7.75	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.50/ 7.63	n.a.
virus control	n.a.	3.0% BSA	n.a.	8.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.50	n.a.
virus control	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.75	n.a.
cell sens. (PBS)	n.a.	3.0 g/l BSA + 3.0 g/l erythrocytes	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.50	n.a.
cell sens. (disinfectant)	1:100	3.0 g/l BSA + 3.0 g/l erythrocytes	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.75	n.a.

n.a. = not applicable

n.d. = not done

**Fig. 1: Adenovirus-inactivating properties
of formaldehyde (0.7 %) at 20°C**



**Fig. 2: Adenovirus-inactivating properties
of Helix ultra (1.0 %) at 20°C**

